Efficacy trial of Double Fortified salt supplementation amongst vulnerable population and feasibility assessment for production

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CERTIFICATE

This is to certify that the thesis entitled "Efficacy trial of Double fortified salt supplementation amongst vulnerable population and feasibility assessment for production" is based on the research work carried out independently by Ms. Kejal Joshi in pursuit of a Doctoral Degree in Foods and Nutrition and represents her original work. This work has not been submitted for any diploma or degree of any other university.

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ABBREVIATIONS

C group	Control Group
C+DW group	Control + dewormed group
CT	Clerical test
DALY	Disability Adjusted Life Years
DFS	Double Fortified Salt
DIT	Diiodotyrosine
DLHS	District Level Health Survey
DMT	Draw-a-man test
E group	Experimental Group
E+DW group	Experimental + dewormed group
FePP	Ferrous Pyropohosphate
GDP	Gross Development Product
GDP	Gross domestic product
Hb	Hemoglobin
hCG	Human Chorionic Gonadotropin Hormone
I	Iodine
I^-	Iodide
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDA	Iron deficiency anemia
IDD	Iodine deficiency disorders
IFA	Iron Folic Acid
IIPS	Indian Institute of population sciences
IOM	Institute of Medicine
IQ	Intelligent Quotient
IRE	Iron regulatory element
IRP	Iron regulatory protein
IS	Iodized salt
KIO_3	Potassium Iodate
MI	Micronutrient Initiative
MIT	Monoiodotyrosine
MUIC	Median Urinary Iodine Concentration
NFHS III	National Health and Family Survey III
NHE	Nutrition Health Education
NIN	National Institute of Nutrition
NNMB	National Nutrition Monitoring Bureau
PII	Plasma inorganic iodide
PWC	Physical work capacity
RCH	Rural Child Health
RCT	Randomized control trial
RDA	Recommended Dietary Allowance
RIC	Renal iodine clearance
RNA	Ribo Nucleotide
SF	Serum ferritin
SHMP	Sodium hexametaphosphate

T3	Triiodothyronine
T4	Thyroxine
TfR	Serum transferrin receptor
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TRH	Thyrotropin-releasing-hormone
TSH	Thyrotropin, Thyroid-stimulating hormone
Tvol	Thyroid volume
UI	Urinary iodine
UIC	Urinary iodine concentration
UIE	Urinary iodine excretion
UNICEF	United Nations Children's Fund
USI	Universal salt iodization
VMT	Visual Memory test
WHO	World Health Organization
ZPP	Zinc protoporphyrin

ABSTRACT

Nutrition is a fundamental base of human life, health and development across the entire life span; from the earliest stages of foetal development, at birth, through infancy, childhood, adolescence and into adulthood and old age. Lack of the essential nutrients- vitamins and minerals- continue to be pervasive and they overlap considerably with problem of malnutrition. Iron and iodine deficiencies are most commonly prevalent among pregnant women, further extended to the developing fetuses in the womb affecting their mental and physical development. These deficiencies are also striking school aged children during their growth spurt and pubertal stages affecting their physical and mental productivity.

Combating these essential micronutrient deficiencies using a single, cost effective and a vehicle with stable formulation is the need of the hour to improve the status of the vulnerable population. Hence, National Institute of Nutrition (NIN) has developed a double fortification strategy with iodine and iron fortification in a same vehicle- Double fortified salt (DFS).

The present study was divided into three phases. Initial two phases were carried out to assess the efficacy of Double Fortified salt (NIN-DFS) supplementation towards combating iron and iodine deficiencies amongst vulnerable population- pregnant women and school children. The study subjects included, N=121 pregnant women (enrolled from a semi government hospital, Vadodara), N=947 school children (Rural villages of Vadodara). The pregnant women were divided into experimental and control groups based on the DFS as supplementation strategy/ non supplementation throughout gestation. However, school children were subdivided into four groups (E+DW, E, C+DW, C groups) including DFS and deworming as dual interventions for 9 months.

An estimation of iron and iodine content of the NIN-DFS revealed 40 ppm and 1050 ppm respectively which remained 37.5 ppm and 979 ppm indicating stability of DFS after one year.

Third phase of the study included medium and small salt producers were advocated and monitored towards technical salt iodization and three of them were further motivated to initiate DFS production at local level. There were N=38 producers enrolled from Anand, Kheda,

Nadiyad, Bharuch and Vadodara districts. Later N=3 producers were further included in the study phase towards assessing feasibility process for DFS production.

Efficacy of DFS supplementation was assessed using biochemical estimations (Hb, UIE, Thyroid hormones) and by cognitive tests (DMT, CT, VMT) of the subjects. Impact of salt advocacy was measured using salt iodine content estimation (Iodometric titration method).

The results at baseline for pregnant women on their nutritional status and biochemical parameters indicated 35% undernutrition, 90% iron deficiency (Hb estimation) and 16.79% iodine deficiency (UI estimation). Towards the end of the study, there was nominal improvement in proportion of non anemic subjects in experimental and reduction in control group. However, mean Hb (Baseling-9.44 g/dl to end-9.86 g/dl) improved significantly ($p<0.001$) among experimental group compared to non significant reduction (Baseline-9.35 g/dl to end- 9.15 g/dl) among control group. Median UIE was observed to be highest during second trimester among both the groups (333.2 μg/L) and it remained >150 μg/L for both the groups throughout gestation indicating sufficient dietary iodine through DFS and iodized salt consumed by experimental and control group respectively. Thyroid hormone analytes (TSH, FT_4, TT_4) remained in the normal ranges and no serious anomalities were observed on the neonatal outcomes. The results on the DFS supplementation or no supplementation including deworming as an additional strategy among school children (5-15 years) were availed. School children (N=947) were enrolled and subdivided into E+DW, E, C+DW and C groups randomly. Overall baseline data on anthropometry indices (CDC standards 2000) revealed 44.60% stunting, 70.78% underweight and 54.16% thinness amongst the children. However, 98% of the children were anemic and 30% were iodine insufficient. Towards the end, mean Hb improved (0.42 g/dl) significantly ($p<0.001$) compared to decrease (-0.54 g/dl) among control groups. Median UIE improved significantly ($p<0.001$) in both the groups and the prevalence of iodine deficiency decreased significantly ($p<0.001$). Thyroid hormones (TSH, FT_4, TT_4) were observed to be normal amongst majority of the children. IQ/cognition scores also improved significantly among experimental groups ($p<0.01$) compared to control groups. Visual memory test scores were observed sensitive towards IDA.

Nutrition health education (NHE) could play a vital role on KAP and dietary intake of the study population. Further, it helped to irradicate inappropriate food taboos and blind believes among

the study population related to food intake during pregnancy. However, NHE provided to the parents of the school children, helped to meet major proportion of RDA of the children. While data from salt iodization units (small scale and medium scale) revealed that, 20.6% producers iodizing at 30 ppm (recommended standards at production level) at baseline which improved significantly to 58.8% towards the end. Conceptualization of DFS has also achieved success, having N=3 producers being motivated to undergo training and production unit upgradation towards DFS production at local level.

Thus, our study concludes that, DFS could improve iron and iodine status of the experimental group compared to control group along with alternative strategies provided. Our NHE could benefit study population remarkably. It could also be stated that, production of DFS at local level could be achieved in nearing future. Further, it is recommended that, DFS shall be used as one of the crucial strategies for controlling iron and iodine deficiencies among all age groups.

CHAPTER 1
INTRODUCTION

Compromised nutrition in any age group has been identified as the world's most serious health problem and the single largest contributor to foetal or child mortality. Under nutrition is a status which affects not only current generation, but it is often extended into future generations while they are in the gestational stages into the wombs of their mothers. Lack of the essential nutrients- vitamins and minerals continue to be pervasive and they overlap considerably with problem of malnutrition (Kotecha PV 2008). Growth faltering observed in foetal stage as well as in the malnourished children which hampers intelligence, psycho motor and cognitive development. These in turn lead to slowing down of socioeconomic growth which can increase poverty and reduces productivity. Therefore economic cost of malnutrition is very high (Mason JB 2003).

India is second after Bangladesh with respect to the prevalence of underweight children in the world. India has 49 % of underweight children which contributes to 39 % of the world's underweight children. Approximately 21.8 % of the country's population consist of school going children. School children aging between 6-14 yrs, carry almost 63-73% prevalence of under nutrition. The prevalence varies from state to state, socioeconomic status of the population and their residential location. The most affected group is rural population (World Bank 2006) and lower strata of urban communities. Maternal nutrition has also the most important determinant influence during the development of foetus. Poor nutritional status during pregnancy is associated with inadequate weight gain, anaemia, retarded foetal growth, low birth weight, still births, preterm delivery, IUGR (intrauterine growth retardation), morbidity and mortality rates (Kansel et al. 2003, Sachdeva 2009). Thus, it may threaten the health and life of the mother and the newborn.

A review of such studies examining the relationship between mental development and severe malnutrition concluded that school-age children who suffered from early childhood malnutrition generally have poorer IQ levels, cognitive function, school achievement and greater behavioural problems than matched controls, and to lesser extent

siblings. The detrimental effect was observed to affect their adolescence and later age (Grantham-McGregor 1995)**.** Malnutrition in early stages has been found to have a long term effect on the growth and development of children, particularly on cognitive development.

Further it has been known to have short and long term effects on disease response, cognitive function, reproductive competence, work output and social behaviour of individuals (Osman et al 1993). It also encompasses micronutrient deficiencies. Among these deficiencies, Iron deficiency is the most detrimental for foetal life and childhood. However, iodine deficiency is been proven to be life threatening during foetal development and hazardous for the developing brains of the children. Thus, WHO has described reproductive age and childhood as the most vulnerable period for malnutrition. Iodine and iron deficiencies are highly prevalent in all the classes of the community in silent forms and thus have been tagged as 'Hidden Hunger'.

Despite recent progress in the fight against hunger and malnutrition in many countries, global food and nutrition security is still a far away goal (Stein A et al 2008). An estimated 820 million people in developing countries are undernourished (FAO 2006). Many more suffer from specific deficiencies in certain micronutrients: 2 billion people are anemic, many due to iron deficiency (WHO 2007) and 2 billion are iodine deficient (ACC/SCN 2004).

IODINE

Iodine is a micronutrient of crucial importance for the health and well being of all individuals. It is a trace element, just 5 gms of which are sufficient to meet the life-time needs of an individual with a life-span of 70 years (Dhaar GM and Robbani I 2008). Iodine deficiency disorders (IDD) is a collective term, which reflects the clinical and subclinical manifestations of iodine deficiency. Thus, making it the most common endocrinopathy in the world and also the most preventable cause of mental retardation (Patrick L 2008). The two major factors responsible for IDD are inadequate iodine intake

(due to inadequate supply from foods) and inadequate iodine utilization (due to consumption of goitrogens).

Hence, sustained insufficiency of iodine for a longer period in the circulation of human body leads to dysfunction of thyroid gland. Thus, it becomes one of the leading reasons for thyroid disorders amongst the population. The global goitre prevalence is more than 2 billion with more than 40 millions in India. The true prevalence and incidence in India of thyroid disorders is difficult to estimate, even conservative estimates put the geographical prevalence between 42 million including cases of iodine deficiency disorders. India is now predominantly iodine sufficient we are nearing the peak prevalence of the autoimmune epidemic. It is estimated that about 7.1 crores Indians are suffering while 20 crores people are at the risk of iodine deficiency disorders (IDD) in our country. As reported by (Sinha 2011), the Union Health Ministry is targeting to reduce IDD prevalence nationally to less than 10% by 2012 and 5% by the end of 2017.

IODINE SUPPLY AND DEFICIENCY DURING PREGNANCY

Over the past several years it has been proven that thyroid dysfunction is more common in adolescent girls than boys and thus may be a factor during reproductive age (Glinoer 1997). Pregnancy is a euthyroid state that is normally maintained by complex changes in thyroid physiology. Adequate iodine supply throughout life span can result into a successful pregnancy outcome.

As per thyroidological terminology, pregnancy is a physiological condition, in which a number of modifications may occur to affect thyroid economy. These events may take place at any point of gestation, leading to transient phase or may persist until term. Sometimes these alterations are permanent (Glinoer D, De Nayer P 1993). The key is to recognize and treat thyroid insufficiency at reproductive age before conception, since it may influence the outcome of mothers and fetus during and after pregnancy. It is associated with fetal loss, placental abruptions, pre-eclampsia, preterm delivery and reduced intellectual function in the offspring.

The prevalence of hypothyroidism during pregnancy is estimated to be 0.3-0.5% for overt hypothyroidism (OH) and 2-3% for subclinical hypothyroidism (SCH). The most contributing cause of hypothyroidism is iodine deficiency, known to affect over 1.2 billion individuals in the world (Ablovich M, Glinoer 2009).

Throughout the life cycle an individual's ability to alter synthesis, secretion or turnover of thyroid hormones in response to the changes in nutrient intake and ambient temperature has a large impact on heat production and body composition. This interaction is most striking during pregnancy and perinatal development when large perturbations in thyroid status within the mother, fetus or neonate may occur. Thyroid hormones are necessary to ensure normal development of brain, lung muscle, nerves, adipose tissue, heart and cardiovascular function in both fetus and neonate, although their role varies with gestational age and maturity at birth. Alterations in thyroid-hormone regulation, therefore, can cause large changes in growth, development and maturation of a number of organs and tissues that ultimately determine if an individual will survive (Symonds 1995).

IODINE DEFICIENCY DURING SCHOOL AGE

There have been many cross-sectional studies comparing cognition and motor function in children from chronically iodine-deficient and iodine-sufficient areas among children from Asia. The results have revealed that, compromised iodine nutrition leads to mean reduction in IQ of 12-13.5 points (Hetzel BS 1983, Zimmerman MB 2009). However, a set of few more studies reviewed by the author also revealed that in children born and raised in areas of iodine deficiency, cognitive impairment is at least partially reversible by iodine repletion (Zimmerman MB 2006).

Iodine status may also influence somatic growth through its effects on pituitary-thyroid axis. Thyroid hormone promotes growth hormone secretion and modulates the effects on the receptors. IGF-I and IGFBP-3 are also dependent of thyroid status. A metaanalysis by (Mason JB et al 2002) found positive correlation between anthropometric indices of the children and iodized salt access of the households.

THYROID PHYSIOLOGY AND IODINE METABOLISM IN HUMAN SYSTEM

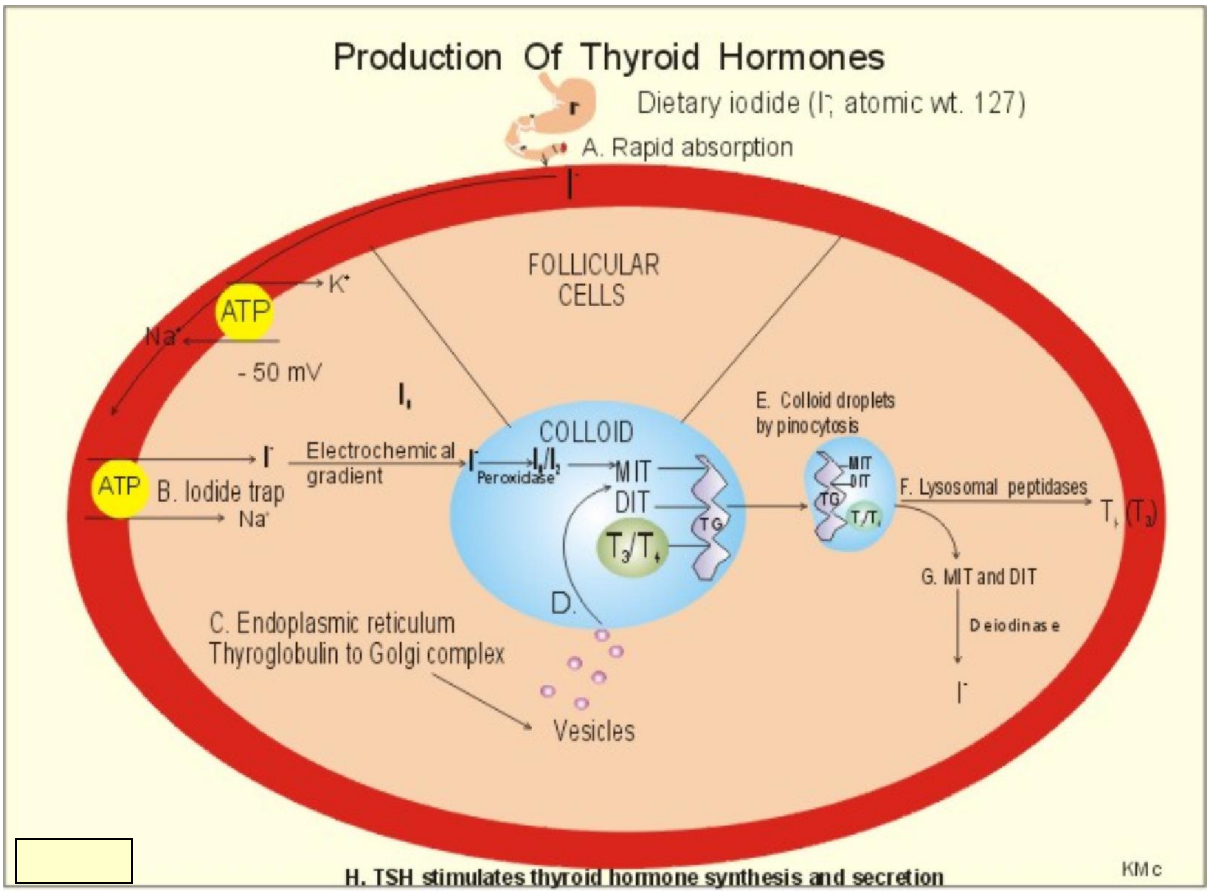
The thyroid gland plays a vital role in the metabolism of iodine. It is the largest of the exclusively function organs as an endocrine gland, weighing about 20 g in an adult. The thyroid gland comprises a unique structure, with a multiple follicles lined by follicular cells resting on a basement membrane. The follicular cells produce colloids. Thyroid follicular cells are cuboidal to columnar, and their secretory polarity is directed toward the lumen of the follicles. Polarity of follicular cells is important for iodine uptake, but the follicle structure is required for the synthesis of thyroid hormones. The luminal surfaces of follicular cells protrude into the follicular lumen and have numerous microvillus projections that greatly increase the surface area in contact with colloid. An extensive network of interfollicular and intrafollicular capillaries provides the follicular cells with an abundant blood supply (Capen 2000).

Follicular cells have long profiles of rough endoplasmic reticulum and a large Golgi apparatus in their cytoplasm for synthesis and packaging of substantial amounts of protein that are then transported into the follicular lumen. Numerous electron dense lysosomal bodies are present in cytoplasm, which are important in the secretion of thyroid hormones.

The individual steps in thyroid hormone formation and secretion (Ahad F. and Ganie S. 2010) may be characterized as follows **(Figure 1.1)**:

1. **Iodine trapping:** It is the first step in the metabolism of iodine. The process commences with the uptake of iodide from the capillary into the follicular cell of the gland by an active transport system. This occurs against chemical and electrical gradients by sodium/iodine symported protein (NIS) found in the basolateral membrane of the follicular cell; the energy required by thus process is linked to the ATPase dependent Na^+-K^+ pump.

Figure 1.1: Thyroid Hormone Synthesis And Secretion



Transport of iodide ion across the thyroid cell membrane is linked to transport of Na^+. The ion gradient generated by Na+-K+-ATPase appears to be the driving force for the active co-transport of iodide. I- is then passively translocated via a putative I- channel across the apical membrane into the colloid, located in the follicular lumen.

Other tissues such as the salivary gland, gastric mucosa, choroid plexus, ciliary body or the eye, and lactating mammary gland also have the capacity to actively transport iodide although at a much lower level than the thyroid. Only the thyroid follicular cells accumulate iodide in a TSH-dependent manner.

2. **Synthesis and Secretion of Thyroglobulin:** It is the second step. It occurs by another independent process within the follicular cell; the synthesis starts on the rough endoplasmic reticulum as peptide units of molecular weight 330,000 (the primary translation product of its messenger RNA). Later these units combine into a dimer,

followed by addition of carbohydrate moieties, after which the molecule moves to the Golgi apparatus. The completed thyroglobulin molecule contains about 140 tyrosine residues, which serve as substrate for the synthesis of thyroid hormones. The thyroglobulin is contained within small vesicles which then move towards the apical surface of the plasma membrane before being released into the follicular lumen.

3. **Oxidation of iodide:** The iodide within the follicular cell moves towards the apical surface of the plasma membrane, to enter into the follicular lumen; this transport by a sodium independent iodide/chloride transporter called pendrin. The iodide ($I^{'}$) is then immediately oxidized to iodine by (I).

4. **Organification of Thyroglobulin:** wherein iodination of the tyrosine residues present within the thyroglobulin molecules occurs. Iodination first occurs at position 3 to form monoiodotyrosine (MIT) and then at 5 to form diiodotyrosine (DIT). Iodination of tyrosine is followed by coupling reaction, whereby, two molecules of DIT couple to form thyroxine (T_4) hormone; and one molecule of MIT couples with one molecule of DIT to form Triiodothyronine (T_3) hormone. The reaction is catalyzed by thyroid peroxidises (TPO). The thyroid hormones are stored inside the thyroid follicles as colloid for several months. The stored hormones can meet the body requirements for 3 months.

The colloid containing iodinated thyroglobulin undergoes endocytosis, whereby it is salvaged from the follicular lumen by the epithelial cells; this is facilitated by TG receptor **megalin** which is present on the apical membrane. The colloid now enters the cytoplasm in the form of colloid droplets, which move towards the basal membrane possibly by way of microtubule and microfilament function. The colloid droplet next fuses with lysosome vesicles which contain proteolytic enzymes. The proteases help digest the thyroglobulin molecule releasing T_4, T_3, DIT and MIT into the cytoplasm. While T_4 and T_3 diffuse via the basal surface into the blood stream, the MIT and DIT get rapidly deiodinated by enzyme deiodinase. This mechanism helps to retrieve iodide for recycling along with tyrosine for recycling.

In the blood stream, T_4 and T_3 may circulate in the bound or free form; whereas 99% of T_4 and T_3 circulate in the bound form. The binding proteins include thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and thyroxine binding albumin (TBA). Binding of hormones apart from serving as a reservoir also helps to prevent urinary loss of hormones. The unbound hormones are biologically active. About 80% of circulating T_3, the most active thyroid hormone is derived from peripheral deiodination of T_4 hormone.

Thyroid secretion is regulated by pituitary gland through TSH which operates on a feed-back mechanism tuned to T_4 level in blood. A fall in T_4 level stimulates the pituitary to increase its TSH secretion which in turn stimulates the thyroid gland to release T_4 in circulation to maintain normal level of the hormone in blood.

Thyroid gland secretes 80 micrograms of iodine in the form of T_3 and T_4 hormones per day; 40 micrograms of iodine secreted appear in extracellular fluid (ECF) per day. T_3 and T_4 are metabolized in liver which releases about 60 micrograms of iodine in ECF and 20 micrograms of iodine into the bile to be excreted in stools. On an average, 480 micrograms of iodine get extracted in urine and 20 micrograms in stools per day.

Regulatory adjustments due to Iodine deficiency

Endemic goitre is an adaptive disease that develops in response to an insufficient supply of dietary iodine. When iodine intake is abnormally low, adequate secretion of thyroid may still be achieved by marked modification of thyroid activity (Zimmerman MB 2009). These adaptive processes include stimulation of the trapping mechanism as well as of the subsequent steps of the intra thyroidal metabolism of iodine leading to preferential synthesis and secretion of T_3. They are triggered and maintained by increased secretion of TSH. The morphologic consequence of prolonged thyrotropic stimulation is the development of goitre, which therefore appears as a mechanism of adaptation to iodine deficiency.

Increased iodide trapping, a fundamental mechanism of thyroid gland adapts to iodine deficiency to increase the trapping of iodide. This results in accumulation within the

gland of a larger percentage of ingested iodide and a more efficient reuse of iodide directly released by the thyroid or generated by the degradation of thyroid hormones. The increased iodide trapping is the result of both TSH stimulation of the iodide pump and perhaps TSH-independent augmentation of membrane iodide trapping involving the thyroid sodium symporter. This in turn must lead to two physiological changes:

1. Decreased amount of iodine excretion in the urine to preserve pre-existing iodine stores.
2. Ensure the accumulation in the thyroid of definite amounts of iodide per day (about 100 µg). This parameter is extremely important because it quantitatively controls all further steps of intrathyroidal iodine metabolism, including the secretion rate of thyroid hormones.

Stimulation of TSH and altered circulatory thyroid hormones- Clinically euthyroid adults in areas with severe iodine insufficiency leads to lower serum T_4, elevated TSH and normal T_3. However, only under conditions of extreme thyroid failure like in myxedematous endemic cretinism that both serum T_4 and T_3 are particularly low and serum TSH is dramatically elevated. In less severe goitre endemics, serum T_4 and T_3 levels are only slightly modified or remain normal.

Due to iodine fluctuating levels state of euthryoidism or hypo/hyperthyroidism precipitates in the human systems, where the up and down regulation of thyroid hormones takes place and leads to various conditions. These conditions are depicted in **table 1.1.**

Table 1.1: Changes in thyroid hormones in a variety of medical conditions

Condition	TSH	TT_4	TT_3	FT_4	FT_3
Euthyroidism	Normal	Normal	Normal	Normal	Normal
Iodine deficiency	Increase	Decrease	Increase	Decrease	Increase
Hyperthyroidism	Decrease	Increase	Increase	Increase	Increase
Hypothyroidism	Increase	Decrease	Decrease	Decrease	Decrease

(Reference: www.thyroidmanager.org)

DIETARY ALLOWANCES FOR IODINE

The dietary allowances of iodine recommended by the World Health Organization (WHO) are 150 µg/day for adolescents and adults, 250 µg/day for pregnant and lactating women, 120 µg/day for children 6 to 12 years of age and 90 µg/day for children below 6 years of age (WHO/UNICEF 2007).

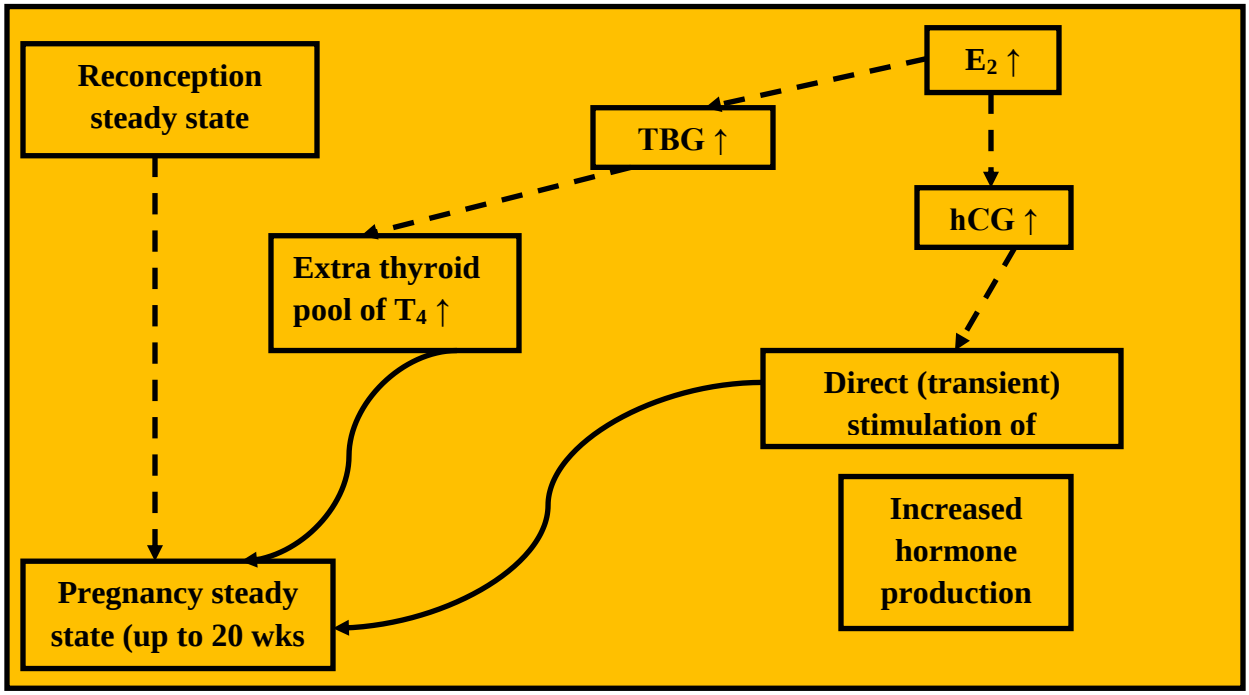
Iodine requirements during pregnancy are increased due to: (1) an increase in maternal T_4 production to maintain maternal euthyroidism and transfer thyroid hormone to the fetus early in the first trimester, before the fetal thyroid is functioning (12^{th} week of gestation); (2) iodine transfer to the fetus particularly in the later gestation and (3) an increase in renal iodine clearance (RIC) (Glinoer 1997). Increased RIC leads to misleading results on iodine deficiency prevalence, since increase renal clearance would increase UIE levels (an indicator of iodine deficiency in epidemiological studies), leading to decreased plasma inorganic iodide (PII) and thus the thyroid gland would lead to conservation mechanism of trapping iodine.

If these requirements are not met for a notable period of time in a given population, then thyroid dysfunctions may occur in a number of ways affecting functional and developmental efficiencies, including thyroid function abnormalities, when severe iodine deficiency occurs then- endemic goitre and cretinism, decreased fertility, increased perinatal death and infant mortality. These complications are preventable by appropriate iodine supply. The socioeconomic, cultural and political limitations may also hinder the sustained programs for iodine supplementation.

THYROID PHYSIOLOGY DURING PREGNANCY

Pregnancy leads to profound changes in thyroid function and iodine requirements (Glinoer D 2007). Increased concentration of estrogen leads to marked increase in the concentration of serum thyroxine binding globulin, which begins during early gestation, reaches a plateau at mid-gestation and is maintained thereafter.

Figure 1.2: Conceptual model of the changes in thyroid economy during pregnancy



E2- Estrogen, hCG- Human chorionic Gonadotrophin, TBG-Thyroxine binding Globulin, T4-Thyroxine, GA-gestational age.

During early gestation, there is an increased renal blood flow and glomerular filtration, which leads to an increased iodide clearance from the plasma and thus to an obligatory loss of iodine. This transition near the end of the first trimester, directs the stimulation of the thyroid gland by an increase in the concentration of human chorionic gonadotrophin that may lead temporarily to a slightly increased concentration of free thyroxine (FT_4) and decreased thyrotropin levels (TSH) due to competency of hCG. Finally, significant changes occur in the peripheral metabolism of maternal thyroid hormones during the second half of gestation, mainly under the influence of placental type 3 iodothyronine deiodinase (Bianco 2002). This further leads to reciprocation in the levels of FT_4 and TSH levels compared to first trimester.

Together, these events represent profound metabolic changes associated with the first half of gestation that constitutes a transition from a preconception steady-state thyroid gland to a pregnancy steady-state thyroid gland (Glinoer D 2004). In order for such metabolic changes to happen, this needs an increase in hormone production by the maternal thyroid

gland of about 50%. As **Figure 1.2** shows, once the new equilibrium has been reached, the increased demand for hormones during pregnancy is sustained until full term.

FETAL THYROID DEVELOPMENT

Thyroid system development in human foetuses can be divided into three phases that roughly correlate with the three classic trimesters of pregnancy.

Phase I: development during first trimester includes embryogenesis of the hypothalamus, the pituitary gland, and the thyroid gland.

Phase II: during second trimester, is a period of continuing foetal growth and relatively quiescent thyroid function.

Phase III: during the third trimester and the neonatal period, includes maturation of hypothalamic-pituitary- thyroid interaction and control. It also includes maturation of thyroid hormone metabolism and actions.

NEURONAL COMPROMISES DUE TO IODINE DEFICIENCY

Thyroid deficiency during the latter two thirds of gestation and the first months after delivery can result in mental retardation, since it would disrupt the migration of neuron in the fetal cortex and hippocampus (Lavado-Autric 2003). Thyroid hormones regulate the process of terminal brain differentiation such as dendritic and axonal growth, synaptogenesis, neuronal migration and myelination (Eayrs and Tylor 1951, Eyers and Horne 1955, Eyers 1955). There is retarded development of neutrophils in cerebral cortex and cerebeller Purkinje cells. Neuronal bodies are smaller and more densely packed, there is diminished dendritic branching and elongation, altered distribution of dendritic spines and delayed cell proliferation and migration (Nicholson and Altman 1972). Deficiencies of myelination are observed in the cerebral cortex, visual and auditory cortex, hippocampus and cerebellum. All these effects can be reversed by iodine supplementation but only if the supplementation is started at early stage (Chan S and Kilby MD 2000).

Thus, the lack of supply at maternal, fetal and young age would lead to thyroid anomalities; lower IQ and cognitive function are observed as the predominant ones with the effects on the developing brains.

IRON

Iron is one of the most essential micronutrient at every stage of human life, other than iodine and Vitamin A. It's deficiency is termed as iron deficiency, which is a major contributor of anaemia amongst the population, computed as iron deficiency anaemia (IDA). One third of the world's population suffer from anaemia. India has continued to be one of the countries with high prevalence of iron deficiency anaemia. According to NFHS III (2005-2006), the prevalence of anaemia is 70- 80 % in children. Anaemia affects the oxygen carrying capacity of the cells and thereby reduces the work capacity of the children. Iron deficiency along with iodine deficiency affects the developing brains, physical and mental growth of the children. However, there were >60% of the pregnant women in India suffered from IDA during NFHS-III (2005-2006). The percentages have remained unchanged till date, respite existing deficiency control programs. The prevalence is irrespective of economic grades of the women. However, severity is observed to be higher amongst lower income groups.

IRON DEFICIENCY DURING PREGNANCY

A high proportion of women in both industrialized and developing countries become anaemic during pregnancy. Estimates from the World Health Organization report (1992) that, from 35% to 75% (56% on average) of pregnant women in developing countries, and 18% of women from industrialized countries are anaemic. However, most of these women are already anaemic at the time of conception, with an estimated prevalence of anaemia at 43% in non-pregnant women in developing countries and 12% in women of wealthier regions. The prevalence of iron deficiency is far greater than the prevalence of anaemia and iron deficiency (low serum ferritin and sparse or absent stainable iron in bone marrow) often develops during the later stages of pregnancy even in women who enter pregnancy with relatively adequate iron stores (NFHS-III, 2005-2006). Hence, it

becomes essential to study the impact of iron supplementation during pregnancy on their own iron status as well as on the pregnancy outcome (Puolakka J. et al 1980).

DIETARY ALLOWANCES AND REQUIREMENTS FOR IRON

At the time of birth, neonates are born with ≈270 mg iron in their body. However, the total iron requirement for pregnancy is much higher than this. The mother's red blood cell count increases, the size of other tissues increases and the placenta itself has a substantial iron requirement. The placenta contains ≈90 mg of iron at term. The maternal red blood cell expansion accounts for ≈450 mg iron and the total basal losses are ≈230 mg. In sum, the total cost of pregnancy in terms of iron is ≈1.2 g (Bothwell 2000). In terms of balance, the mother can recover ≈600 mg of iron from cessation of menses and the recovery of red blood cells synthesized during pregnancy. This leaves a net requirement for iron of ≈600 mg. However, according to Indian data (NFHS III, 2005-2006) with 60-70% prevalence of anemia during pregnancy and mean hemoglobin concentration to be 9.17-9.19 g/dl, the requirement of iron is been set as per the health status and weight gain in all three trimesters. NIN has provided **(Table 1.2)** average calculation towards iron requirements during each trimester of pregnancy.

Table 1.2: Iron requirements during pregnancy

Trimester	Requirements of iron (mg)	
	10 kg GWG	**12 kg GWG**
1st trimester	130	138
2nd trimester	320	372
3rd trimester	310	351
Total	760	861

GWG- Gestational weight gain* **(Source: NIN 2009)

The recommended daily allowance for young children 6-9 years is 16 mg/day. The requirement increases with age. Hence, it goes about 21mg/d and 27mg/day for boys and girls of (10-12 years) respectively, it is 32 mg/d and 27mg/day for children 13-15 years (adolescents) of both the genders respectively.

MECHANISMS OF IRON UPTAKE AND TRANSFER TO THE FETUS

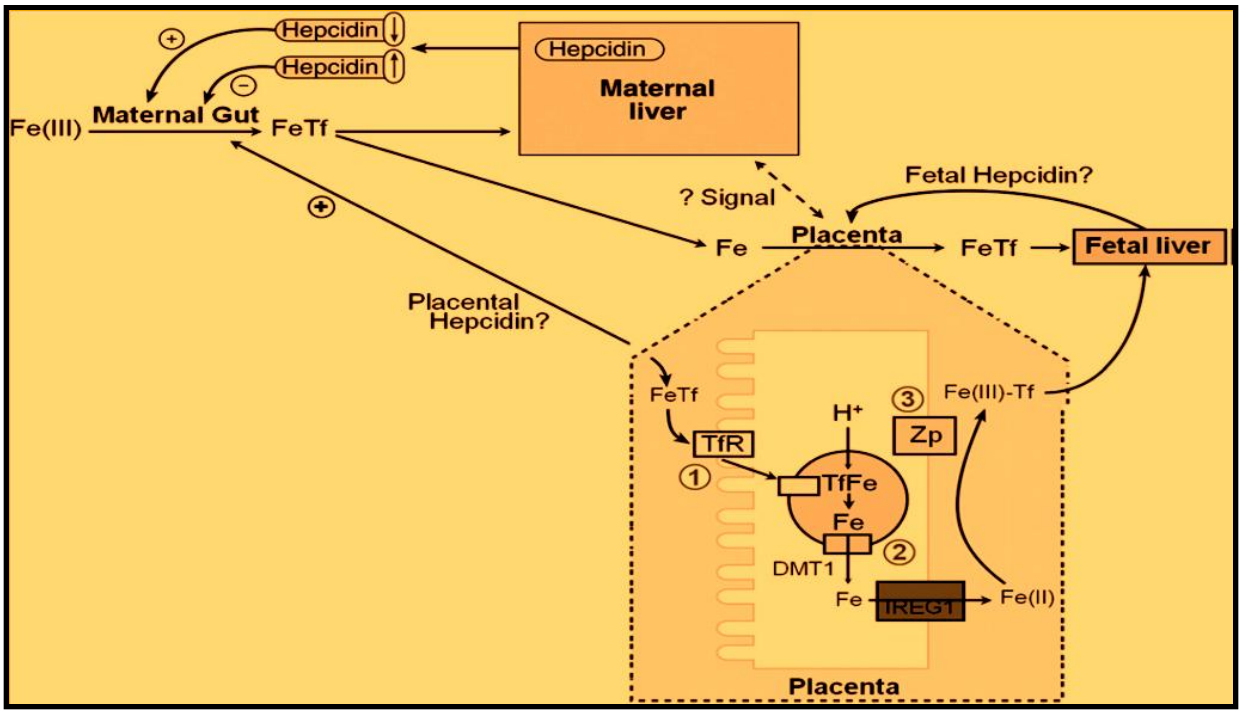
Gambling L. (2011) has demonstrated the possible mechanism in a very simple and understandable manner between feto-maternal complex. It reveals that, after it is absorbed across the maternal gut, iron is carried to the liver. In the serum, iron is bound to transferrin. Transferrin has 2 iron-binding sites with approximately equal affinities for iron. It is glycosylated, and, of interest, the glycosylation patterns change during pregnancy (Van Dijk JP, van der Zande FG, Kroos MJ et al 1993). The functional consequence(s) of these alterations is unknown (Jeschke U, Wang X, Briese B et al 2003).

In non pregnant conditions, 40% of the iron is taken up by the liver on the first pass through the portal circulation. Whether this is so in pregnancy is not known, but the liver still plays an important role in iron homeostasis. Iron stores in the liver is transferred to the fetus has not been studied directly. However, concentrations decrease significantly during pregnancy, so the process does occur, and presumably it is mediated by signals from the developing fetus. The nature of these signals is not yet known (Millard KN, Frazer DM, and Wilkins SJ et al 2004).

Although the release of iron from ferritin has been studied extensively, the underlying mechanism is still a matter of discussion. After it is released, the iron [as Fe(II)] is oxidized by ceruloplasmin to Fe (III) and incorporated into transferrin. The site of this interaction is also not clear, but presumably it occurs at the hepatocyte cell surface. Thereafter, transferrin is carried in serum to the placenta, where the steps outlined **(Figure 1.3)** take place. The transferrin binds to the transferrin receptor on the placental microvillar membrane surface.

After binding is completed, the complex is incorporated into clathrin-coated vesicles and internalized. The pH inside the vesicle is reduced, probably by an H+-ATPase. This has a very interesting effect: the iron is released from the transferrin. At pH 7.4, apo-transferrin (transferring with no iron on it) has a relatively low affinity for the receptor; therefore, apo-transferrin will not bind on the cell surface.

Figure 1.3: Diagrammatic presentation of feto-maternal iron transfer complex



(Source: Gambling L. 2011)

At pH 5.5 (the approximate pH inside the vesicle), the affinity of transferrin for Fe is greatly decreased; consequently, the iron is released from the protein and the protein becomes apo-transferrin. At this low pH, the receptor affinity reverses and apo-transferrin has a much higher affinity than diferric transferrin. Hence, in the clathrin-coated vesicle, as the pH drops and the iron is released, the transferrin protein stays bound to the receptor (Gambling L 2011).

This complex will eventually recycle back to the surface, where the apo-transferrin will be released, as the pH returns to 7.4. Inside the vesicle, iron [as Fe (II)] moves through a channel known as **divalent metal transporter 1 (DMT1)** (now formally classified as slc11a2) into the cytoplasm (Chong WS, Kwan PC, Chan LY et al 2005). Its transfer to the fetal side of the cell is not known. It is possible that carriers are used to ensure that the iron stays as Fe (II) and is not reoxidized, and also to prevent it from being involved in chemical reactions; however, to date, none have been identified.

Of interest, DMT1 may not be essential for the intracellular transport process. (Gunshin et al 2005) showed that the channel is not essential for iron transport across the placenta. This is a very intriguing observation that has some important implications. Either there is an alternative pathway of iron transport, or there is redundancy that allows iron to escape from the vesicle without a specific channel requirement.

The iron is released from the cell through a protein called ferroportin. Fetal transferrin (in fact, all transferrins) bind Fe as Fe (III), and hence it must be oxidized once it is released in order for it to bind to its carrier protein. This is carried out by a protein called zyklopen, a copper ferroxidase from a family of ferroxidases that are central to iron release (Chen H, Attieh ZK, Syed BA, et al. 2010). The mechanisms and steps involved are presented in Figure 1.3 (McArdle HJ, Danzeisen R, Fosset C, et al 2003 and McArdle HJ, Andersen HS, Jones H et al 2008).

REGULATION OF UPTAKE AND TRANSFER BY FETUS

Iron is a very reactive element, with the capacity to accept and donate electrons. This ability is central to its function, but it can also cause problems. Uncontrolled reactions can generate free radicals, with consequent peroxidation of lipids and membrane damage. A series of regulatory steps have evolved to minimize the risk of this happening. At the same time, because iron is essential, systems have evolved to make sure iron supplies are adequate for optimal function. This is especially true during pregnancy.

The amount of iron that is transferred from mother to fetus rises as gestation proceeds (McArdle HJ, Douglas AJ, Bowen BJ, et al 1985). The number of transferrin receptors on the placenta increases in parallel with iron accumulation, implying that it is the availability of transferrin- binding sites on the surface of the placenta that limits iron transfer (Gambling L, Danzeisen R, Gair S, et al. 2001).

The number of receptors rises as the amount of iron in the maternal diet decreases, meaning that a larger proportion of the iron absorbed is carried to the fetus. Most of the transferrin receptors are located on the microvillar membrane of the placenta (Bradley J,

Leibold EA, Harris ZL, et al 2004). When a cell accumulates excess iron, it stores it in ferritin. Ferritin and transferrin are regulated in an exceedingly elegant manner.

Each of the 2 mRNAs has an iron regulatory element (IRE) at either the 5' (ferritin) or 3' (transferrin receptor) end (Rouault TA et al 2006). This is a loop of RNA to which the iron regulatory protein (IRP) binds. When iron is present, it binds to the IRP and causes its release from the IRE. This release has different effects depending on where the IRP binds. Increased iron means increased iron stores, and releasing the IRP from transferrin receptor (TfR) mRNA destabilizes it so that it is degraded, whereas removing the IRP from ferritin mRNA releases it from being blocked from translation, so that ferritin protein is produced (Rouault TA et al 2006).

This very simple but neat system of interdependent regulation also applies to other iron-regulated proteins, such as DMT1, but it is important to note that there are other proteins that are not regulated like this. In the case of the placenta, ferroportin and zyklopen are 2 of the iron proteins that are not regulated by the IRE/IRP system. All of these systems are modulated by fetal liver iron concentrations. In fact, the fetal liver seems to regulate the whole process of iron absorption, from transfer across the maternal gut to storage in the maternal liver, concentrations in the plasma, and transfer across the placenta (Gambling L, Czopek A, Andersen HS, et al 2009).

NEURONAL COMPROMISES DUE TO IRON DEFICIENCY

During fetal development, iron is prioritized to red cells at the expense of other tissues, including the brain (WHO 1998, Lozoff B 2007). When iron supply does not meet iron demand, the brain is at risk even though the infant may not be anemic, since iron distributions are sensitive to stages of neurodevelopment, metabolic activity and neurigenerative pathologies of central nervous system (Beard J 2003). The most common etiology of reduced iron supply to the fetus is maternal iron deficiency (Lozoff B 2007).

Iron is required for proper myelination of the spinal cord and white matter of cerebellar folds (Kwik-Uribe CL et al 2000; Larkin EC and Rao GA 1990) and it is a cofactor for a

number of enzymes involved in neurotransmitter synthesis (Yehuda S and Youdim MBH 1989) as well as neurotransmitter catabolism. Iron is also a cofactor in a ratelimiting step in DNA synthesis.

The predominent brain cell type containing iron are Oligodendrocytes, plays a role in myelin formation. Lack of iron leads to decreased amount of these cells and composition of myelin (Aoki et al 1989; Morley R et al 1999; Siddapa et al 2002). Deficiency of iron is also been reported to affect energy metabolism of brain. Iron deficiency inutero is associated with significant decrease in GABA transaminase activities and thus affects hippocampus, striatum and globus pallidus (Beard JL 2003).

These damaging effects of iron deficiency lead to the birth of neonates with lower IQ, cognitive functions and motor skills, which progresses with the age. There has been a significant correlation established between iron deficiency and lower cognition. Thus, it also leads to hampered scholastic performance and active participation in the school children. This has also been proven by many research studies.

IRON DEFICIENCY DURING SCHOOL AGE

Iron is required by the body in very minute quantities, and yet plays a leading role in the production of enzymes, hormones and other substances, helping to regulate growth, activity, development and the functioning of the immune and reproductive systems. In IDA, physical work capacity (PWC) is reduced because the decrease in haemoglobin reduces the availability of oxygen to the tissues, which in turn affects the cardiac output (Beaton, Corey and Steel 1989). Further, in iron deficiency, changes in brain iron content and distribution, and in neurotransmitter function may affect cognition (Beard JL 2001). Anemia may produce scholastic under-achievement and behavioural disturbances in school children (Pollitt and Liebel 1976).

Research on preschool children has shown that iron deficient children performed lower on psychomotor tests than did non-anemics (Bhatia and Sheshadri 1992). However, little

is known as regards impact of anemia among children entering adolescence and those undergoing the pubertal growth spurt.

INTERRELATION BETWEEN IRON AND IODINE DEFICIENCIES

As reported by WHO 2001, Multiple micronutrient deficiencies coexists in developing countries at a higher rate due to monotonous diets (Zimmerman MB et al 2005) based on staple foods of low nutrient density. Along with iodine, other essential micronutrient deficiencies like iron, Vitamin A and Selenium may adversely affect the thyroid. Deficiencies of these micronutrients can act in concert with iodine deficiency to impair thyroid function and modify the response to prophylactic iodine (Arthur et al 1999; Zimmerman MB et al 2002; Zimmerman MB et al 2004). It is assumed that, IDA may induce alterations in central nervous system control of the thyroid axis (Beard JL et al 1998) and reduce T_3 binding to hepatic nuclear receptors (Smith SM, Johnson PE and Lukaski HC 1993).

IDA may also impair thyroid metabolism by decreasing oxygen transport, similar to the thyroid impairment in hypoxia (Galton 1972, Surks MI 1969). Chronically hypoxic children have lower level of circulating T_4 and T_3 and increased concentration of rT_3.

A study suggests the mechanism for impaired thyroid hormone metabolism in IDA is reduced activity of the iron-dependent enzyme, thyroid peroxidise (TPO). TPO is glycosylated heme enzyme active at the apical membrane of the thyrocyte (Yavuz et al. 2004). It catalyzes the two initial steps of thyroid hormone synthesis- iodination of thyroglobulin and coupling of the iodotyrosine residues **(Figure 1.4)** (Dunn JT and Dunn AD 2001). The justification suggests impaired thyroid function in IDA is due to reduced TPO activity, likely caused by decreased intracellular heme concentrations (Hess et al 2002).

Thus, pregnancy and school age are two crucial stages of life cycle, where the future generations are going to be affected.

Figure 1.4: The role of iron dependent thyroid peroxidise in iodine pathway

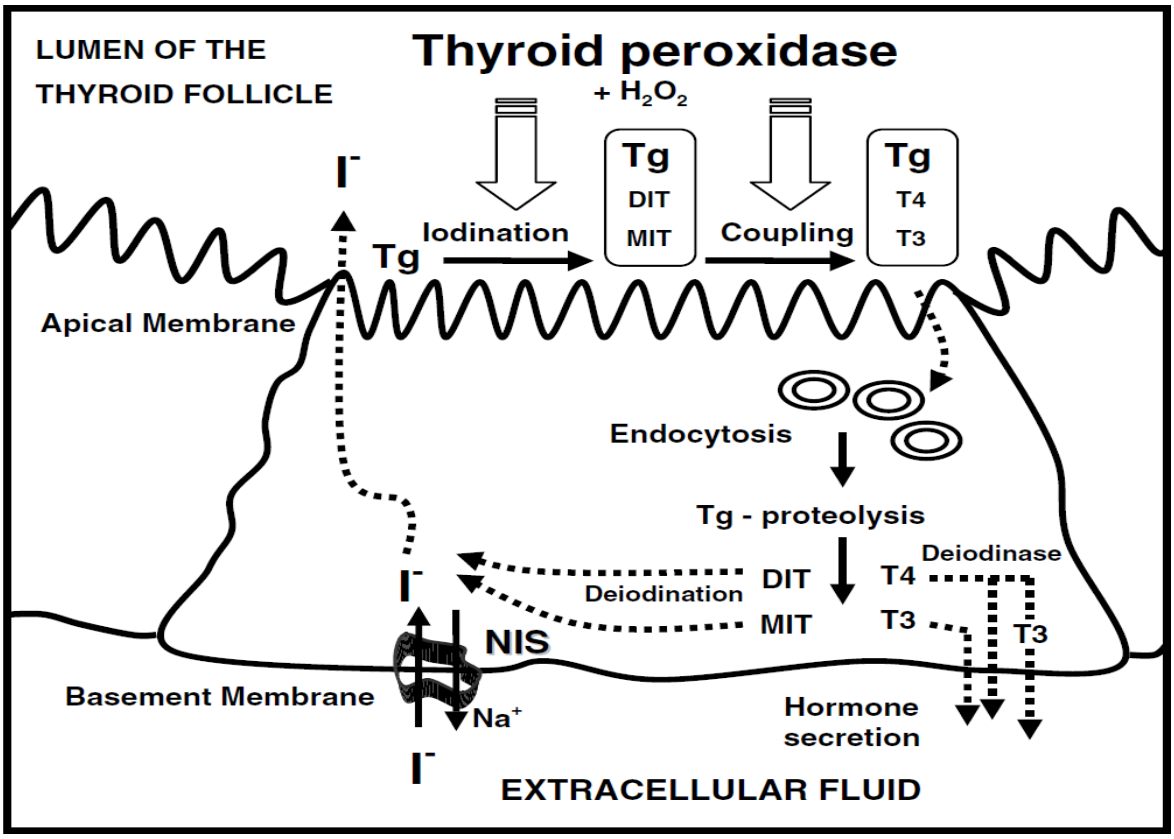


Figure 1.4 *The role of thyroid peroxidase in the iodine pathway in the thyroid cell.Iodide (I−) is transported into the thyrocyte by the sodium iodide symporter (NIS) at the basal membrane and migrates to the apical membrane. The I− is oxidized by thyroid peroxidase (TPO) together with hydrogen peroxidase (H2O2) and attached to tyrosyl residues in thyroglobulin (Tg) to produce the hormone precursors iodotyrosine (MIT) and diiodotyrosine (DIT). In a second step catalyzed by TPO, the residues then couple to form thyroxine (T4) and triiodothyronine (T3) within the Tg molecule in the follicular lumen. Tg enters the cell by endocytosis and is digested. T4 and T3 are released into the circulation, and nonhormonal iodine on MIT and DIT is recycled within the thyrocyte.*

PUBLIC HEALTH DETERMINANTS OF IODINE AND IRON DEFICIENCIES

Iron and iodine deficiencies may adversely affect each and every age group of human life cycle. The targeted effect at each level has been described in **Table 1.3** and **1.4**, indicating the importance of these essential micronutrients for humans.

Table 1.3: Consequences of IDD amongst humans

Pregnant women and their foetuses	Neonates	Infant/child/ Adolescent	Adult
Spontaneous abortions	Goitre	Goitre	Goitre and its compilations
Stillbirths	Overt or subclinical hypothyroidism	Subclinical or overt hypothyroidism	Hypothyroidism
Congenital anomalies	Cretinism	Mental retardation	Endemic mental retardation
Increased perinatal and infant mortality		Retarded physical development	Decreased fertility
Neurological cretinism: Mental deficiency, deaf mutism, spstic displegia and squints		Increased susceptibility of the thyroid gland to nuclear radiation	Spontaneous hyperthyroidism in the elderly
Myxedematous cretinism: Mental deficiency, hypothyroidism and dwarfism			Increased susceptibility of the thyroid to nuclear radiation
Psychomotor defects			

(Source : Marwah RK 2011)

Table 1.4: Consequences of IDA amongst humans

Consequences of Iron Deficiency	
Decreased maximum aerobic capacity	Impaired cognitive functioning and memory
Decreased athletic performance	Decreased school performance
Lowered endurance	Compromised growth and development
Decreased work capacity	Increased lead and cadmium absorption
Impaired temperature regulation	Increased risk of pregnancy complications, including prematurity and fetal growth retardation
Depressed immune function	
Increased rates of infection	

(Source: Provan D. 1999; Frith -Terhune AL, Cogswell ME, Khan LK 2000; Beard J, Tobin B. 2000)

STRATEGIES TO COMBAT MICRONUTRIENT MALNUTRITION

The main strategies suggested for improving community nutrition include: food-based strategies like dietary diversification and food fortification, for ensuring adequate

nutrition at household level; addressing behaviour modification to bring about dietary change in the population. This can be achieved through community-based nutrition interventions, using a social marketing approach, behaviour change through communication and mobilizing families and communities; control of micronutrient deficiencies; regular nutrition assessment and counselling; care during pregnancy and postnatal period and intersectoral linkages at community.

Food-Based strategy includes:

1. ***Dietary Diversification***
2. ***Food fortification***
3. ***Nutrition Education, Public health and Food safety measures***
4. ***Supplementation***

MICRONUTRIENT FORTIFICATION

Food fortification as the practice of deliberately increasing the content of an essential micronutrient, i.e. Vitamins and Minerals (including trace elements) in a food, in order to improve the nutritional quality of the food supply and provide a public health benefit with minimal health risk. Food fortification, sometimes called 'enrichment', refers to the addition of one or more vitamins or minerals to a food product or ingredient (WHO 2004).

IODIZED SALT- AS AN EFFECTIVE TOOL

India being a land of dietary contrasts, due to various dietary culture in between and within regions, salt iodization has been the most simplest, traditional and widely practiced method. Salt has been proven to be the best vehicle for fortification. The reasons for the salt iodization as a strategy are,

- It is one of the few commodities that come closest to being universally consumed by almost all sections of a community irrespective of economic levels or geographic locations.

- In India, there is a well established network of production, distribution and sale for common salt is existing.

- It is consumed at an approximate same level every day, throughout in a given region. Average intake in India 10gms/day/person. Thus a micronutrient like iodine introduced through salt will be administered to each individual at a uniform dosage every day, throughout in ones lifetime.

- In many remote areas of the world, that incidentally are also severely affected by IDD, salt is one of the few commodities that comes from outside the area thereby lending itself to processing on an economic scale under controlled conditions.

- The mixing of iodine compound Potassium Iodate (KIO_3)/ Potassium Iodide (KI) or Sodium Iodate ($NaIO_3$)/ Sodium Iodide (NaI) with salt is a simple operation. It produces no chemical reaction.

- The equipment required for salt iodization is simple, easy to operate and maintain.

- The addition of iodine to salt doesn't impart any colour, taste or odour to the salt. In fact iodized salt is indistinguishable from uniodized salt.

- It is economically lesser costly than any other food commodity as the cost is approximately 5 US cents/person/year- less than the price of a cup of tea.

Even when considering economic cost which includes cost of land, building, labor, equipment and other operating cost, addition of KIO_3 is only 10% of total economic cost.

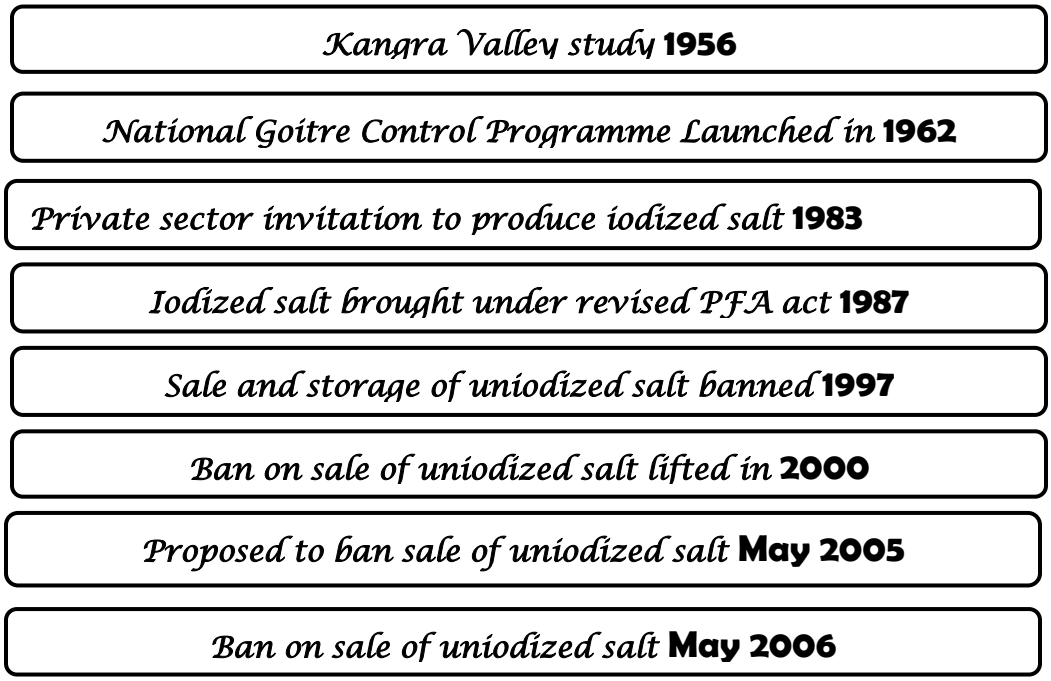
Therefore salt iodization is the preferred strategy for elimination of IDD and is being currently practiced in more than 130 countries. In India over the past few years considerable progress has been made in improving the availability and accessibility of quality of iodized salt to the population across the country (IDD Newsletter 2005).

"By consuming iodized salt, families can better protect themselves against IDD and can provide their children with an improved chance of physical and mental development. It will not only improve children's health, but it will have a significant impact on the

development of the nation itself. A generation as a prerequisite to a prosperous and productive nation!!"- (UNICEF 2004)

Thus, to prevent the detrimental implications to the vulnerable population, salt fortification has been proven to be the best strategies, than the others, since it makes it reach the fortified food ingredient to each and every member of the community along with their daily diet. Thus, the legislation for procurement and consumption of iodized salt by every human and livestock's has promised to improve iodine nutrition in the population. Salt iodization program has also undergone many political and community level ups and downs for universal acceptance, which had hampered its sustained functioning by making government to lift the ban for a while and again the scientific committees has provided sufficient evidences for the benefits of iodized salt consumption for the community **(Figure 1.5)**.

Figure 1.5: Overview of salt iodization program in India



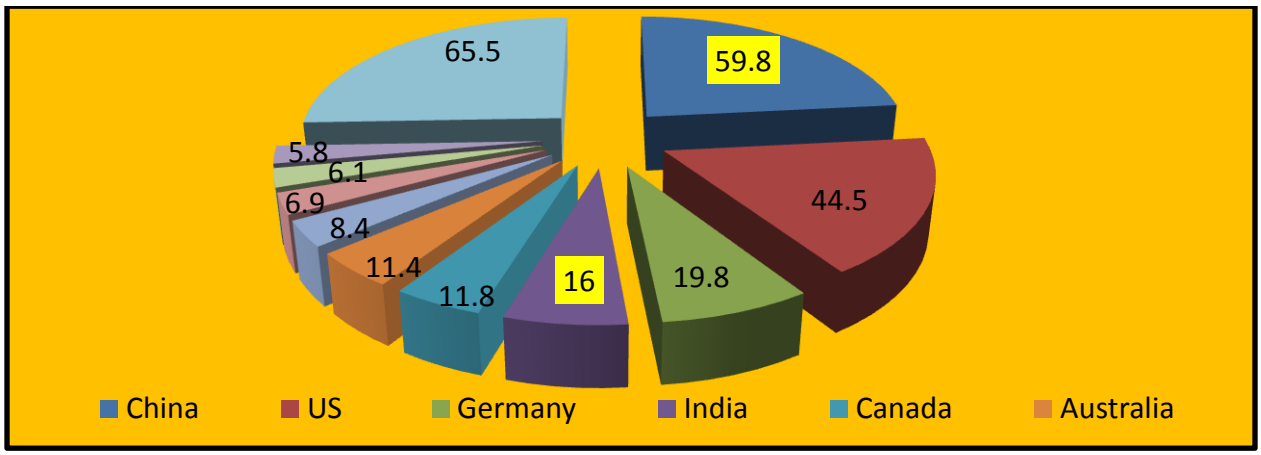
Salt iodization program in India, has passed through decades of acceptability and rejection by the community and political influences, though it is a 100 percent gain for the community. However, the struggle for the survival has brought favourable results with successful implementation of salt iodization program in current times and being headed towards increased iodized salt production and consumption since 2006. However,

small scale salt producers/ crushers are posing a main challenge. According to the field experiences and discussions it was gathered that, the cost of KIO_3 is assumed to compromise on their small margin of profit and thus their gain ends with almost nil. However, the lack of political commitment and unawareness of the democratic population on the incredible benefits of iodized salt consumption in the country are opined to be major reasons for the current status.

It is understood that, salt iodization process elicits 10 paisa/kg cost for the small scale salt industry and thus, the producers started escaping the iodization step. This resulted in closing of many of the salt plants due to very narrow profit margin. On the other hand malpractices of mislabelling for brand and iodine content started taking place. Thus, the small scale salt producers, who were unregistered with the salt department were became the target groups, to improve the salt iodization production and distribution in the community.

India has also been fortunate enough to have coastal regions and desserts, which are rich with suitable climatic conditions for quality salt crystal formations. Thus, in global map India has achieved and maintained its position in top five countries from last few decades.

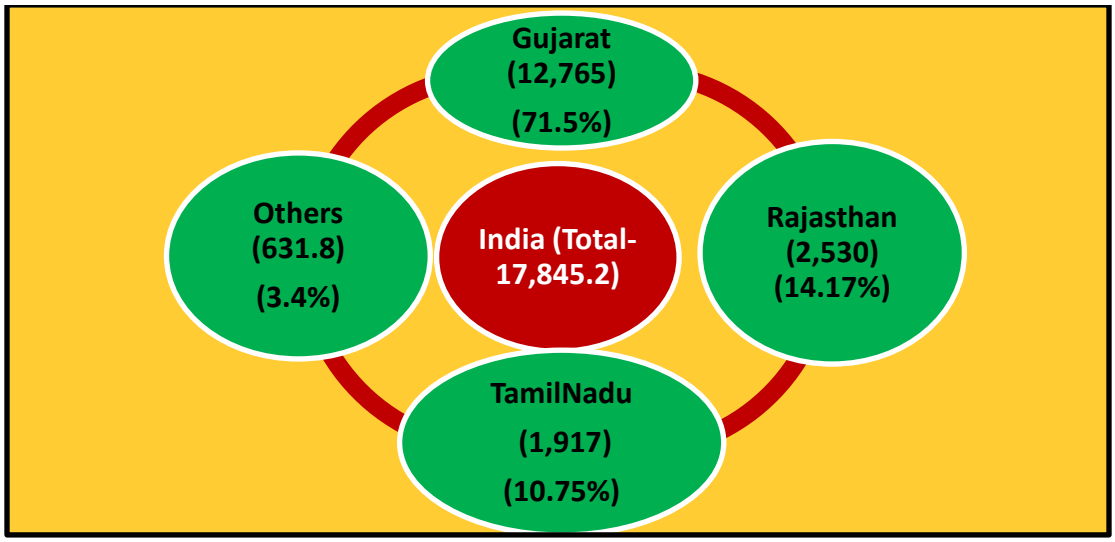
Figure 1.6: Global salt production for year 2007 ('000 Metric tonnes)



Global database on salt production for year 2007-2008 **(Figure 1.6)** has revealed salt production in India was 16 million tonnes, securing forth highest rank in the world.

Gujarat, the largest manufacturer of salt in the country, contributes 71% of the total salt Production. Thus, Gujarat should be the major area of focus considering its current production and its potential to increase quality production according to the demand of iodized salt.

Figure 1.7: Statewise production of salt in India (Production-'000 tonnes)



(Source: Annual Report 2007-2008, Salt Dept., GOI, Jaipur)

India is poised to show unprecedented economic growth by 2050. However in the South East Asia region, India is the worst performer after Pakistan and Afghanistan in consuming adequately iodized salt as per ICCIDD, 2008. The introduction of USI in deficient population can increase the average IQ of the population as much as 13.5 points. As a consequence, cognitive development and school performance are enhanced, leading to greater economic productivity for the population as a whole. Iodine deficiency can be eliminated for pennies per day. The cost benefit is enormous using iodized salt.

India is one of the 18 priority countries of United Nations who are yet to achieve USI. Despite the presence of an adequate programme and legislation, India is lagging behind in the consumption of iodized salt with 51% (NFHS-III). Thus India's effort to keep its prestigious role in shaping the global economy will face detrimental obstacles and will be unable to defeat the chronic deficiency of iodine by 2015 (Pandav CS 2008).

Unless iodine nutrition is maintained, the symptoms and signs of iodine deficiency will recur in a short period of time. Hence a steady progress up to 90% consumption of iodized salt, by all households is essential. Therefore sustenance of the programme is a major aspect to be focused on. It requires active commitment and advocacy on the part of the member states, based on national investments by salt producers, the public and the body politic (Hexton D. 2007).

To achieve this, an awareness campaign has to be generated and strong public private partnership to be forged with a firm commitment to resolve the issue soon (MI 2006).

Strengthening the salt iodization process in India is one of the most critical interventions needed. While the relatively easier task of getting the large and medium scale units to comply is on the way to being achieved, compliance by small and some medium scale salt producers continues to pose the main challenge.

Thus, the study of economics of salt trade becomes an important element of the strategy for USI. It would assist in identifying the trade related aspects which hamper salt iodization and at the same time help in promoting consumption of adequately iodized salt through better targeting. It is our hope that, the study would serve to initiate a meaningful dialogue with producers for quality production.

However, medium and large scale industries are doing well with quality iodized salt production and thus meeting the requirements of the all urban and few rural settings of the country. I can be expected that, the vigorous efforts towards upgraded of the iodized salt production at small scale industries would also earn the success in a nearing future.

Along with iodine deficiency disorder (IDD) prevalence of iron deficiency anaemia (IDA) is also one of the greatest challenges faced by vulnerable age groups especially the pregnant mothers and young children. The provision of iron supplements to pregnant women is one of the most widely practiced public health measures, yet the net results of attaining sufficient iron stores remain a challenge. Hence, the need for an intervention, which can target both the micronutrient deficiencies, was conceptualized using salt as a vehicle.

CONCEPTUALIZATION OF DOUBLE FORTIFIED SALT (DFS)

Typical Indian diets contain adequate amounts of iron, but the bioavailability of iron from rice and wheat, the staple cereals of Indians goes down, since it is affected by phytates and other inhibiting factors. In addition to that, the intake of meat products which are rich in heme iron is low due to low-socioeconomic status. Thus only 2-5% of the iron intake is absorbed, and it is one of the major causes of widespread iron deficiency.

As effectively advocated public health approaches towards the control and prevention of iron deficiency are the distribution of supplements of medical iron and fortification of foods with a suitable iron compound. Medical input of iron is recommended for a short-term measure for the correction of anemia, while fortified foods are used to improve the iron balance over a period of time and build up iron reserves. Since, India already has a program to supply iodized salt, double fortification of salt with iron and iodine makes eminent sense (Vinodkumar M. and Rajgopalan S. 2007). In India the efforts towards producing a stable formula containing iron first and later merging iodine and iron together, were pioneered by Dr. Narsinga Rao in early 70s.

As a sequel to the introduction of universal iodization of edible salt as a National Policy in the country, NIN evolved the concept of double-fortification of salt (DFS) with iodine and iron for controlling the deficiencies of both these micronutrients in a single measure as "one intervention controlling two problems." NIN-DFS was developed with good-quality food grade salt and chemicals. SHMP is a permitted food additive (JECFA 1992) and is extensively used in the food industry. SHMP (Sodium hexametaphosphate) in NIN-DFS is intended to protect and prevent the interaction of iodine from undesirable reactions with iron and other constituents of the salt in DFS. Good-quality food grade common salt (magnesium < 0.10%, moisture < 1.5%, NaCl > 98%) and food grade chemicals are used in the production of NIN-DFS. Higher levels of magnesium or moisture in salt are detrimental to the stability of iodine in DFS (Ranganathan S. et al 1996).

COMPARISON BETWEEN VARIOUS DFS FORMULATIONS IN INDIA

Table 1.5: Comparative features of different formulations of DFS

Characteristic	NIN formulation	MI formulation	Nutrisalt
Chemical composition			
Iodine (ppm) source	30-40 KIO_3	50 KI	30, KIO3
Iron (ppm) source	1000, Ferrous sulphate	1000, Ferrous Sulphate	1000, Iron salt
Stabilizer	SHMP	Encapsulation of iodine by dextran	Stabilizer and promoter
Laboratory studies			
Stability/ salt quality	Stable upto 9 months with the salt commercially used for iodization	Stable for 12 months. Salt used- refined quality.	Published data claims good stability. Iodine quality not indicated
Acceptability	Full fledged acceptability/ organoleptic properties described. No colour or smell. Good acceptability.	Develops slight yellow or brown colour with the time. Found less acceptable with some foods in all population studied compared to local salt	Reports claim acceptability and stability during cooking.
Bioavailability	Demonstrated on human and rat models. Mean absorption with rice based diet in humans is 6.1%. Urinary iodine excretion increased significantly equal to iodized salt	Demonstrated on human and rat models. Iron mean 13.5% absorption and 4.0% inhibition. Urinary iodine excretion increased significantly equal to iodized salt	Not reported
Production	Large-scale production	Not reported	Not known.

(Source: Sivakumar B and Nair S 2002)

The current status of DFS revealed by The Ministry of Health & Family Welfare, Govt. of India, constituted a Technical Committee under the Chairmanship of Dr.M. K Bhan, Secretary, Dept. of Biotechnology, Govt. of India, and Prof. N. K. Ganguly, Director-General, Indian Council of Medical Research, as Co-Chairperson on "Formulations of guidelines for use of double-fortified salt as a measure to reduce prevalence of anemia" The main Committee and the Sub-Committees met as per requirements and analyzed the data available on different formulations of DFS and finally approved only NIN-DFS because of convincing evidence from NIN-DFS based on (1) scientific publications, (2)

formulation, (3) nutrient level, (4) process, (5) ultrastructure, (6) salt quality, (7) stability, (8) organoleptic studies, (9) acceptability, (10) factory production, (11) community acceptance, (12) safety evaluation, (13) bioavailability, (14) iron impact, (15) iodine impact, and (16) cost (Ranaganathan S. and Sesikeran B 2008). Furthermore, the Dr. Bhan Committee recommended the introduction of NIN-DFS in nutrition programs (Bhan Committee 2006).

OPERATIONAL FEASIBILITY OF NIN-DFS

The operational feasibilities of NIN-DFS were successful, which are described as below:

Production- The technology of NIN-DFS is based on a simple method of dry mixing salt with iodine and iron compounds and does not involve elaborate or expensive measures (Ranganathan S. et al 1996). Large-scale production of DFS (9 to 60 metric tons) was successful in salt factories located at different cities throughout the country.

Transportation- Packing of NIN-DFS in 0.5-kg or 1-kg low-density polyethylene pouches and long-distance transportation by road to different parts of the country was found to be feasible and smooth (Brahmam GNV et al 1994, 2000; Rao N. 1994; Ranganathan S. et al 1996, 2005, 2007; Interim Report 2003).

Distribution- NIN-DFS was distributed to households periodically in 1-kg pouches in the community study in tribal areas of the East Godavari district of AP, while it was supplied in 50-kg sacs to the residential schools in Hyderabad for over 2 years in each study (Brahmam GNV et al 1994, 2000; Sivakumar B. et al 2001).

Biosafety – SHMP (Sodium hexametaphosphate) is an internationally permitted food additive (JECFA 1992). Furthermore, the daily ingestion of phosphorus is 30 mg through the intake of 10 g NIN-DFS. Nevertheless, the biosafety of NIN-DFS was reevaluated as an item for daily consumption through foods. The SHMP being a polyphosphate, perhaps could alter calcium/phosphorus turnover and thus bone metabolism. Therefore, the safety of long-term (9 months) feeding of DFS in relation to Ca and P metabolism was tested in rats. In addition, the hemoglobin regenerating ability of diet with DFS was compared

with both iron-fortified salt (IFS) and unfortified salt using a depletion-repletion rat model. The results at the end of 4 week revealed that the amounts of hemoglobin regenerated in both the fortified-salt-fed groups (DFS: 13.0 ± 1.4 and IFS: 11.7 ± 1.4 g/dL) were significantly higher than that in the unfortified salt group (7.6 ± 4.0 g/dL); at the end of 9 months, the hemoglobin levels increased to 15g/dL in both DFS and IFS groups; no untoward effect was observed on the integrity of bone and the histopathology of various tissues in experimental rats (Nair M et al 1998a). It was concluded from the results that long-term feeding of NIN-DFS containing SHMP does not apparently impair Ca and P balances in rats and is relatively safe in day-to-day use in the diets. Similar results were obtained for Ca and P balances in children (Nair M et al 2000). Thus, the daily consumption of DFS was proved to be safe.

Efficacy **-** NIN-DFS has provided sufficient evidences on the efficacy to improve iron and iodine status of the consumers at its best. The community based interventional studies have provided the scientific proofs in the form of biochemical parameters.

Cost **-** All the ingredients to manufacture NIN-DFS are readily available in the country at low cost. From the current cost of the materials, the approximate cost of production works out to be Rs. 4.85 (0.121 US$) per kg and when profits and transport costs are included it would be Rs. 6.85 (0.171 US$) per kg. Thus, the expenditure would be about a paisa (0.00025 US $) per head per day (Technical Report 2005).

BENEFIT: COST RATIO OF FORTIFIED FOODS

The World Bank estimated that the combined economic costs of iron deficiency, iodine deficiency and Vitamin A deficiency in developing countries could waste as much as 5% of gross domestic product (GDP). On the same lines (Murray and Lopez 1996) calculated the ‘global burden of disease” in which iron-deficiency anaemia, iodine deficiency and Vitamin A deficiency accounted for 2.4% of the overall disease burden of developing countries. However, four times higher percentage (9% to 10% of the disease burden in developing countries with high mortality) to iron-deficiency anemia, Vitamin A deficiency and Zinc deficiency were reported by WHO in 2002. GDP loss due to single

micronutrient deficiency- IDA was calculated to be 4.5% in India (Horton S and Ross 2003).

Thus, the concept of biofortification of the foods gains the importance to defeat the prevalent deficiencies affecting India's economy and obstructing its way to become super economy by 2015. However, the circle again comes to the starting point, indicating a need for a universally consumed food item or ingredient for Indian population and the only answer is "Double Fortified Salt".

Salt as a vehicle to supplement iodine also has been proven to be an effective fortificant to provide iron also. NIN has ventured with a stable formula of Double Fortified Salt providing Iodine- 40 ppm and Iron- 1000 ppm / gm of salt. Efficacy trials in rice based population have been carried out and have given motivating results by improving iodine and iron status of the subjects. It has also showed a good stability of both micronutrients in DFS. Hence it was necessary to run an efficacy trial amongst wheat consuming population in rural and urban scenario.

Thus, the present work was undertaken to assess the impact of double fortified salt supplementation at life cycle approach and putting a step ahead towards the possibilities of production at local level.

Thus, this study was conceptualized as an attempt and determination to improve micronutrient status of the target groups, using multiple approaches inculcating with the supply of an incredible food ingredient-DFS. The broad objective of the research work carried out was **"To study the efficacy of Double Fortified Salt supplementation amongst pregnant women and school aged children on iodine and iron status and feasibility assessment for DFS production at local level".**

SPECIFIC OBJECTIVES

The study was comprised of three phases:

Phase I: Impact assessment of Double Fortified Salt supplementation amongst pregnant women

- Screening of pregnant women of urban Vadodara for iodine and iron deficiencies.
- To assess nutritional status through anthropometry.
- To assess socioeconomic status (SES), knowledge-attitude-practices (KAP) and dietary consumption pattern.
- To assess iodine and iron content of Double Fortified Salt.
- To supplement DFS amongst pregnant women and assess the impact on iodine and iron status.
- To provide nutrition health education and behavior change communication for iodine and iron nutrition.
- To assess impact on neonatal anthropometry and cord blood thyroid hormone analytes.

Phase II: Impact of Double Fortified Salt supplementation amongst rural school children

- To assess nutritional status through anthropometry of the school children.
- To map the prevalence of iodine and iron deficiency.
- To assess parental SES, KAP and food consumption pattern of the families.
- To assess cognitive parameters of the school children.
- To assess iodine and iron content of Double Fortified Salt.
- To supplement DFS to experimental group
- To provide NHE and BCC, to mothers and family members regarding the usage, storage and cooking practices using iodized salt or double fortified salt.

- To assess the impact of DFS supplementation or encouraged iodized salt consumption on anthropometry, iodine, iron and cognitive parameters of the children.
- To assess the impact of NHE and BCC on the KAP of the mothers of the children.

Phase III: Feasibility for Double Fortified Salt Production at local level

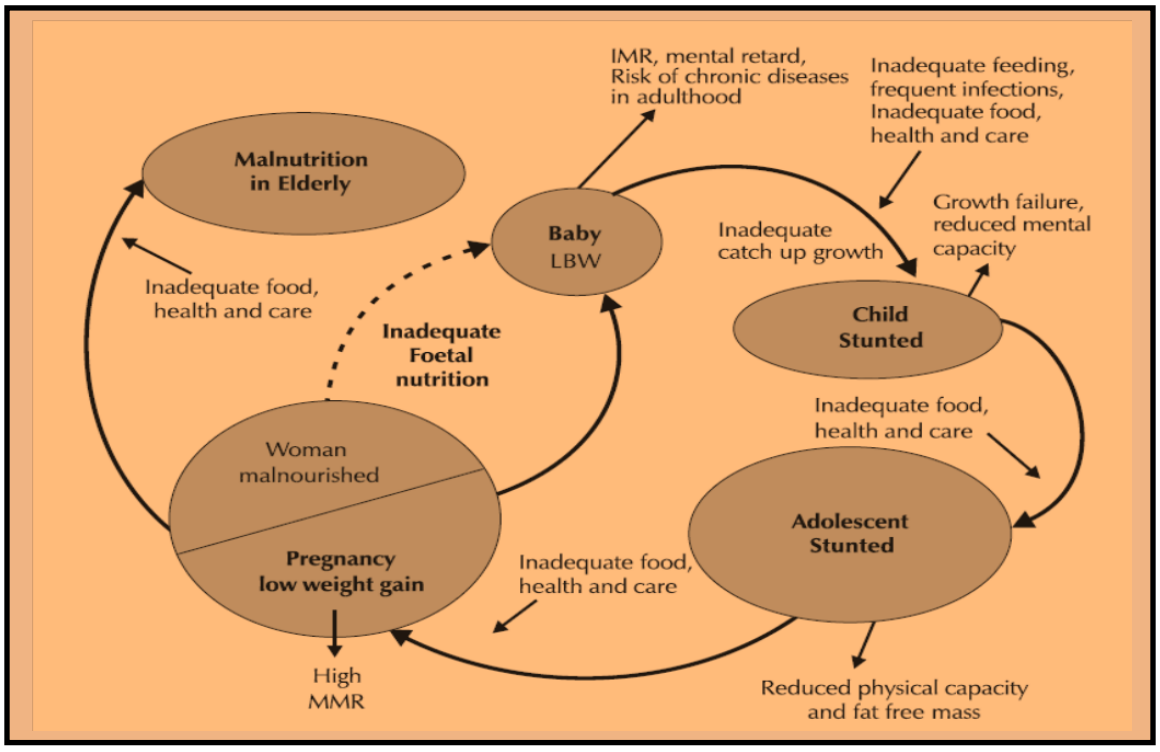
- To map the small scale salt units and assess the salt iodization levels in Anand, Nadiyad, Vadodara, Bharuch and Kheda districts of Gujarat.
- To build up the capacity of small scale salt producers of these districts for salt Iodization.
- To provide technical support for optimal iodization.
- Selecting salt producers for initiation of double fortified salt at medium scale and large scale.
- To initiate the conversation on feasibility for DFS production at local level.
- Laisoning efforts for the producers.

CHAPTER 2
REVIEW OF LITERATURE

Nutrition is a fundamental base of human life, health and development across the entire life span; from the earliest stages of foetal development, at birth, through infancy, childhood, adolescence and into adulthood and old age. Appropriate food and adequate nutrition are essential for the survival, physical growth, mental development, performance and productivity, health and well-being (WHO 2000; Ijarotimi O.S. et al. 2007).

Poor nutrition starts before birth, and generally continues into adolescence and adult life and can span generations. Chronically malnourished girls are more likely to remain undernourished during adolescence and adulthood, and when pregnant, are more likely to deliver low birth-weight babies. Epidemiological evidence from both developing and industrialized countries now suggests a link between foetal under-nutrition and increased risk of various adult chronic diseases (ACC/SCN, 2000). It is thus imperative to prevent malnutrition at every stage of the life cycle.

Figure 2.1: Nutrition throughout life cycle (Source: ACC/SCN 2000)



More than half of the world's undernourished population lives in India (Krishnaswami 2000) and more than half of Indian children are undernourished (Measham and Chatterjee 1999). About 39% of the adolescents were stunted irrespective of the sex in study conducted in rural areas of India.

2.1 IODINE DEFICIENCY

Iodine deficiency has multiple adverse outcomes on growth and development in animals and humans lives. Iodine plays a critical role in neuropsychological development of the foetus throughout gestation and in the first two years of life (NHMRC Australia 2009).

2.1.1 CAUSES OF IODINE DEFICIENCY

- **Environmental factors**

The main cause of iodine deficiency in soils is leaching by glaciation, floods or high rainfall. Mountainous regions have some of the highest prevalence of iodine deficiency. Iodine deficiency also occurs due to flooding, which leads to lower iodine content of the vegetation and crops cultivated in the land with lower iodine content.

Unclean drinking water may contain humic substances that block thyroidal iodination, and industrial pollutants, including resorcinol and phthalic acid, may also be goitrogenic (Gaiten E 1989).

Perchlorate is a competitive inhibitor of thyroidal iodine uptake (Blount BC, et al.2006). It appears that most of these goitrogenic substances do not have a major clinical effect unless there is coexisting iodine deficiency.

- **Consumption of Goitrogens**

Dietary substances that interfere with thyroid metabolism can aggravate the effect of iodine deficiency; they are termed goitrogens (Gaitan E 1989). Many vegetables and staple foods consumed in developing countries contain cyanogenic glucosides that can liberate cyanide. Cyanide is converted to thiocynate in the body. This is a goitrogen, as it blocks the uptake of iodine by the thyroid. With the exception of cassava, cyanogenic

glucosides are located in the inedible portion of plants. Cruciferous vegetables, including cabbage, kale, cauliflower, broccoli, turnips etc. contain glucosinolates. Similarly cassava, lima beans, linseed, sorghum and sweet potato contains cyanogenic glucosides; both these contents can be converted to thiocynate.

Cassava, contains linamarin- a thioglycoside. Thus, it must be soaked before consumption, if not soaked adequately or cooked to remove the linamarin, it is hydrolyzed in the gut to release cyanide, which is metabolized to thiocynate (Ermas AM, et al. 1972). The adverse effects can also be overcome by increasing iodine intake.

Soy and millet contain flavanoids that may impair TPO activity. Soy-based formula without added iodine has been observed to affect infants, but in healthy adults, soy-based products appear to have negligible effects on thyroid function (Messina M and Redmond G 2006).

- **Smoking**

Cigarette smoking is associated with higher serum levels of thiocynate that may compete with iodine for uptake via the NIS into both the thyroid and the secretary epithelium of the lactating breasts; thus smoking during lactation is also associated with reduced iodine levels in breast milk (Lauberg P, et al. 2004).

- **Other micronutrient deficiencies**

Deficiencies of selenium, iron and Vitamin A exacerbate the effects of iodine deficiency. Selenium is an essential component of the enzymes, Gluthione peroxidase and type I deiodinase. Glutathione peroxidase deficiency may lead to accumulated peroxides and may damage thyroid. However, type I deiodinase, catalyzes the conversion of thyroxine (T_4) to triiodothyronine (T_3). Combined iodine and selenium deficiencies are thought to cause the myxoedematous form of goiter (Zimmerman MB 2002).

Iron deficiency impairs thyroid hormone metabolism because the two first steps in thyroid hormone synthesis are catalyzed by thyroperoxidise, which are iron requiring enzymes. Iron deficiency lowers plasma T_3 and T_4 concentrations, reduces the rate of conversion of T_4 to T_3, and increases thyrotropin concentrations. Because of these

impairments in iodine metabolism, goiter in anemic individuals may be less responsive to iodine treatment (Zimmerman MB, et al. 2006). Pregnant women are highly vulnerable to iron deficiency anemia and poor maternal iron status predicts both higher TSH and lower T_4 concentrations during pregnancy and in area of borderline iodine deficiency (Zimmerman MB 2007).

Vitamin A deficiency in iodine-deficient children increases TSH stimulation and risk for goiter through decreased Vitamin A mediated suppression of the pituitary TSH ß gene (Zimmerman MB, et al. 2004, Zimmerman MB, et al. 2007).

- **Physiological changes**

Pregnancy- a stage where metabolic changes takes place and maternal requirements of majority of the nutrients increases. Iodine is one of them and the increased requirement (1 ½ to 2 folds increase), if not met may lead to decreased serum iodine pool and leads to thyroid dysfunctions.

Some of the metabolic disorders or conditions, where the uptake of iodide or release of iodide into the circulation is affected, also become a reason for prevalent iodine deficiency.

2.1.2 IODINE DEFICIENCY AND PREGNANCY

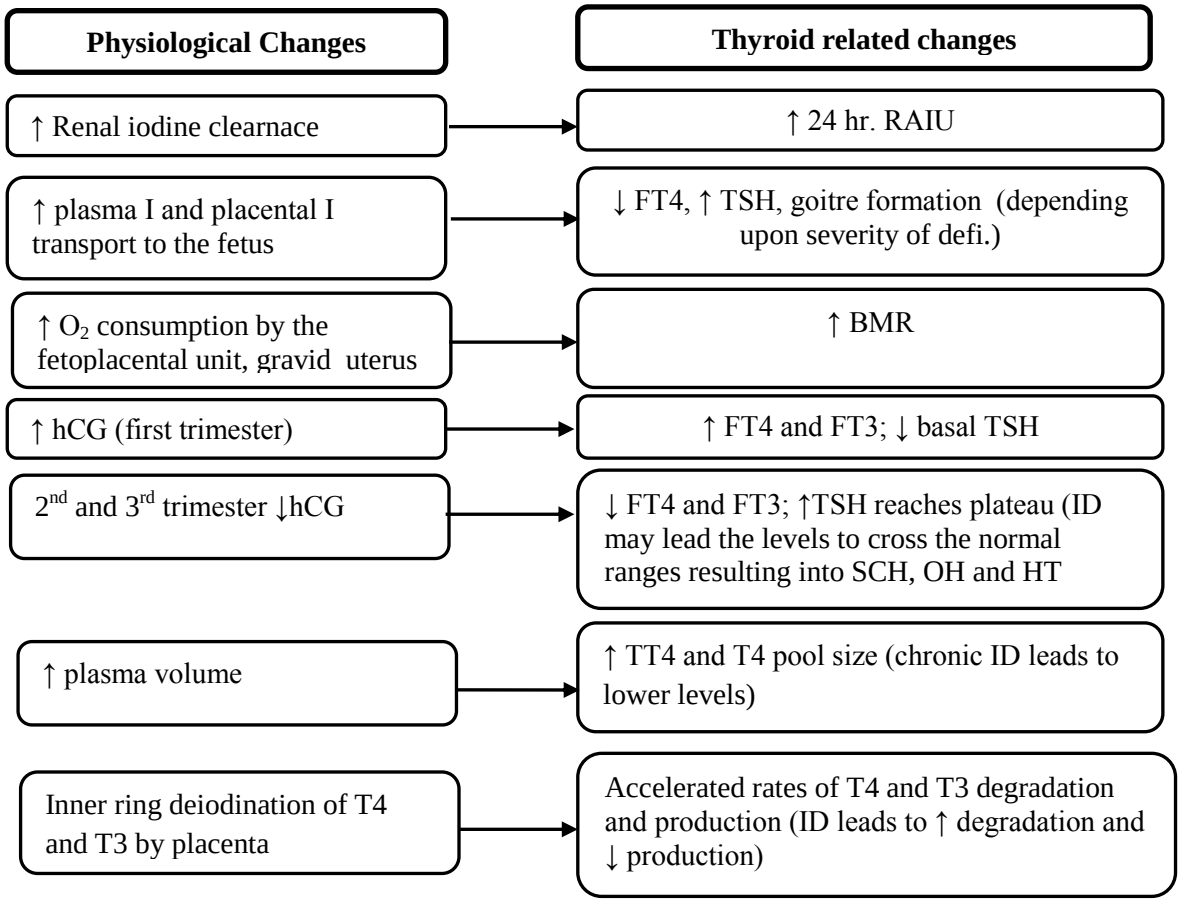
Iodine uptake by the thyroid is higher during pregnancy and the iodine reserve in the thyroid can decrease to approximately 40% of preconception levels. The increased clearance rate can lead to an increase in thyroid volume in pregnant women in iodine deficient areas. There is no or little change in thyroid volume observed in pregnant women in areas with sufficient dietary iodine intake (Glioner D. 1997, Perez-Lopez 2007, Zimmerman M. 2009).

The thyroid stores iodine from the diet. However, maternal iodine status is not entirely dependent on the current dietary intake during gestation. If preconception iodine nutrition is adequate there, then only there will be sufficient stored thyroid hormone to support the mother and fetus, at least in the first trimester. However if preconception dietary intake is deficient the increasing demands of later pregnancy may produce a deficit which

untreated can result in a hypothyroxinaemic state (Smyth 2006). There is some evidence suggesting that in areas of mild to moderate iodine deficiency, the maternal thyroid is able to adapt to meet the increased thyroid hormone requirements of pregnancy (Zimmerman M. 2009). Although, during the first two trimesters of pregnancy the foetus is entirely dependent on the maternal thyroid hormone supply as the foetal thyroid does not develop until 13-15 weeks gestation (Smyth 2006, Glioner D. 1997). As the fetus progresses into the third trimester, it develops the ability to produce its own thyroid hormones but it is still dependent on maternal iodine for hormone synthesis (Becks and Burrow 2000).

There are a series of changes taking place during pregnancy related to iodine metabolism either normal or in an iodine deficient state. The schematic representation has been given below **(Fig 2.2)**. These changes are observed during normal pregnancy also.

Figure 2.2: Physiological and thyroid related modifications during pregnancy



The changes during pregnancy may lead to various physiological conditions together termed as 'Hypothyroidism'. However, at an individual level they can be classified as overt, subclinical hypothyroidism and hypothyroxinemia.

Hypothyroxinemia, elevated serum TSH, enlargement of the thyroid (by 10–50%), and goiter are the most obvious consequences for the pregnant woman. They can be prevented by adequate iodine supplementation (Glinoer D. et al. 1995, Berghout A. et al 1998). Iodine deficiency poses additional reproductive risks, including overt hypothyroidism, infertility, and increased abortions. Hypothyroidism causes anovulation, infertility, gestational hypertension, increased first trimester abortions, and stillbirths; all are common in iodine deficiency. Lack of iodine also has cultural and socioeconomic consequences for the mother. Infertility and fetal wastage may compromise her quality of life and her role in the family and community. If she produces a defective child, she will most likely be responsible for its long-term care, diverting her time and resources from other needs.

Vanderpump and Tunbridge (2002) reported prevalence of overt hypothyroidism between 1% to 2% and subclinical hypothyroidism in 8% of women. Women with hypothyroidism have relatively increased infertility, miscarriage rates and carry an increased risk for obstetric and fetal complications (Poppe K, Glinoer D, 2003). The main obstetric complications are anemia, pre-eclampsia, cardiac dysfunction, placental abruption and postpartum hemorrhage. Fetal complications include prematurity, low birth weight, fetal distress in labor, fetal death, perinatal death and congenital hypothyroidism (Mestman J et al. 1995, Leung AS et al. 1993).

Overt hyperthyroidism complicates 0.2% of all pregnancies. Subclinical hyperthyroidism is found in 0.4% of pregnancies (Mestman JH, 1998). Maternal and fetal complications of hyperthyroidism include congestive heart failure, thyroid storm, preterm delivery, fetal growth restriction, still birth, fetal and neonatal thyrotoxicosis (Miller LK et al.1994). There is now increasing understanding of the association between not only overt, but also subclinical thyroid disorders and dysfunctions with adverse consequences on both obstetric outcome and long term neurological development of the offspring.

2.1.2.1 EFFECTS OF MATERNAL IODINE DEFICIENCY ON IQ OF THEIR CHILDREN

It is unequivocal that severe iodine deficiency in pregnancy results in significant impairment in cognition in the child. It has been suggested that cretinism is at the far end of a spectrum of effects that iodine deficiency can have on the central nervous system, and that varying degrees of intellectual impairment can occur across this spectrum concordant with the degree of iodine deficiency (Skeaff 2011).

A number of clinical trials published globally on pregnant women living in moderate to mild iodine deficiency and correlation with the mental development of their children. The review quotes few of them below:

A reduction of up to 13.5 Intelligence Quotient (IQ) points has been observed in moderate to severe iodine deficient regions (APHDPC 2007). A study conducted in US to compare IQ scores of seven to nine year old children of mothers with subclinical hypothyroidism during pregnancy found IQ scores of seven points lower than controls (Zimmerman MB 2009). A meta-analysis of 37 studies (Qian et al 2005) of IQ in iodine deficient regions in China found a reduction of 12.45 IQ points when compared to iodine sufficient regions. This loss was reduced to 8.7 IQ points when iodine supplementation was introduced during pregnancy and after birth. A 12 point increase in IQ was found 3.5 years after the introduction of iodine supplementation. The study concluded that iodine supplementation before and during pregnancy to women in iodine deficient areas could prevent their child from intellectual deficit.

Haddow et al. (1999) tested the neuropsychological development of children whose mothers were hypothyroid during pregnancy. Although none of the children were hypothyroid as newborns, their full-scale intelligence quotient scores at the age of 7 to 9 years were points lower than those of the matched controls. These results indicate that maternal hypothyroidism has adverse effects on the child's development even without immediate clinical manifestation. As thyroid hormones are transferred from mother to fetus, both before and probably even after the onset of fetal thyroid function (Glinoer &

Delange, 2000), maternal thyroid sufficiency might therefore be most important in early pregnancy.

Fierro-Benitez et al. (1988) compared 8 and 15 year old school children of mothers who had received iodized oil during pregnancy to children of a neighboring comparable community whose mothers had not received iodized oil. Statistical significant differences in tests of intellectual function were not found, but results showed distinct differences in maturation of psychomotor function between the two groups. A case-control study in Bangladesh, comparing mental retardation according to maternal history of goiter, found an increased risk of reduced intelligent scores in children of goitrous mothers (Durkin et al., 2000).

Berbel et al. (2009) recruited three groups of pregnant women living in Spain at different phases of gestation; the first group of women had T_4 concentrations >20th percentile at recruitment (i.e., >0.92 ng/dL at 4–6 weeks gestation), while the second and third groups of women had T_4 concentrations <10th percentile (i.e., <0.83 ng/dL) at 12–14 weeks gestation and near term, respectively. All three groups of women were supplemented with 200 μg of iodine until the end of lactation. When the children were 18 months old, the development quotient of children in mothers supplemented in the first group (i.e., 4–6 weeks) was significantly higher than that of children whose mothers received supplements from 12–14 weeks gestation and near term. A limitation of this study is the small numbers of children tested, with less than 20 children in each of the three groups. Furthermore, the women supplemented later in pregnancy or at term were specifically selected because they had low FT_4 (i.e., <10th percentile) in pregnancy, while the women supplemented earlier in pregnancy had a higher FT_4 (i.e., >20th percentile), thus a difference in fT_4 rather than the iodine supplementation may account for the findings.

Another Spanish study conducted in an area of moderate iodine deficiency (i.e., UIC of pregnant women in this area was <100 μg/L) by Velasco et al. (2009) found that children of mothers supplemented with 300 μg of iodine in the first trimester had higher psychomotor development scores than children from mothers who did not start

supplementation until the last month of pregnancy. A limitation of this study was that children were tested at different ages in this study (5.5 months vs. 12.4 months).

Finally, both studies were not randomized, double-blind, placebo-controlled trials, and although they suggest that neurodevelopment in the child may be adversely affected by moderate iodine deficiency, they are certainly not definitive. The consequences of milder iodine deficiency (i.e MUIC 100-150 μg/L) in pregnancy have yest to be elucidated. It is possible that in mild iodine deficiency adaptive mechanisms conserve iodine in the mother, such that the mother can supply the infant with sufficient thyroid hormones for normal brain development; the parasitic nature of the fetus in pregnancy is well known.

2.1.2.2 IODINE SUPPLEMENTATION DURING PREGNANCY

A review of six randomized control trials (RCTs) (Zimmerman MB and Delange F.2004) found a significant increase in UIC with iodine supplementation. Iodine dose in the studies varied from 50 to 230μg/d and produced variable increases in UIC. These studies also found that supplements are generally effective at minimizing increases in thyroid volume during pregnancy (Zimmerman MB 2009). However, there was no clear effect on total or free thyroid hormone concentrations (Zimmerman MB. and Delange F. 2004, Zimmerman MB. 2009). Iodine supplementation was considered efficacious for both maternal and newborn iodine status.

The review of iodine status in pregnant women in Europe (Zimmerman MB and Delange F. 2004) concluded that, supplements appear to be generally safe and recommends that pregnant and lactating women and women planning to become pregnant, take an iodine supplement of 150μg/d.

Iodine supplementation, at the recommended doses of 50 to 250μg/d, will not lead to an iodine intake in excess of the UL of 1100μg/d. Currently, recommendations for iodine supplementation in areas of mild to moderate deficiency vary from 50 to 250μg/d. (Perez-Lopez 2007, Skeaff et al 2005, WHO/UNICEF/ICCIDD 2007a, Zimmerman MB and Delange F. 2004).

2.1.2.3 PREVALENCE OF IODINE DEFICIENCY DURING PREGNANCY

Many researchers have carried out studies to assess the iodine nutrition status of pregnant women globally at different point of gestation **(Table 2.1)**. Globally the prevalence of iodine deficiency during pregnancy was and still is the area of concern from the most of the developing countries and few of the developed countries. In developed and developing countries the prevalence of iodine deficiency from last few decades was reported by many researchers using thyroid volume as an indicator. This reported 14-30% increase during pregnancy compared to no change in iodine sufficient countries.

Table 2.1: Iodine nutrition in pregnant women worldwide

Author; year; location	N	Design	Trimester	UIE (µg/L)
Glinoer et al; (1990); Belgium	230, 265, 370	C	1, 2, 3	58, 58, 53
Pedersen et al; (1993); Denmark	26, 26	S S	2, 3	51, 40
Nohr et al.; (1993); Denmark	98	C	5 d PP	35
Mocan et al; (1995); Turkey	90	C	1,2,3	91
Liesenkotter et al; (1996); Germany	89, 89	S S	1 11 d PP	53, 50
Caron et al; (1997); France	306, 224	S S	1, 3	50, 54
Smyth et al; (1997), (1999); Ireland	38, 38, 38, 84	S S S C	1, 2, 3, 40 d PP	150, 120, 115, 74
Mezosi et al;(2000);Hungary	119	C	1,2,3	57
Hess et al.; (2001);Switzerland	511	C	2,3	138
Antonangeli et al; (2002); Italy	67	C	1,2	74
Rajatanavin R; (2007); Thailand	125	S	3	103
Mehdi T. et al; (2009); Bangladesh	60 60 60	S S S	1, 2, 3	143 132 120

Table 2.2: Summary of studies on iodine nutrition in pregnant women in India

Studies	N	Setting	Location	Adequately iodized salt consumption	MUIC (µg/L)	<150 MUIC (µg/L)	TGR	TSH
Singh MB et al. (2009)	384	Community based	Rajasthan	77.3%	117.5	58.8%	3.1%	-
Ategbo E A (2008)	349	Community based	Rajasthan	59.5%	127	56%	-	-
Chakraborty I. (2006)	267	Hospital based	West Bengal	-	144	54.6%	-	4.1 mIU/ml (median)
Srinath S (2004)	400	Hospital based	Haryana	64%	178	38.3%	22%	2.3% (>5 µIU/ml)
Pathak P. (2003)	151	Community based	Uttaranchal	-	95	67.2%	14.6%	-
Kant S.(2003)	149	Community based	Delhi	95%	139	52.7%	-	-
Kapil U (1999)	768	Hospital based	Delhi	89%	-	44.4%	1.9%	-
Kapil U. (1997)	137	Hospital based	Himachal Pradesh	-	-	30.4%	-	-
Dodd NS (1993)	429	Community based	Mumbai	-	-	95.3%	45%	1.8% (>5.1 µg/ml)

A number of the studies have also been carried out in India **(Table 2.2)** and the data suggests that the prevalence of iodine deficiency based of urinary iodine excretion is comparatively higher than the developed countries.

2.1.3 IODINE DEFICIENCY IN CHILDREN

Evidence based studies have reported that, **physical growth** and **cognitive development** in children are faster during early years of life, and that by the age of four years, 50% of the adult intellectual capacity has been attained and before thirteen years, 92% of adult intellectual capacity is attained (Vernon 1976). However, it is affected significantly due to iodine deficiency.

2.1.3.1 EFFECTS ON THE COGNITION OF THE CHILDREN

Certain specific cognitive and intellectual functions, eg, verbal and perceptual performance, memory and mathematical abstractions are particularly vulnerable to iodine deficiency as suggested by serious impairment of these capacities in cretins (Tiwari B. et al 1996). The available data does not indicate any gross specific defects at physiologic or functional levels in severely iodine deficient (SID) population other than cretins; however, it is generally recognized that the spectrum of clinical features extend from mild hypothyroidism to frank cretinism in affected populations. The subclinical effects of iodine deficiency on central nervous system (CNS) development and function that are more subtle in nature may be difficult to detect by commonly used intelligence tests (Connolly KJ and Pharoah POD 1994).

There have been many cross-sectional studies comparing cognition and motor function in children from chronically iodine-deficient and iodine sufficient areas, including children from Asian and European backgrounds (Zimmerman M. 2009). These cross-sectional studies, with few exceptions, report reduced intellectual function and motor skills in children from iodine-deficient areas. However, observational studies are often confounded by other factors that affect child development.

Tiwari B. et al (1996) reported that, the severely iodine deficient (SID) children were slow learners. This was proven by the battery of tests performed amongst the children

aged 8-18 years. Maze test results favored mild iodine deficient (MID) children compared to their SID counterparts ($p<0.01$), since SID made higher number of errors in maze learning and the time taken to learn the maze ($p<0.01$) was also higher amongst SID. Test on pictorial learning also revealed that, the children from MID group performed better ($p<0.01$) compared to SID. Achievement of motivation revealed that, the children from MID group, particularly older ones, were significantly ($p<0.01$) more motivated to achieve than were SID children. Thus, the correlation analysis between test scores and severity of iodine deficiency using UIE,TSH and FT_4 as indicators revealed that, there was a significant negative correlation of errors in Maze to UIE (r= -0.58) and FT4 (r= -0.53). However, TSH showed a significant positive correlation with errors (r= 0.18). The negative correlation of these indexes with urinary iodine excretion and thyroxine and their positive correlation with TSH indicate a significantly poorer rate of learning in the SID group.

A recent 28 week New Zealand study (Gordon et al 2009) on children aged 10-13 taking a 50mg/d iodine supplement vs. placebo found significant improvement in UIC (baseline 66mg/L, post treatment 145mg/L, $p<0.001$)) and Tg (baseline 16.5mg/L, post treatment 8.5mg/L, $p<0.001$). The children were also tested on cognitive ability before and after the 28 weeks. The study found significant improvement in picture concepts (treatment effect 0.81, p 0.023) and matrix reasoning tests (treatment effect 0.63, p 0.040) representing perceptual reasoning, a higher-order cognitive ability. There was not a significant difference in symbol search (treatment effect 0.16, $p<0.608$) or letter-number sequencing (treatment effect 0.18, $p<0.480$). This has shown that mild iodine deficiency prevents children from attaining their full intellectual potential and that iodine supplementation improves UIC, Tg and cognitive performance.

A meta-analysis including 21 observational studies and experimental studies including a control group of the effect of iodine deficiency on mental development (Bleichrodt N, Born MP 1994). The age ranged between 2-45 years. Of these total studies, 16 studies were on children. At the final analysis, 2214 participants (mainly children) and IQ was the main outcome measure. The studies were carried out in moderate to severe iodine deficiency. The IQs of non-iodine deficient groups on average 13.5 points higher than

those of the iodine-deficient groups. This, clearly suggests the detrimental effect of iodine deficiency on IQ of the children.

It is hypothesized that iodine deficiency may be associated with Attention Deficit Hyperactivity Disorder (ADHD). A study conducted in a moderately iodine deficient area in Sicily found that 11 of 16 participants were diagnosed with ADHD by age 10, as compared to none in the marginally iodine sufficient control group (Vermiglio et al 2004).

2.1.3.2 EFFECT ON SOMATIC GROWTH

The effects of subclinical IDD are less known than those of the severe syndrome. Intellectual abilities are known to be affected, probably into at least two ways: by development damage in utero, which is largely irreversible and by iodine deficiency in early childhood or later life, with lower thyroixine levels leading to reduced intellectual and physical vigor,which can be improved by iodine supplementation (Bleichroft N et al 1996;Delange 1994;Lofti M and Mason J 1993).

In five Asian countries , household access to iodized salt was correlated with increased weight-for-age and mid-upper-arm circumference in infancy (Mason 2002). However, controlled intervention studies of iodized salt alone (Bautista A 1982; Shreshtha RM 1994) and iodine given with other micronutrients (van Stuijvenberg 1999; Rivera 2001; Moreno-Rayes 2003) generally have not found child growth to be affected.

Data from studies on iodine intake and child growth are mixed. However, most studies have found positive correlation between iodine and growth (Bautista A 1977; Ali O 1994; Neumann CG 1994; Lal RB 1996). Iodine status may influence growth through its effects on the thyroid axis. Administration of T_4 to hypothyroid chidren increases their growth (Hernandez-Cassis 1995). Thyroid hormone promotes GH secretion and modulates the effects of GH at its receptor (Crew MD 1986; Samuels MH 1989; Hochberg 1990). IGF-I and IGF binding protein (IGFBP)– 3 are also dependent on thyroid status (Burstein 1979; Nanto-Salonen 1993). In human, hypothyroidism decreases circulating IGF-I and IGFBP-3 levels, and thyroid hormone replacement increases them (Miell JP 1994; Iglesias P 2001). In iodine-deficient children, impaired thyroid function and goitre are inversely

correlatwd with IGF-I and IGFBP-3 concentrations (Wan Nazaimoon 1996; Aydin K 2002; Alikapifoolu 2002). However, in an uncontrolled trial, oral iodized oil paradoxically decreased IGF-I and IGFBP-3 concentrations in Turkish Children (Ozon A 2004).

In order to detect somatic and psychomotor disturbances in children and adolescents residing in areas of iodine deficiency, schoolchildren from three areas with different degrees of iodine deficiency, (Azizi F et al 1993) carried out a cross-sectional study. In Randan, the prevalence of severe endemic goiter was accompanied by alteration in thyroid function, increased thyrotropin levels and retardation of both bone and psychomotor age and decreased intellectual quotient. In Tehran, where iodine deficiency is mild, visible goiter was present in 1 5% of schoolchildren but no alterations in thyroid function, serum thyrotropin, somatic or psychomotor development could be detected. In Zagoon, where the prevalence and severity of goiter was less than Randan but more than Tehran, thyroid function was normal but slightly decreased as compared to Tehran; somatic development was unaltered, but retardation in psychomotor development was evident and the mean intellectual quotient was less than that of Tehranian schoolchildren. These findings indicate the occurrence of physical and psychomotor disturbances in apparently normal schoolchildren from areas of iodine deficiency. Thus, the study concluded that the alteration in psychomotor development may occur in children with normal physical growth, due to iodine deficiency.

A study conducted by (Zimmerman MB 2007) to determine whether iodine repletion improves growth in school-age children and to investigate the role of IGF-I and IGFBP-3 in this effect. Three prospective, double-blind intervention studies were done in areas of varying iodine deficiency: in severly iodine deficient Moroccon children; in moderately iodine-deficient Albanian children and in mildly iodine deficient South African children. In all three studies, iodine treatment increased median UI in the conrols remained unchanged. In South African children, iodine repletion modestly improved IGF-I but did not have a significant impact on IGFBP-3, total T_4 or growth. In Albenian and Moroccon children, iodine repletion significantly increased total T_4, IGF-I, IGFBP-3, weight-for-age Z scores, and height-for-age Z scores. These controlled studies clearly demonstrate that,

iodine repletion in school age children increases IGF-I and IGFBP-3 concentrations and improves somatic growth (Zimmerman M 2009).

Another study to investigate whether serum thyroxine concentration, within the normal reference intervals were age-related among children and adolescents. This investigation was carried out on 243 subjects who were chosen randomly from all those patients who were referred during the one-year period from to the Danesh Medical Diagnostic Laboratory in Gorgan, northern Iran was carried out by (Mansourian AR and Ahmedi AR 2010). The sample population were divided into 4 age groups of 1-5 years, 6-10 years, 11-15 years and 16-21years. The results of this study showed that, there is an inverse age correlation ($p<0.05$) between thyroxin concentrations among children and adolesctnts in this region. This in turn indirectly suggested that, in normal condition growth is regulated by thyroid hormone, indicated by increased TSH with age. However, during iodine deficiency, increased TSH at early stage compared to standard age may lead to insufficient growth of the children.

Further, A total of 859 prepubertal euthyroid Danish children aged 4–9 yr undertaken for thorough clinical investigation by (Boas M et al 2009), including anthropometrical measurements and determination of TSH, thyroid hormones, autoantibodies, urinary iodine excretion, and thyroid volume (TV) by ultrasound. Longitudinal growth data from birth were available. Results revealed that, TV increased significantly with age ($r <0.487$; $P < 0.001$). Mean TV ± SD for different age groups were as follows: 4 yr, 2.2 ± 1.4 ml; 5 yr, 2.5± 1.3 ml; 6 yr, 2.8 ± 1.3 ml; 7 yr, 3.2 ± 1.3 ml; 8 yr, 3.5 ± 1.3 ml; 9 yr, 3.7 ± 1.3 ml. A significant positive association between IGF-I and TV ($P < 0.001$) was observed. Furthermore, in multiple regression analyses, TSH ($P<0.013$), free T_4 ($P < 0.001$), lean body mass ($P <0.001$), and body surface area ($P<0.001$) as well as other anthropometrical measurements were identified as factors significantly associated with TV. Family history of thyroid disease and presence of incidental abnormal ultrasound findings were also positively associated with TV ($P <0.025$ and 0.022, respectively).Thus, in this cohort of prepubertal Danish children, the GH/IGF-I-axis was positively correlated with thyroid size, suggesting a role in the regulation of thyroid growth. Moreover, anthropometric measurements, in particular body surface area, were the best predictors of TV.

2.1.3.3 PREVALENCE OF IODINE DEFICIENCY IN CHILDREN

Globally the prevalence of iodine deficiency has been oberved affecting millions of school aged children in developing and developed countries equally. The effects were observed to be less severe amongst countries where salt iodization programme is existing and consumption of iodized salt is remarkable. However, this is not the only criteria to assure adequately iodized salt consumed by the community.

Table 2.3 depicts the prevalence of iodine deficiency amongst school aged children belonged to different countries at diffent point of time (before or after initiation of salt iodization program).

Thus, the reviewed studies in thes section suggests that, iodine deficiency is the world's single greatest cause of preventable mental retardation. It is specifically damaging during the early stages of pregnancy and developmental years of the children. There are almost no countries in the world where iodine deficiency has not been a public health problem.

2.1.4 COMBATING IODINE DEFICIENCY

About thirty eight million newborns in developing countries every year remain unprotected from the lifelong consequences of brain damage associated with IDD. This shorcoming affects a child's ability to learn, and later in life, to earn, therefore, preventing children, communities and nations from fulfilling their potentials. International support for elimination of iodine deficiency dates from the World Summit for children in 1990 **(Table 2.4)** (UNICEF 2007).

2.1.4.1 SALT IODIZATION STRATEGY

Globally salt iodization has been proven to be one of the most renowned, workable and controversial strategy to combat iodine deficiency disorders. However, many of the countries have achieved the goal of reaching >90% iodization and some are still striving to achieve the same.

Table 2.3 : Summary on the iodine nutrition in school children worldwide

Author; year; location	N	Age-group	Adequately iodized salt consumption	MUIC (µg/L)	<100 MUIC (µg/L)	TGR	Thyroid volume	Conclusion
Biswas A. et al.; 2002; India	2392	8-10 years	85.1%	150	14.7%	11.3%	-	Transition phase of ID
Copeland D. et al;2002;Bangladesh	399	6-10 years	-	73	-	27%	-	Deficient
Copeland D. et al;2002;Guatemala	458	6-16 years	-	181	-	15%	-	Deficient
Copeland D. et al;2002;USA	284	6-12 years	-	282	-	2%	-	None
Zimmerman M. ;2004; WHO regions	2.84 millions	6-12 years	-	-	36.4%	-	-	Severe Deficiency
Andersson M. et al; 2005; WHO regions (UNPD)	848 millions	6-12 years	-	-	36.5%	15.8%	-	Deficient
Abuye C. et al;2007;Ethiopia	10,958	6-12 years	4.2%	24.5	83%	39.9%	-	Deficient
De Benoist et al.; 2008; WHO regions (47 countries)	266 millions	6-12 years	-	-	31.5%	-	-	Deficient
Andersson M et al; 2012; Europe	240.9 millions	6-12 years	-	-	29.8%	-	-	Deficient

Thus, data suggests that, India is one of them respite showing a tremendous economical growth and development within a last two decades, along with the iodized salt consumption reaching 71% (MI-ICCIDD 2011).

Table 2.4: Major United Nations milestones for elimination of iodine deficiency

Year	Milestone	Programme progress
1990	Declaration of the World Summit for Children includes goal of virtual elimination of IDD 43th World Health Assembly accepts IDD elimination by 2000 as a major public health goal for all countries	Accelerated programme initialtion and a shift from supplementation to salt iodization
1994	UNICEF-WHO Joint Committee on Health Policy endorses universal salt iodization as a safe, cost-effective and sustainable strategy to ensure sufficiency intake of iodine by all individuals	IDD prevention and control through expansion of salt iodization programme
2002	UN General Assembly Special Session on Children adopts A World Fit for Children, the declaration that set the goal of sustainable elimination of IDD by 2005	Programme maturation with improvement in enforcement, public education and advocacy, monitoring and partnership with salt industry.
2007	A World Fit for Children commemorative session reviews progress in achieving and sustaining IDD elimination through USI programmes	Enhancements in programme sustainability

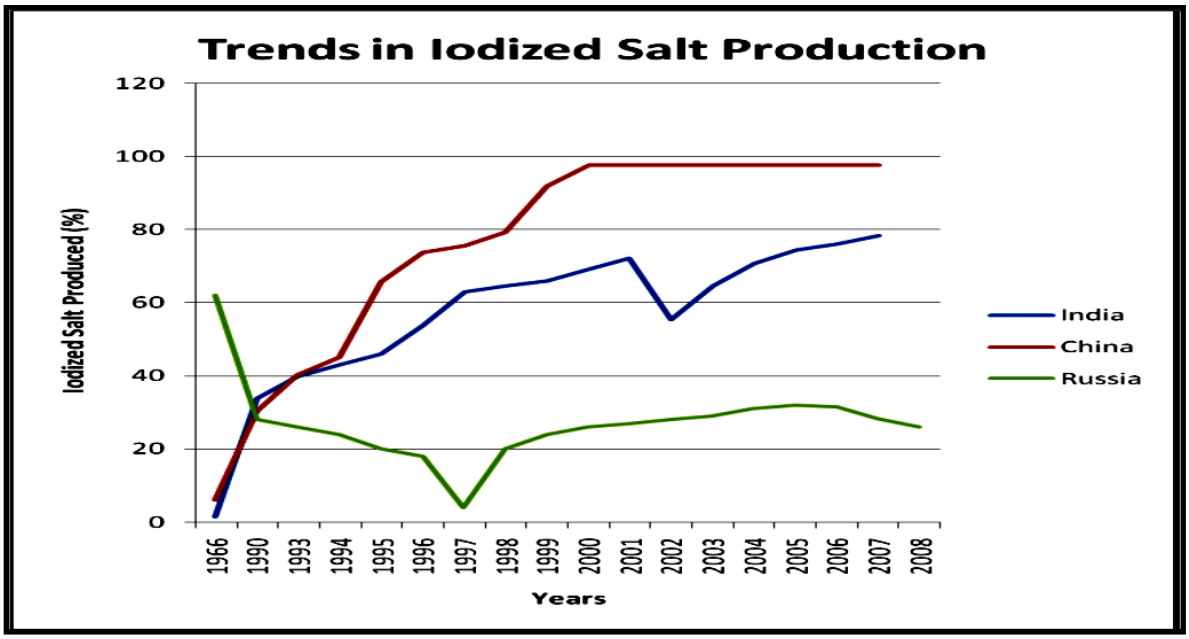
(Source : UNICEF 2007)

It is evinced that legislation is a corner-stone to sustenance of Universal salt Iodization (USI) strategy. India, Russia and China demonstrate the effect of government support upon USI. Salt has a significant place in India's political history, notably Gandhi's salt march to Dandi in protest of taxation on salt in 1930. However, NGCP in 1962 and later NIDDCP have provoked the strategy towards achieving USI in India (Sundaresan, 2008).

A proven fact is that the commitment to IDD elimination by a national government is essential to firmly root a USI program. Evidence of political commitment to USI and elimination of IDD usually comes in the form of legislation that mandates that all salt for human and animal consumption be iodized; a national coalition or oversight body responsible for the programme that reports to the Minister of Health; and the appointment

of a responsible executive officer for the IDD elimination programme. (WHO, UNICEF, ICCIDD, 2007, Third ed.)

Figure 2.3: Trend in iodized salt production in India, China and Russia



Trends lines extrapolated from available data **(Source : Mannar V and Bohac L 2010).**

2.1.4.2 SALT IODIZATION AS A UNIVERSAL NORM

As reported by MI (2010), the salt industry has been entrusted with the responsibility of dovetailing iodization into the prevailing salt production and distribution system, creating a standard of adequate iodization at minimum cost and disruption. In large streamlined processing plants iodization is a relatively simple step. Iodization in medium/ small operations poses more significant challenges in countries where salt manufacturing techniques and product quality vary over a wide range from cottage scale units producing a few hundred tons a year to very large fully automated plants producing several million tons. The strategies used to achieve the first 50-60% coverage of iodized salt in several countries may not necessarily result in addressing the challenge for the remaining 40% of the population. New strategies will need to systematically identify the bottlenecks or constraints that impede universal iodization and address them through a combination of advocacy, technical support, monitoring and enforcement.

The key indicators of effectiveness and sustainability of salt iodization (and its integration into the provision of salt for human and animal consumption) in a country include: (WHO, UNICEF, ICCIDD, 2007, Third ed.)

- Quality assurance of iodized salt production,
- On-going gathering and analysis of data relating to salt importation, production and iodization process, distribution, major companies involved, the role small scale producers/salt farmers, association of salt producers, prices of products and the market situation,
- Working relationships and practices between regulatory authorities and salt producers.

The stability of iodine in salt and levels of iodization and packaging are also related to issues of quality assurance. Conditions of high humidity result in rapid loss of iodine from iodized salt, with iodine loss ranging anywhere from 30 to 98% of the original iodine content. (Diosady, Alberti, Mannar, & FitzGerald, 1998) By refining and packaging salt in a good moisture barrier, such as low density polyethylene bags, iodine losses can be significantly reduced, during storage periods of over six months. However, the issue of refining and quality packaging is the obstacles with the small scale industries.

2.1.4.3 ROLE OF SMALL SCALE SALT PRODUCERS IN CONTROLLING THE PREVALENT IODINE DEFICIENCY

Large scale producers account for nearly 75% of all salt for edible consumption in salt producing countries, a small but significant proportion of the salt is produced by many small producers. The smaller units often operate with a minimum of organization and little or no quality control. Being geographically scattered, the small units do not lend themselves to regulation by the government. Very often precise figures regarding even their location, extent of holdings and production statistics are not available.

As reported by (Jooste P. 2009), salt producers are obliged to meet the legal requirements related to iodized salt in the countries with legislation for iodized salt, but their level of iodine knowledge is likely to have a crucial influence on their dedication and efficiency

in meeting these requirements. They work and produce at the interface between a commercial enterprise and a health system, often without an understanding of the health issues involved in the iodized salt production.

Further, the producers have limited financial means and lack access to technical or financial assistance to institute quality iodization processes and to monitor quality. Additionally they have poor packaging practices or do not package the salt at all. As a result, the salt produced in these units is often of poor quality. Nevertheless, these small salt producers are often the main salt source to the communities that are not reached by the conventional iodized salt suppliers and therefore most at risk of IDD (Mannar V and Bohac L 2010).

Few pilot initiatives in different developing countries on recognizing the role of small scale salt producers have been undertaken to integrate them into the overall USI strategy of their respective countries.

In Senegal, which has more than 10,000 operating small producers, the prospect of financial returns motivated those producers to be involved in the pilot project to join into associations of producers rather than the ban on uniodized salt production. These associations were provided with iodization machines, internal quality assurance, production tools and training to enable them to produce a quality of iodized salt that complied with national standards while increasing their overall productivity (Ndao, Ndiaye, Miloff, Toure, & A., 2009).

Shortcomings in the level of knowledge of iodine nutrition were apparent in an assessment among salt producers in South Africa (Jooste P 2003). Instead of merely enforcing the legal specifications, the iodine knowledge level of these producers was improved through an educational campaign that consisted of intermittent mailing of IDD brochures, pamphlets and other materials, workshops and personal visits to the production units. The benefit of this approach showed up in a reassessment of the iodine content of salt at the production sites, as well as in the iodine status of women and children in a subsequent national survey.

In Rajasthan, India, where small salt producers account for 88% (1.32 metric tons) of the state's total production for human consumption, the pilot project aimed not only to build the iodization capacity of small salt producers through the provision of technical inputs such as machinery and equipment but to sustain that capacity by creating awareness and demand for iodized salt in the community, teaching good business as well as quality assurance practices, and by establishing a revolving fund operated through their newly formed cooperatives to provide the salt producers with the financial support to upgrade their facilities, leverage other loans and expand their capacity (Gulati & Jain, 2009).

In Gujarat, India, similar efforts were carried out with extensive advocacy and technical support towards implementing salt iodization at optimal levels among small scale salt producers and results were observed to be motivating with 100% salt iodization achieved compared to 86% at baseline and 60% of the small scale producers meeting the recommended standards for salt iodization (30 ppm) at production level compared to negligible proportion at baseline (Nair S. and Joshi K., Unpublished 2006-2007).

In all cases, the support has been intensive in the initial phases with equipment and technical assistance provided, but built into the projects is a scheme to first, promote the economies of scale (sharing of equipment and facilities) and second, to support the sustainability of the operation and transfer the ownership of the production of iodized salt to the small producers. Thus, to make an incredible contribution to the community by providing a pinch of iodized salt.

Recent reports by the Copenhagen Consensus, which rate salt iodization as one of the top investments with a benefit: cost ratio of $30:$1, provide a strong argument to be directed at national policy makers in countries where national commitment has not been made. (Horton, Alderman, & Rivera, 2008) In addition, in those countries which have existing USI programs, a reaffirmation - in the form of commitment of both human and financial resources for salt iodization programs - would not only assure sustainability but also mark the national ownership of the program and the goal.

In 1998 India instituted legislation which banned the sale of non-iodized salt. This legislation was revoked in 2000 amidst political turmoil, and subsequently resulted in a

drop of adequately iodized salt production from 70.3% on 1997 to 29.6% in the period 2000- 2004. (Vir S. 2009) In 2006, Government of India reinstituted the ban on the sale of non-iodized salt for human consumption. Thus, India's production of iodized salt went from 4.1 million tons in 199 to 1.69 million during the course of the interruption in legislation and then increased to 5.1 million tons in 2007 (Sundaresan 2008; Vir S. 2009).

2.1.4.4 SUCCESS OF SALT IODIZATION

Our review suggests that increased production and consumption of iodized salt would enable the world to achieve very crucial sections of the millennium development goals. Elimination of iodine deficiency also contributes to six of the eight millennium development goals agreed by UN member States in 2000. Meeting these goals would transform the lives of millions of children during next 10 years **(Table 2.5)**.

Table 2.5: IDD and the Millenium Development Goals (UNICEF 2007)

Goal 1- *Eradicate extreme poverty and hunger*	Eliminating IDD increases learning ability and intellectual potential, leading to better educated citizens earning higher wages.
Goal 2 – *Achieve universal primary education*	Improved cognitive development and learning potential leads to improved school performance and reduced dropout rates
Goal 3- *Promote gender equality and empower women*	Eliminating IDD in children reduces women's childcare burdens, frees up household resources and allows women more time for income generating work.
Goal 4- *Reduce child mortality*	Reducing iodine deficiency lowers rates of miscarriages, stillbirth and other pregnancy complications and neonatal deaths
Goal 5- *Improve maternal health*	Lower rates of thyroid disease and other clinical results of iodine deficiency improve the health of women of reproductive age.
Goal 6 – *Develop a global partnership for development*	Programmes for sustainable elimination of iodine deficiency strengthen partnership at global, regional and commitments through alliances of public organizations, civil society and private sector.

The strategy of salt iodization has emerged as a successful strategy to combat iodine deficiency from the study carried out and recommended by many researchers globally.

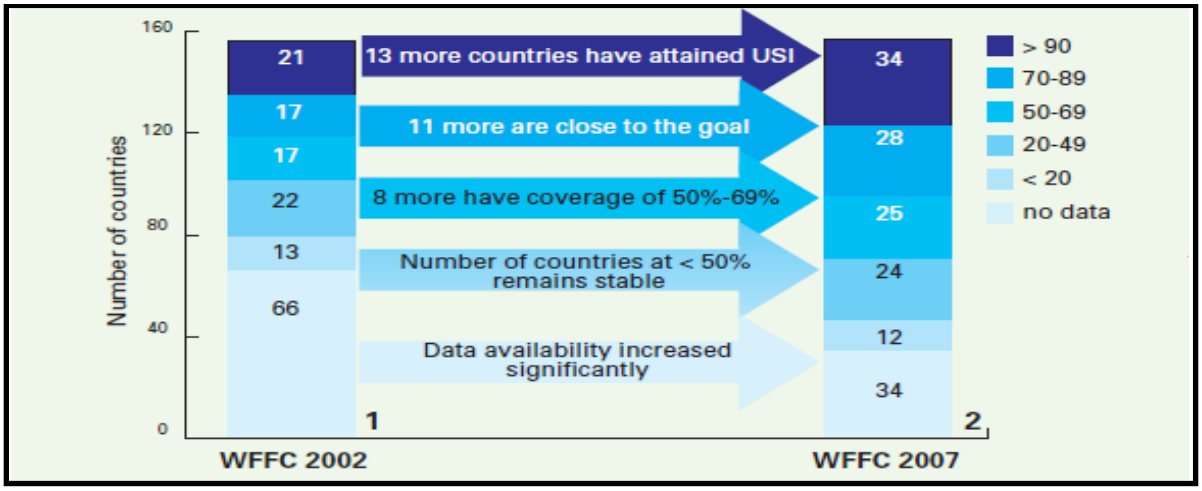
The overall global status of iodine deficiency has improved since 2003, reflecting the fact that current strategy of salt iodization is effective **(Table 2.6)** and **(Figure 2.5)**. There are now fewer countries where iodine deficiency is considered to be a public health problem in 2007 than there was in 2003. However, 47 countries continue to have problem with iodine deficiency (de Benoist et al. 2008).

Table 2.6: Global Scorecard 2010 on improved iodine nutrition worldwide

Country	Total Population 2008 (in thousands)	% Households consuming Iodized salt (2003-2008)	MUIE (µg/L)	Prevalence of low UIE (<100µg/L) (%)	Survey year	Total goitre rate (%)
Afghanistan	27,208	28	49	71.9	2004	-
Bangladesh	160,000	84	163	33.8	2005	17.2
Bhutan	687	96	230	24.0	1996	14
China	1,337,411	95	246	15.7	2005	6.5
India	1,181,412	51	-	-	-	-
Nepal	28,810	63	188	27.4	2005	40
Pakistan	176,952	17	-	63.6	2001	6.7

(Source: WHO, ICCIDD, MI, Iodine network 2010)

Figure 2.4: Global distribution percent households consuming iodized salt



(Source: UNICEF 2007)

2.1.4.5 IMPACT OF IODIZED SALT CONSUMPTION

There are number of studies published of iodine supplementation in pregnancy. Undertaking randomized control trial studies on 450 pregnant women in European countries, it has been proven that the iodine supplementation through iodized salt has brought about significant improvement in urinary iodine levels of pregnant women indicating sufficiency levels belonged to mild-moderate iodine deficient countries **(Table 2.7)**.

Table 2.7: Randomized, controlled trials of iodine supplementation in pregnancy: effect on urinary iodine concentration in iodine deficient countries

Author; Year; Location	Iodine dose/day	Urinary Iodine Excretion(UIE)(µg/L)	
		Pre-treatment	Post-treatment
Romano et al (1991);Spain	120–180	37 mg/day	100 mg/day
Pedersen et al (1993);Denmark	200	55 mg/l	90–110 mg/l
Glinoer et al (1995); Belgium	100	36 mg/l	80–90 mg/l
Liesenko¨tter et al (1996);Germany	230	53 mg/g cr	104 mg/g cr
Nohr et al (2000); France	150	50 mg/l	105 mg/l
Antonangeli et al (2002);Italy	50	65 mg/g cr	128 mg/g cr
Antonangeli et al (2002); Italy	200	91 mg/g cr	230 mg/g cr

Recently Moleti *et al*. (2008) reported the effects of long-term and short-term iodized salt use on the risk for maternal thyroid failure in a cohort of pregnant women from Sicily. Women were enrolled during the first trimester of pregnancy, and their iodized salt use for the preceding 2 yr was retrospectively ascertained. Among 38 women who started using iodized salt only when they became pregnant, the prevalence of thyroid failure during pregnancy was remarkably high at 36.8%. Most of this thyroid failure consisted of hypothyroxinemia with normal serum TSH values. Although long-term iodized salt use reduced the risk for maternal thyroid failure 6-fold, 6.4% of the 62 women who consistently used iodized salt for at least 2 yr before pregnancy also developed thyroid failure during pregnancy. First-trimester median urinary iodine excretion in both of these

groups (115 μg/liter in those with long-term iodized salt use and 63 μg/liter in those with short-term iodized salt use) was below the World Health Organization's optimal pregnancy range of 150–249 μg/liter.

However, the improvement in iodine status of the children has also been reported by a number of researchers.

Although programs to control iodine deficiency, such as salt iodization, have been effective for decades, iodine deficiency remains a major threat to the health and development of populations around the world, particularly among children and pregnant women in low-income countries. Other than the production the prevalence of iodine deficiency also fluctuates due instable or no regulation for iodized salt production and consumption. Review quoted below reveals the global overview of prevalence of iodine deficiency and reemergence due to reasons like non existence, poor monitoring or opposition of the salt iodization strategies.

2.1.5 IODINE DEFICIENCY RE-EMERGENCE

In 1992 the median UIC levels in Australia were 200mg/L (Eastman 1993), which is on the border between sufficient and above requirement. More recent studies in Tasmania, New South Wales and Victoria have shown median levels <100mg/L indicating mild deficiency (Seal et al, 2007, Li et al 2006). In pregnant women, levels have been found to be well below 100mg/L indicating inadequate iodine intake in these states. In 2009 WHO, based on the national median urinary iodine concentration of 96μg/L (Li et al 2008a), has classified Australia as a mildly iodine deficient country. This depicts the reemergence of iodine deficiency in the country.

A Study by Rathod S. et al. (2011) was conducted among 2,940 School children in the age group of 6-12 years in rural schools of Bhavnagar District Gujarat. Urine samples were collected from 15% of selected children and salt samples from 43% of sub-sample. Clinical examination revealed an overall goitre prevalence of 34.19% in the region. Females had a prevalence of 32.9% and males 35.4%. The median urinary iodine excretion in the region was 11.0 μg/l (range: 29.0-190.0 μg/l). Ninty-seven percent of

subjects had biochemical iodine deficiency with 73.87% having severe deficiency, 21.38% having moderate and 4.04% mild iodine deficiency. In Bhavnagar region, only 34.27% households consumed powdered salt having an Iodine content of greater than 15 ppm. Thus, the study concluded that severe goiter prevalence in primary school children in Bhavnagar district after 6 years of mandatory salt iodization in the region.

After two years of mandatory salt iodization in the country, Ategbo et al. (2008) assessed the progress in iodization in Rajasthan and revealed the iodine status of pregnant women and school age children. The results revealed median UIE amongst school children was observed to be 139 µg/L and 127 µg/L in pregnant women, depicting the transition phase of iodine deficiency to sufficiency. However, the levels of iodization at 30 ppm were emphasized. This also suggests indirect need for strengthening the iron supplementation program since the prevalence of ID was observed to be 56% amongst pregnant women, where the need for iron also increases along with iodine.

Another very crucial reference study to our concept of need for dual fortification with iodine and iron was carried out by Das et al (2011) in Chandigarh, India. The study focused on persistence of iodine and iron deficiencies amongst the school children (n=2148), post iodization phase. The results depicted the prevalence of goitre in the studied subjects was 15.1 per cent (13.9% in 6 to 12 yr and 17.7% in 13 to 16 yr age group, P= 0.03). Median urinary iodine excretion in both the groups was sufficient and comparable (137 and 130 µg/l). 3.2 per cent children with goitre and 2.4 per cent without goitre had hypothyroidism (subclinical and clinical) and only one child with goitre had subclinical hyperthyroidism. Nine (4.9%) children in the goitre group and 3 (1.9%) in control group had anti-TPO antibody positivity. The median serum selenium levels were not different in both the groups (181.9 and 193.5 µg/l). Seventy one (37.4%) of the goitrous children had anaemia (haemoglobin <12 g/dl) as compared to 41 (24.8%) of the control group ($p<0.01$). More number of goitrous children (39, 20.6%) were depleted of tissue iron stores (serum ferritin <12 µg/l) as compared to controls (11, 6.4%; $P<0.001$). Serum ferritin level negatively correlated with the presence of goitre ($r = - 0.22$, $p=0.008$) and had an OR of 2.8 (CI 1.20 - 6.37, $p=0.017$). Thus, it concluded that there was a high prevalence of goitre in young children despite iodine repletion and low thyroid

autoimmunity. The concurrent iron deficiency correlated with the presence of goiter. However, the cause and effect relationship between iron deficiency state and goitre requires further elucidation.

Thus, the above studies suggest the concept of co-existence of iodine and iron deficiencies and their eradication to improve iodine status of the subjects gains more importance in the further review.

2.2 IRON DEFICIENCY ANEMIA

Unlike iodine deficiency, scarcity of another essential micronutrient- iron is also endangering the survival of many pregnant women, their fetuses, neonates and malnourished children globally. Hence, the focus on the remedies for combating micronutrient deficiencies has been shared by both of these deficiencies.

Anaemia is the most common nutritional deficiency disorder in the world. WHO (2010) has estimated that prevalence of anaemia in developed and developing countries in pregnant women is 14 per cent in developed and 51 per cent in developing countries and 65-75 percent in India. About one third of the global population (over 2 billion) is anaemic. Prevalence of anaemia in South Asian countries is among the highest in the world.

Anemia is not a disease but may be considered a syndrome caused by malnutrition in its widest sense. It is a condition in which the hemoglobin content of the blood is lower than the normal due to deficiency of either a single or more essential nutrients regardless the cause of such deficiency. Iron deficiency anemia is an important public health problem in most developing countries (WHO 1992).

2.2.1 CAUSES OF IRON DEFICIENCY ANEMIA

School age children are considered to be a nutritionally vulnerable segment of the population, since they includes preadolescents and adolescents in a group. Thus, the range of nutritional requirements varies within a wide range. A rapid growth rate

combined with a marginal nutrient intake increases the risk of micronutrient deficiencies in the population.

The vulnerability towards iron deficiency is more due to inadequate iron intake, reduced bioavailability of dietary iron, increased need for iron, chronic blood loss and parasitic infections. Iron deficiency is a major cause of anemia in all developing countries, where consumption of iron is limited because dietary sources of iron are not affordable by most families (World Bank Report 2003).

- **Dietary inadequacy**

Iron requirements are high during pubertal growth and pregnancy. Fetal and purbetal growth implies a corresponding increase in the total hemoglobin mass, and for this formation of new hemoglobin, iron is needed. The peak need for iron has shown to be more closely related to maximal growth spurt and maturation than age (Sjolin 1981). Thus, not meeting iron requirements through diet or the problem of food insecurity-unequal distribution of foods in the families increases the incidence of iron deficiency anemia in the women and girl child of the families in India.

- **Dislike for iron rich foods**

These foods include green leafy vegetables as one of the major contributor as far as Indian vegetarian families are concerned. Especially amongst school aged children and adolescents, it is one of the major reason for poor dietary iron intake. Poor intake may be associated with lack of knowledge in the groups. ICMR reported that the average intake of iron is only about 50% of RDA in India (Toteja and Singh 2003).

- **Bioavailability**

The bioavailability of dietary iron in our body is an important to iron nutrition as the amount of iron consumed. The average iron absorption varies extremely, ranging from 1-5% for plant origin foods and 10-25% for animal origin foods (Monsen 1998). The bioavailability of iron is low in predominately cereal-based diets, as in India, because of their high phytate and phosphate content. Tannates present in tea are also known to inhibit the absorption of iron of when consumed with meals (Sheshadri et al 1989).

- **Reproductive health**

Repeated pregnancies within less than 2 years interval does not give time to the women to get back to normal hemoglobin levels due to the earlier pregnancy. Further, anemia at preconception stage leads to detrimental effects to the fetal growth and development. It can extend upto the unpredictable survival chances of the fetus during severity of maternal anemia.

- **Parasitosis**

Hookworm infection load can include iron deficiency anemia, especially in women of reproductive age and children, whose dietary iron intake is low and whose body iron stores are exhausted due to increased demand of iron for growth. Even normal levels of dietary iron intake may not be sufficient to protect from anemia in the situation of high hookworm load.

Evidences have revealed that the IDA affects brain development (Stoltzfus et al 2004; Pinero et al 2000; Nelson et al 1997) and that leads to measurable effects on children's behavior, motor development and cognition (Lozoff et al 1998; De Andraca et al 1997; Idjradinata and Pollit 1993). Variations in cognition test performances are equivalent to a six-month delay in development can typically be attributed to heavier infections of the sort experienced by around 60 million school age children (Partnership for child development report 1998).

2.2.2 IRON DEFICIENCY ANEMIA DURING PREGNANCY

Studies to define the effect of maternal anaemia on the foetus indicate that different types of decompensation occur with varying degrees of anaemia. Most of the studies suggest that a fall in maternal haemoglobin below 11.0 g/d1 is associated with a significant rise in perinatal mortality rate (Ramchandran P.1989; 1992; Prema K 1982). There is usually a 2 to 3-fold increase in perinatal mortality rate when maternal haemoglobin levels fall below 8.0 g/d1 and 8-10 fold increase when maternal haemoglobin levels fall below 5.0 g/dl (Prema K. et al.1981; Lister VG et al.1985). A significant fall in birth weight due to increase in prematurity rate and intrauterine growth retardation has been reported when

maternal haemoglobin levels were below 8.0 g/d1 (Prema K. et al.1981; Lister VG et al.1985).

2.2.2.1 EFFECTS OF ANEMIA ON MATERNAL MORTALITY AND MORBIDITY

The major concern about the adverse effects of anemia on pregnant women is the belief that this population is at greater risk of perinatal mortality and morbidity (WHO 1968, CDC 1998). Maternal mortality in selected developing countries ranges from 27 (India) to 194 (Pakistan) deaths per 100 000 live births (WHO 1968; Abouzahr 1991). Some data show an association between a higher risk of maternal mortality and severe anemia, although such data were predominantly retrospective observations of an association between maternal hemoglobin concentrations at, or close to, delivery and subsequent mortality. Such data does not necessarily prove that maternal anemia causes higher mortality because both the anemia and subsequent mortality could be caused by some other condition.

In a large Indonesian study, the maternal mortality rate for women with a hemoglobin concentration < 100 g/L was 70.0/10 000 deliveries compared with 19.7/10 000 deliveries for nonanemic women (Chi I 1981). However, the authors believed that the relation of maternal mortality with anemia reflected a greater extent of hemorrhage and late arrival at admission rather than the effect of a prenatal anemic condition.

In another study, often cited as showing an association between maternal anemia and subsequent mortality, approximately one-third of the anemic women had megaloblastic anemia due to folic acid deficiency and two-thirds had hookworm. The standard cutoff for anemia was extremely low (< 65 g hemoglobin/L), and the authors stated that although anemia may have contributed to mortality, it was not the sole cause of death in many of the women (Llewellyn-Jones D. 1965).

2.2.2.2 MATERNAL ANEMIA AND BIRTH WEIGHT

In several studies, a U-shaped association was observed between maternal hemoglobin concentrations and birth weight (Murphy JF et al. 1986). Abnormally high hemoglobin

concentrations usually indicate poor plasma volume expansion, which is also a risk for low birth weight (Steer PJ. 2000, Garn SM et al 1981).

Lower birth weights in anemic women have been reported in several studies (Hemminki E et al. 1991; Agarwal K. et al 1991, Singla PN et al 1997). In a multivariate regression analysis of data from 691 women in rural Nepal, adjusted decrements in neonatal weight of 38, 91, 187, and 153 g were associated with hemoglobin concentrations ≥20, 90–109, 70–89 and < 70 g/L, respectively. The odds for low birth weight were increased across the range of anemia, increasing with lower hemoglobin in an approximately dose-related manner (1.69, 2.75, and 3.56 for hemoglobin concentrations of 90–109, 70–89, and 110–119 g/L, respectively) (Dreyfuss M 1998). Trials that included large numbers of iron-deficient women showed that iron supplementation improved birth weight (Agarwal KN et al. 1991, Hemminki E 1978).

Lone FW et al (2004) studied 626 pregnant women and found that preterm birth risk was 4 times, low birth weight risk was 1.9 times, low APGAR score was 1.8 times and intrauterine fetal death was 3.7 times more common in anemic pregnant women compared to non anemic.

Levy A et al. (2005) in their retrospective study, evaluated the preterm birth and birth weights of the anemic pregnant women and determined the maternal anemia as an independent risk factor for preterm birth and low birth weight, no association was found with bad perinatal outcome in their study.

Bondevik GT et al. (2001) in their case control study on 1400 pregnant women, used the first antenatal visit hematocrit levels as parameter, and concluded that low birth weight and preterm birth rates were significantly higher when the maternal hematocrit was under 24%.

Malhotra M et al. (2002) grouped 447 pregnant women into 4 groups according to their anemia levels, compared them for maternal and perinatal outcome and postpartum complications. They reported that severe anemia increased the risk for low birth weight, and mild anemia had the best maternal and perinatal outcome.

Patra S et al. (2005) reported the maternal and perinatal outcomes of 130 severely anemic pregnant women who had 5 gr/dl or lower hemoglobin. The hemoglobin levels were acquired at the 3 rd trimester and 81 % of their population were multiparas. Pregnancy intervals for multiparas was found to be 16. 5-/+ 0.5 months, and following outcomes were reported: preterm birth rate 69.2%, preeclampsia 17%, eclampsia 4%, placental ablation 3%, fetal distress 23%, low birth weight 24.6 % and neonatal death rate 35%.15 They concluded that especially in multiparas when the pregnancy intervals were short and nutritional support was insufficient, pregnancy complications associated with maternal anemia were more commonly encountered.

Some investigators reported a negative association between maternal serum ferritin and birth weight and a positive association with preterm delivery (Goldenberg RL et al. 1996; Tamura T. et al 1996; Rondo PH et al. 1997). These findings probably indicate the presence of infection, which elevates serum ferritin.

2.2.2.3 MATERNAL IRON DEFICIENCY ANEMIA AND DURATION OF GESTATION

There is a substantial amount of evidence showing that maternal iron deficiency anemia early in pregnancy can result in low birth weight subsequent to preterm delivery. Welsh women who were first diagnosed with anemia (hemoglobin <104 g/L) at 13–24 wk of gestation had a 1.18–1.75-fold higher relative risk of preterm birth, low birth weight, and prenatal mortality (Murphy JF 1986).

After controlling for many other variables in a large Californian study by Klebanoff et al (1991) showed a doubled risk of preterm delivery with anemia during the second trimester but not during the third trimester. In Alabama, low hematocrit concentrations in the first half of pregnancy but higher hematocrit concentrations in the third trimester were associated with a significantly increased risk of preterm delivery (Lu ZM et al. 1991). When numerous potentially confounding factors were taken into consideration, analysis of data from low-income, predominantly young black women in the United States showed a risk of premature delivery (< 37 wk) and subsequently of having a lowbirth-

weight infant that was 3 times higher in mothers with iron deficiency anemia on entry to care. There was no such increased risk for mothers who were anemic but not iron deficient at entry to care, or for those who had iron deficiency anemia in the third trimester (Scholl TO et al. 1992).

Similar relations were observed in women from rural Nepal, in whom anemia with iron deficiency in the first or second trimester was associated with a 1.87-fold higher risk of preterm birth, but anemia alone was not. (Dreyfuss M 1998). In an analysis of 3728 deliveries in Singapore, 571 women who were anemic at the time of delivery had a higher incidence of preterm delivery than did those who were not anemic, but no other differences in either pregnancy complications or neonatal outcomes were observed (Singh K et al. 1998). Thus, the results of several studies are consistent with an association between maternal iron deficiency anemia in early pregnancy and a greater risk of preterm delivery. The apparent loss of this association in the third trimester is probably because a higher hemoglobin concentration at this time may reflect poor plasma volume expansion and an inability to discriminate between low hemoglobin caused by iron deficiency from that caused by plasma volume expansion.

2.2.2.4 MATERNAL ANEMIA AND INFANT HEALTH

An association between maternal anemia and lower infant Apgar scores was reported in some studies. In 102 Indian women in the first stage of labor, higher maternal hemoglobin concentrations were correlated with better Apgar scores and with a lower risk of birth asphyxia (Rusia U. et al. 1995). When pregnant women were treated with iron or a placebo in Niger, Apgar scores were significantly higher in those infants whose mothers received iron (Preziosi et al. 1997). A higher risk of premature birth is an additional concern related to the effect of maternal iron deficiency on infant health; preterm infants are likely to have more perinatal complications, to be growth-stunted, and to have low stores of iron and other nutrients.

In the Jamaican Perinatal Mortality Survey of > 10 000 infants in 1986, there was an <50% greater chance of mortality in the first year of life for those infants whose mothers

had not been given iron supplements during pregnancy (Greenwood R. et al 1994), although the iron status of these infants and their mothers was not assessed.

Apart from this survey, there is little known concerning the effects of maternal iron status during pregnancy on the subsequent health and development of the infant.

2.2.2.5 IRON SUPPLEMENTATION AND MATERNAL IRON STATUS

There is little doubt that iron supplementation improves maternal iron status (Lindsey A. 2000). Even in industrialized countries, iron supplements have been reported to increase hemoglobin, serum ferritin, mean cell volume, serum iron, and transferrin saturation (Dawson EB et al. 1987; Svanberg B et al. 1976; Milman N et al. 1991; Simmons WK et al. 1993; De Benaze C et al. 1989; Tylor DJ et al. 1979; Fleming AF et al. 1986). These improvements are seen in late pregnancy, even in women who enter pregnancy with adequate iron status (Puolokka J. et al 1980; Svanberg B et al. 1976; Milman N et al. 1991; De Benaze C et al. 1989). When compared with unsupplemented pregnant women, differences in iron status due to supplementation usually occur within <3 months of the time supplementation begins (Puolokka J. et al 1980; Milman N et al. 1991; IOM 1993). Supplementation can reduce the extent of iron depletion in the third trimester (Svanberg B et al. 1976).

However, for women who enter pregnancy with low iron stores, iron supplements often fail to prevent iron deficiency. Well-nourished Danish women were given either a placebo or 66 mg Fe/d as ferrous fumarate beginning week 16 of pregnancy. At term, in the placebo group, 92% of women had no bone marrow iron, 65% of women had latent iron deficiency, and 18% of women had iron deficiency anemia. Even in the group supplemented with iron, iron stores at term were exhausted in 54% of women, although only 6% of women had latent iron deficiency and no women had iron deficiency anemia (Milman N et al. 1991; IOM 1993). Iron supplements also failed to replete iron stores fully in other studies (Puolokka J. et al 1980; Svanberg B et al. 1976). Low compliance may explain some of this problem.

The benefits of iron supplementation on maternal iron status during pregnancy become even more apparent postpartum. This is illustrated by a Swedish study in which all pregnant women who did not take iron supplements had less than "sufficient" iron stores in late pregnancy compared with 43% of supplemented (200 mg Fe/d) women (Svanberg B et al. 1976). Two months after iron supplementation began, these differences were even more striking: 90% of unsupplemented women but only 20% of supplemented women, still had sparse iron stores.

Several intervention studies showed that iron supplementation, beginning during the second trimester of pregnancy, resulted in higher maternal hemoglobin concentrations for <2 months postpartum and higher serum ferritin concentrations for as long as 6 mo after delivery than observed in unsupplemented control subjects. In Denmark, for example, serum ferritin concentrations at 2 months postpartum in women supplemented during pregnancy were twice those of women who did not receive iron (Milman N et al. 1991;).

A Finnish study showed that iron supplementation during pregnancy improved maternal serum ferritin, but not hemoglobin, concentrations for ≥6 months postpartum (Puolokka J. et al 1980). Compared with a placebo group, women in Niger who were supplemented with iron during pregnancy had higher concentrations of hemoglobin, serum iron, and serum ferritin; higher mean cell volumes; and lower erythrocyte protoporphyrin at 3 months postpartum. At 6 months postpartum, erythrocyte protoporphyrin was still significantly lower in the iron-supplemented group (Preziosi et al. 1997). These benefits on postpartum maternal iron status may be especially important when interpregnancy intervals are short because the supplemented mother will enter a subsequent pregnancy with better iron status. In addition, many women are anemic in the postpartum period because of blood loss during delivery. Although a similar benefit could be obtained if women were supplemented during lactation, pregnancy is a time when iron absorption is particularly efficient and when there is usually more opportunity to provide, encourage, and monitor the use of supplements.

2.2.2.6 BENEFITS OF MATERNAL IRON SUPPLEMENTATION ON IRON STATUS OF THE FETUS AND INFANT

It is generally assumed that the iron status of the fetus, and subsequently the infant, is quite independent of maternal iron status during pregnancy (IOM 1993), except perhaps when infants are born to severely anemic women. A review of the literature on this issue indicates that indeed, with rare exceptions (Gasper MJ et al 1993), there is no significant association between maternal hemoglobin concentrations at or near term and cord blood hemoglobin concentrations. This lack of an association was reported in countries as diverse as Niger (Preziosi et al. 1997), India (Agarwal RMD 1983), China (Lao TT et al. 1991), Japan (Hokama T et al.1996), and Ireland (Barton DPJ et al. 1994).

A lack of association between maternal and cord blood hemoglobin was also found in France (De Benaze C et al. 1989) and Denmark (Milman N. et al.1994), even when half of the women were provided with iron supplements. However, although there was no relation between low hemoglobin concentrations in unsupplemented British women in the third trimester and hemoglobin concentrations in infants 3–5 d postpartum, infants born to nonanemic mothers had distinctly higher blood volumes, red cell volumes, and circulating hemoglobin mass than those of infants born to anemic mothers (Sisson TR et al. 1958). Cord blood ferritin was, however, related to maternal hemoglobin or maternal ferritin in most of these and other nonintervention and intervention studies (De Benaze C et al. 1989; Gasper MJ et al 1993; Agarwal RMD 1983; Hokama T et al.1996; Milman N. et al.1994;Ajayi OA 1988; Tchernia G et al.1996) with few exceptions (Barton DPJ 1994; Zittoun J. et al. 1983; Rusia U el. 1995).

In the study by Rusia et al (1995), serum transferrin receptor concentrations were higher in infants born to anemic mothers. De Benaze et al (1989) found the relation between the iron status of French pregnant women and serum ferritin concentrations of their infants to still be apparent 2 months postpartum.

Similarly in Turkey, maternal hemoglobin at delivery was correlated with serum ferritin in 2-months-old infants (Tekinalp G. et al. 1996). Colomer et al (1990) analyzed the relation between the hemoglobin concentration of pregnant women and the risk of anemia

in their infants at 12 months of age. Infants born to anemic mothers were more likely to become anemic themselves (odds ratio: 5.7), when feeding practices, morbidity, and socioeconomic status were controlled for.

Preterm delivery associated with iron deficiency could also contribute to lower fetal iron stores. Nonetheless, the effect of the mother's iron status on her infant's iron stores postpartum needs to be clarified because of the known detrimental effects of iron deficiency anemia on the mental and motor development of infants.

2.2.2.7 PREVALENCE OF IRON DEFICIENCY ANEMIA DURING PREGNANCY

WHO estimates that even among the South Asian countries, India has the highest prevalence of anaemia. What is even more important is the fact that about half of the global maternal deaths due to anaemia occur in South Asian countries; India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia (Ezzati M. 2002). It is obvious that India's contribution both to the prevalence of anaemia in pregnancy and maternal deaths due to anaemia is higher than warranted by the size of its population **(Table 2.8)**. Available estimates also suggest that the magnitude of reduction in the prevalence of anaemia during nineties in India is lower than that in neighbouring South East Asian countries.

Table 2.8: Prevalence of anaemia and its contribution to maternal mortality

Country	Prevalence of anaemia in pregnant women %	Maternal deaths from anaemia
Bangladesh	74	2600
Bhutan	68	<100
India	87	22,000
Nepal	63	760
S. Asia Region Total		25,560
World Total		50,000

(Source: Kalaivani K. 2009)

Globally there have been many prevalence data nationwide, statewise or districtwise has been generated by number of researchers, a few of them have been depicted in the **Table**

2.9, revealing the global scenario of iron deficiency anemia amongst pregnant women in developed and developing countries.

Table 2.9: Prevalence of iron deficiency anemia amongst pregnant women

Author; year; Location	N	Mean Hb(g/dl)	Serum ferritin	Percent Prevalence of IDA	Deficiency
Karaoglu L et al; 2010; Turkey	823	11.5	-	27.1%	Moderate
Hanif J. et al; 2007; Malaysia	1180	11.46	-	35%	Moderate
Xing Y. et al; 2009; Tibet	380	12.7	-	>40%	Severe
Ghazala I. et al; 2011; Pakistan	60	-	14.6-18.5 (all three trimesters)	66%	Severe
Toteja GS et al; 2006; India	6923	-	-	84.9%	Severe
Hanif J. et al; 2007; Malaysia	1072	-	-	35%	Mild
Chellan R, DLHS-RCH (2002-2004); 2010; India	21,233	-	-	96.2%	Severe
Elzahrani S; 2012;Saudi Arabia	190	-	-	22.6%	Mild

2.2.3 IRON DEFICIENCY DURING CHILDHOOD

It is estimated that approximately 600 million preschool and school-aged children are anaemic worldwide, and it is calculated that at least half of the cases are due to iron deficiency. In general, low-income countries have a higher prevalence of anaemia (WHO/CDC 2008). This association is also true in high-income countries where people of low socioeconomic status are especially susceptible to iron and other vitamin and mineral deficiencies (Cole 2010).

2.2.3.1 EFFECTS OF IRON DEFICIENCY ANAEMIA DURING CHILDHOOD

The condition includes growth retardation, reduced school achievement, impaired motor and cognitive development, and increased morbidity from a variety of causes including diarrhoea and acute respiratory infections (WHO 2001). Specifically, iron deficiency can lead to deficits in memory and behavioural regulation as iron is required to make neurotransmitters such as dopamine, epinephrine and serotonin (Iannoti 2006; Moy 2006; Beard 2008), while impaired myelination contributes to deficits in motor function. Long-term effects of early iron deficiency include decreased work capacity and impaired cognitive and behavioural development (Lozoff 2000; Lozoff 2007). Some of these impairments are thought to be irreversible if they occur at an early age and the consequences may continue even after treatment, reinforcing the importance of prevention (Siddiqui 2004; Iannoti 2006; Lozoff 2007).

2.2.3.2 IRON DEFICIENCY AND GROWTH OF THE CHILDREN

Iron requirements depend on body needs for tissue growth and tissue maintenance, which may vary with the life cycle and certain environmental factors (Beard, Dawson and Pinero 1996). Total iron requirements for adolescent are computed from the increased iron requirements for the expansion of the total blood volume (0.18 mg/d in boys and 0.14 mg/d in girls on an average) and the increase in the total body essential pool with the increase in the lean body mass (0.55 mg/d in boys and 0.33 mg/d in girls medial additional requirements). The increase in the iron requirements for the red blood cell mass includes both the increase in total blood volume as well as the increase in the mean hemoglobin concentration from the early adolescent years through the adolescent growth spurt.

Further, adolescent iron requirements are ever higher in developing countries due to infectious diseases and parasitic infestations that cause iron loss and because of low bioavailability or iron from diets limited in heme iron. Low iron status among adolescents may limit their growth spurt (Brabin and Brabin 1992).

One symptom associated with iron deficiency is loss of appetite-anorexia (Judisch et al 1966; Pollitt 1976). IDA is reported to suppress appetite in anemic children, leading to poor food intake, which again may adversely influence growth.

Chawang et al (1988) found myeloperoxidase activity to be reduced and morbidity to be increased in anemic children compared to normal. They found a significant reduction in the level of morbidity in rural Indonesian children (8.2 to 13.5 years) receiving iron supplements as compared to children receiving the placebo.

2.2.3.3 EFFECTS OF IRON DEFICIENCY ON COGNITION OF THE CHILDREN

There are many pathways through which iron deficiency can affect cognition. Briefly it is proposed that even at the very early stages of iron deficiency, a decrease in iron dependent dopamine D2 receptors in the cortex alters dopamine neurotransmission, which, in turn, impairs cognitive function (Leibel et al 1979, Youdin 1990). The alternative hypothesis is less explicit and refers to move advanced stages of iron deficiency when hemoglobin concentration is compromised. It postulates that in the presence of anemia there may be systematic effects that interfere with cognition (Leibel et al 1979).

In iron deficiency, changes in brain iron content and distribution, and in neurotransmitter function may affect cognition. Attention processing of environmental information is highly dependent on appropriate rates of dopamine. Alteration in dopamine is associated with altered perception, memory and motivation (Beard 2001). Therefore, iron deficiency impairs cognitive function thus promising learning abilities (Kurz and Johnson- Welch 1994). Anemia may produce scholastic under- achievement and behavioral disturbances in school children (Pollitt and Liebel 1976). Studies show that iron deficient children perform less well on psychomotor tests than do non-anemics (Bhatia and Seshadri 1993). However, little is known regarding the impact on children entering adolescent and those undergoing the pubertal growth spurt.

There is consistent evidence showing how level and quality of dietary intake influences both the development and function of the central nervous system. Early IDA may have relatively permanent effects on brain myelination, thus restricting the level of gains that can result from early iron supplementation (Lozoff 1998). Alternatively IDA may also act to reduce the number of D2 dopamine receptors, and thus disrupt function mediated by the dopamine neurotransmitter system.

In a longer-term study of recovery from iron deficiency in infancy, Lozoff and colleagues (2000) reevaluated a group of Costa Rican children who had been tested and treated for iron deficiency when they were infants. This reevaluation occurred at both 5 and 12 years of age. Of the original 191 participants, 87% were reevaluated in early adolescence. Those who had chronic, severe iron deficiency in infancy were compared with those who had good iron status before and/or after iron therapy in infancy. Children who had been iron deficient in infancy scored lower in arithmetic, writing, reading, school progress, and motor function, and experienced more anxiety, depression, and social problems. They performed more poorly on a spatial memory task and were slower on the Tachistoscopic Threshold subtest of the Computerized Abilities Test. Both cognitive tasks likely involve hippocampal and prefrontal cortex-striatal neural systems. This study is critical because it is of the longest duration to date and provides very strong evidence that iron deficiency during early life is associated with a path of cognitive and behavioral development that is significantly below that of non iron-deficient anemic children.

A long term follow-up study on infants who had been treated with iron at 6–18 months of age and then examined at 6–7 years of age was carried out by Cantwell and colleagues. These formerly anemic children had difficulty in motor control tasks and were rated as inattentive, but the failure to include a control group in the study precludes strong conclusions regarding proof of effect (Beard J 2003).

An Indian study in Pondicherry, anemic young adolescent girls (10-12 years) had significantly lower grades measured using Raven's Progressive Matrics Intelligence scale and had impaired learning and school achievements (Many and Rajini 2006). In Jamaica, anemia in adolescent girls (13-14 years) was associated with poor school

performance, which remained after controlling for social background and attendance (Walker et al 1996).

2.2.3.4 IRON SUPPLEMENTATION IN CHILDREN

A number of intervention trials examined effects of iron deficiency on neural functioning in school-age, pre-adolescent, and adolescent boys and girls (Groner JA 1986; Kashyap P 1987; Politt E 1989; Sheshadri S 1989; Soemantri 1985).

The study from India (Seshadri 1989) on stratified subjects by age and involved matching at baseline for hematological status, income, maternal education, height and weight, and a number of other variables prior to assignment to iron or placebo treatment. After two months, the investigators observed greater improvements in verbal and mathematical test results in the iron-treated groups than in the placebo-treated groups.

Pollitt and colleagues (1989) used a four-month intervention trial in nine-year-old Egyptian children and measured matching familiar figures tests with inclusions of placebo controls. Iron-deficient children did less well than controls at baseline and improved significantly if they received the iron treatment. In a study of slightly older pre-adolescent Indonesian individuals, Soemantri and colleagues (1985) used a three-month intervention trial to determine if learning and problem solving were adversely affected by iron status. The iron-treated group improved significantly, but not enough to make them equivalent to the non iron-deficient controls. The finding of a "response to iron therapy" was not replicated several years later by investigators using similar age groups and design in Thai children and with a longer period of intervention (Politt E 1989). Children were randomly assigned to treatment prior to determination of iron status. As is seen in most of these studies, iron-deficient anemic children had lower global intelligence scores at baseline. Four months of treatment did not significantly change these scores. Some of the issues regarding the failure to observe consistent benefits to iron therapy include specificity of tests, cultural validity of the tests, duration and severity of the preexisting iron deficiency, and confounding factors within the microenvironment of the study that are not controlled for in the analysis. Investigators in nearly all of these studies, however,

did lengthy evaluations of potential confounding variables and in the case of pair-matching, tried to control for these confounds prior to treatment assignment.

In order to test the hypothesis that "anemia" was an essential component, the majority of subjects selected by researchers in this age group had iron-deficiency anemia, and some had iron deficiency but no anemia. Bruner et al (1996) specifically selected only iron-deficient, but not anemic, adolescents to determine if iron therapy had any significant impact on cognitive processes. She administered a wide range of tests of attention, learning, and memory to inner-city adolescent girls in an intervention trial. Two months of iron therapy resulted in improvement in iron status and in a memory task but showed no differences from controls in three different measures of attention or vigilance.

The reviewed literature revealed that iron deficiency during school age years and into adolescence can have adverse effects on cognitive functioning. Importantly, in most cases the interventions for two to four months were sufficient to return performance in these tasks to levels that are similar to those in controls. While some studies showed strong effects on attentional process, others demonstrated effects on learning and memory, but not on attention. The lack of identical conclusions in all of these studies likely involves test specificity, cultural validity, duration and severity of iron deficiency, and the presence of confounding variable.

2.2.3.5 PREVALENCE OF IRON DEFICIENCY ANEMIA AMONGST SCHOOL CHILDREN

Globally the prevalence of anemia has observed to be very high amongst vulnerable groups including early childhood to lactation. However, adolescents and school aged children were considered to be not at risk groups in early 90's, but the data generated by many researchers has proved this age group to be most vulnerable after pregnancy compared to rest of the age groups. This could be due to increased iron requirement during growth spurt in both the genders and onset of menstruation amongst adolescent girls. **Table 2.10** reveals the prevalence of anemia globally depicted by various research groups.

Table 2.10: Global prevalence of iron deficiency anemia amongst school children

Author; year; Location	N	Mean Hb (g/dl)	Serum ferritin (µg/l)	% Prevalence of IDA	Deficiency
Bhanusali M et al; 2011; India	104	9.34	-		Moderate
Patel H et al;2009; India	65	11.29	-	63.1	Mild
Handa R et al; 2008; India	150	-	-	65.33	Mild
Ali A. et al; 2011; Egypt	400	9.8	-	55%	Moderate
Hioui M. et al; 2008; Morocco	295	12.4	26.8	12.2%	Mild
Keskin Y et al; 2005; Turkey	1041	13.1	26.3-34.2	3.9%	None
Odeh M; 2006; Palestine	290	-	-	12.7%	Moderate
Goyle A., Prakash S.; 2009;India	109	9.43	-	96.3%	Moderate

2.2.4 PROGRAMMES FOR PREVENTION AND MANAGEMENT OF ANAEMIA

India was the first developing country to take up a National Programme to prevent anaemia among pregnant women and children. **The National Anaemia Prophylaxis Programme** of iron and folic acid distribution to all pregnant women in India through the primary health care system was evolved and implemented from 1972, so that the vast majority of pregnant women who never seek health care, could benefit from this outreach programme. It was hoped that this programme will bring about a reduction both in the prevalence and severity of anaemia in pregnancy. There were two major components of the anaemia prophylaxis programme − pre-school children were to receive 20 mg elemental iron and 100 mg folic acid and pregnant women to receive 60 mg elemental iron and 500 µg of folic acid.

Of the two components, the coverage under the component for children had always been very poor. Comparatively the component for pregnant women has fared better. Under the program an attempt was made to identify all pregnant women and give them 100 tablets containing 60 mg of iron and 500 µg of folic acid. However all the national surveys (IIPS

1998-99; IIPS 2005-2006; DLHS-RCH 2002-2004; ICMR 2004; NNMB 2002) indicated that coverage under all these programmes was very low and there has not been any change either in the prevalence of anaemia or the adverse consequences associated with anaemia. Two decades after the initiation the National Anaemia Prophylaxis Programme, an ICMR study confirmed that most women received 90 tablets without Hb screening. Many did not take tablets regularly. Even among small number of women who took over 90 tablets, rise in Hb was low and mean Hb levels were no more than 9.1 g/dl (ICMR 1989). The study conducted in 1989 by ICMR indicated that coverage under the National Anaemia Pregnancy Programme was low and that 60 mg of ferrous sulphate was perhaps inadequate to treat anaemia.

The Programme was revised and renamed as **National Anaemia Control Programme (NACP)**. The Programme envisaged that all pregnant women will be screened for anaemia. Non anaemic women would get iron (100 mg) and folate (500 µg) and those with anaemia should get two tablets daily (Ramchandran P. 1992).

2.2.4.1 PROBLEMS FACED DURING IMPLEMENTATION OF ANAEMIA PREVENTION AND CONTROL PROGRAMMES

- The DLHS 2 (1998-99) showed that pregnant women were not being screened for anaemia and given appropriate therapy.
- Most women in poorly performing States did not come for antenatal check up. Many of those who came for antenatal check up (ANC) did not get IFA throughout pregnancy nor did they get 100 tablets (DLHS-RCH 2002-2004, NNMB 2002, ICMR 1989, DLHS 2008).
- Majority of those who got the tablets did not consume all the tablets (DLHS 2002-2004, DLHS 2008).

NNMB surveys (2002) showed that the proportion of pregnant women who receive IFA tablets is not high even among well-performing States like Tamil Nadu, Kerala and Maharashtra. DLHS 2 (2006) showed that there was some improvement in the coverage and content of antenatal care. About 40 per cent women had blood examination done

which included Hb estimation. DLHS 2 also showed that there has been some improvement in % of pregnant women receiving IFA tablets.

There has been a significant reduction in the percentage of women who received but did not consume the tablets. These data suggest that, if all pregnant women are screened for anaemia and provided appropriate therapy it might be possible to achieve substantial reduction in prevalence of anaemia in pregnancy.

2.2.4.2 CERTAIN CONSIDERATIONS FOR IRON DEFICIENCY CONTROL PROGRAMS

Increasing iron intake of the targeted population in the community is the primary motto of the entire preventive and control programs for IDA. These concerns are more focused during pregnancy and growth spurt of the children. However, certain criteria to be understood before initiating blanket coverage (Nair M 2001).

- **Iron supplementation during pregnancy**

There is a concern that supplementation programmes would not substantially improve birth weight. Further, there is little evidence to show that iron supplementation during pregnancy can minimize the risk of adverse pregnancy outcomes. Although studies (Zhou et al. 1998; Steer et al. 1995) suggest that pregnant women with anemia - Hb , 100 g/L. have a higher risk for preterm birth and low birth weight, it is not clear to what extent anemia is responsible for these outcomes and whether iron supplementation substantially ameliorates these problems.

- **Absorption of iron**

A large fraction of the daily supplemented large doses of iron remains unabsorbed, since iron absorption from the intestine is a highly regulated process. Continuous exposure of the intestine to iron is believed to reduce iron absorption from subsequent doses (Fairweather-Tait et al. 1985). Thus, the issue of exposing the intestine to large amounts of supplementary iron generating free radicals via the Fenton reaction and thus leading to oxidative damage of the tissues has come into focus (Slivka et al. 1986). This is

particularly important, considering the meager intake of antioxidants by the target groups in developing countries.

- **Mucosal response to iron supplement**

Though there is evidence of a positive impact of iron supplementation in properly conducted clinical trials, the results obtained with large scale public health programmes have been variable (Baynes & Cook, 1996; Yip, 1996). Gastrointestinal side-effects to oral iron leading to poor compliance is believed to be the main reason.

Although there are many factors that could contribute to the ineffectiveness of iron supplementation in public health programmes, it is unclear to what extent the failure of such `real-life' programmes is due to the response of the intestinal absorptive surface to repeated high doses of iron, particularly in the nutritionally compromised intestine of the populations in developing countries (Nair M 2001).

- **Poor compliance to the supplement**

Poor compliance to iron supplements during any age groups leads to decreased dietary iodine intake. It is also known that, consuming tablets as a source of iron becomes unacceptable to the affected population on daily basis. However, there are some other determinants also leading to poor compliance.

Some studies indicated that forgetfulness was a significant barrier for consumption of iron tablets. Researchers have suggested that direct supervision helped pregnant women adhere to the iron tablets consumption (Elder LK. 2000).

In developing countries like India, there are various causes that contribute to decreased adherence to iron supplementation including, misunderstanding of instructions, side effects, cultural beliefs, and inconvenient dosing regimens (Galloway R. 1994).

- **Intestinal iron absorption**

The possible role of transferrin receptor mediated uptake of iron (Vasantha Lakshmi, 1998) might be playing a role as a vital mechanism for intestional absoption. Iron

deficient intestines exhibited greater concentration of the transferrin receptor. Functionally this is expected to increase iron absorption through a luminal transferring ± mucosal transferrin receptor system. It is not known whether there is any increased burden on the receptor during daily therapeutic regimen of iron.

- **Iron supplementation and oxidative damage of the gastrointestinal tract**

Research studies have demonstrated that repletion of iron deficient rats with iron promotes oxidative stress, damages the absorptive cells and brings about functional and ultra structural derangements in the intestine (Nair M 2001). The causative factor responsible for such effects was identified as the hydroxyl radical produced by excess iron at the site of iron absorption (Srigiridhar & Nair M 1998 and unpublished observations). The findings on the role of food per se (natural diet) in reducing the effects of iron mediated oxidative stress have practical relevance (Srigiridhar & Nair 1997).

2.3 IODINE AND IRON DEFICIENCIES INTERACTIONS AND THEIR EFFECTS

Fetal growth (size and composition) are dependent on two determinants namely maternal nutrition and micronutrient status. Iron, iodine calcium, folate, Vitamin A and Vitamin C all influence the offspring size. A study by (Yajnik 2002), designed to examine the relationship between maternal nutrition, fetal size at birth and postnatal growth, showed that maternal circulating folate and Vitamin C concentration predicted larger offspring size, while higher ferritin levels predicted smaller sized babies.

Nutritional deficiencies of micronutrients can also affect development through childhood. Certain stages are more vulnerable than others, depending upon the particular nutritional deficiency. The intrauterine period is the most vulnerable to long-term neurological and cognitive function deficits due to iodine deficiency during pregnancy. The subsequent postnatal period is also vulnerable to this deficiency and iron deficiency as well (Grantham-McGregor and Ani 2001).

Early development of the child affected by iodine and iron deficiency leads to poor life quality. The shortage of theses essential micronutrients, its duration and severity may

alter the outcome. In developing countries where the prevalence of these deficiencies is still considerably high, social and economic development are also likely to be affected (Grantham-McGregor and Ani 2002).

Second and third trimester are the stages where pregnant women are highly vulnerable to iron deficiency anemia because of their increased iron needs, which are rarely met by dietary sources (Zimmerman MB and Hurrell RF 2005; Scholl TO 2005). The prevalence of anemia and iron deficiency anemia during pregnancy among industrialized countries ranges from 6-28% and 24-44%, respectively (Zimmerman MB and Hurrell RF 2005; Scholl TO 2005; Milman N 2006; Carriaga MT et al 1991). However, in developing countries, the majority of the women are anemic during second half of pregnancy (Carriaga MT et al 1991; UNICEF, UN, WHO 2001). Although several studies on adult population have reported higher prevalence of anemia in hypothyroid patients depicting the intercorrelation between both conditions due to iron and iodine deficiencies (Das KC et al 1975; Horton L et al 1976). Serum ferritin concentrations and total iron binding capacity may be lower in hypothyroid adults compared with euthyroid controls (Duntas LH et al 1999).

In hypothyroid iron deficient anemic population, Hb concentrations increase with T4 replacement, but the Hb increase is greater when T4 is given with iron (Horton L et al 1976). Poor iron absorption in hypothyroidism due achlohydria was reported by (Marqusee E, Mandel SJ. 2000; Seino Y et al 1976).

2.3.1 EFFECTS ON FETO-MATERNAL HEALTH

A study conducted among pregnant women residing in Switzerland, with the mildly iodine deficiency revealed that, poor maternal iron status predicts higher TSH and lower TT_4 concentrations. It was indicated by a lower relative risk of having TT_4 concentration less than 100 nmol/L in the group with negative body iron stores was 7.8. It is reasoned out that, iron deficiency blunts the thyrotropic response to TRH, decreases serum T_3 and T_4 levels slows turnover of T_3 and may reduce T_3 nuclear binding (Zimmerman MB 2006). A central mechanism in this effect is impairment of the heme-dependent enzyme TPO, limiting synthesis of thyroid hormone, and reducing circulating TT_3 and TT_4 (Hess

SY et al 2002). In young anemic women, treatment with iron may increase circulating thyroid hormone concentration (Beard JL, Borel MJ and Derr J 1990; Eftekhari MH et al 2006). In patients with subclinical hypothyroidism, iron repletion modestly increases circulating thyroid hormones and lowers TSH levels (Duntas LH et al 1999).

A study on the assessment of thyroid status of anemic pregnant women residing in goiter endemic conditions in the Republic Kazakhstan was reviewed by (Zel'tser et al 1994). In total N=120 pregnant women were examined; half of them had goiter and rest showed absence of goiter. The control group consisted of 20 healthy pregnant women. Clinical and ultrasonic examinations, thyroid puncture biopsy, TSH, FT_3 and T_4, TT_4, TBG, as well as peripheral RBC counts, levels of hemoglobin, serum Fe and TIBC of serum ans saturation coefficients were assessed. Healthy pregnant women were from a focal point of endemic goitre were found to be at risk for anemia in the third trimester of pregnancy. The study concluded that, in anemic pregnant women, endemic goitre aggravates anemia and chronic iodine deficiency promotes subclinical hypothyroidism, while in the presence of anemia its severity increases, more so if anemia is present along with goitre.

2.3.2 EFFECTS ON CHILDREN

A study conducted by Eftekhari MH et al 2006 amongst Iranian adolescent girls on interrelation between iron and iodine deficiency revealed that, using a stepwise regression procedure, only ferritin contributed significantly to the rT_3 concentration ($r=-0.34$, $P<0.01$); thus, subjects with lower iron stores also had a higher reverse triiodothyronine concentration. A positive and significant association between the serum ferritin level and the TT_4 concentration, and a negative and significant association between ferritin and the T_3/T_4 ratio, as well as a negative and significant association between ferritin and TSH, suggests that thyroid status alterations could be due to a deficiency in iron-dependent enzymes such as thyroperoxidase that impairs thyroid metabolism. Thus the study stated that, an increase in rT_3 is related to changes in iron status and that the increased level of rT_3 is inversely correlated with changes in plasma ferritin concentration. Iron deficiency decreased plasma concentrations of T_3 and T_4 and increased in vitro hepatic rT_3 deiodination, suggesting that iron-deficient animals tend to metabolize thyroid hormone via a deactivating pathway (Smith SM et al 1993).

A study conducted by Mohammed H et al (2008) also studied relation between iron deficiency and persistent goiter amongst Iranian school children aged between 8-13 years. Iron status was assessed using serum ferritin and iodine status using (FT_4, TSH and TT_4) thyroid hormones. It was reported that, the iron status did not vary with age or gender. However, it was observed that, children with <15 ng/ml serum ferritin demonstrated decreased FT_4 and increased TSH ($p<0.001$) levels at the time of assessment. This pattern suggested a clear correlation between the metabolisms of both the micronutrients.

Another study conducted by Eftekhari MH et al (2006) on randomized control trial with supplementation of ferrous sulphate (60 mg elemental iron) and lipoidal tablet 400 mg for 5 tablets/week amongst iron+iodine group and rest were only iron, only iodine and control groups. This study results conducted with Iranian adolescent girls revealed non significant changes in the level of TSH during the study and differences between groups at the end of the study. Levels of tT_4 in iron + iodine and iron-treated groups increased significantly (+11.5 and 10.5%, respectively, $P<0.001$), resulting in significantly higher levels than in both the iodine and control groups. The direction of change in tT_3 was similar to that of tT_4, in that the level of tT_3 in iron+iodine and iron groups at the end of study showed a significant rise (+3.5 and 4%, respectively, $P<0.05$), and final levels were significantly greater than in iodine and control groups. Although levels of fT_4 in the iron+iodine and iron groups showed a, significant rise at the end of the study (+8.6 and 9.6%, respectively, $P <0.001$ for both), final levels showed no significant difference compared with the iodine and control groups.

The relationship between iron and iodine deficiency amongst adolescents residing in iodine-deficient area of Turkey was studied by Yavuz et al (2004). Total n=330 school aged children were included with mean age 14 years. FT_3, FT_4 and TSH, Hb and mean corpuscular volume, Fe and total Fe-binding capacity concentration and ferritin levels were determined. They observed that, there was non significant difference between iron sufficient and deficient the children on thyroid hormone profile. As a result study reported no significant difference between iron status and thyroid hormone levels.

Data from the few available cross-sectional studies which have investigated the correlation between IDD and IDA are equivocal.

A survey in Ethiopian children found no correlation in goiter rate or thyroid hormone levels and iron status (Wolde-Gebriel et al., 1993b). Also no significant difference was found in the prevalence of anemia between goitrous and non-goitrous subjects in the Philippines (Florentino et al., 1996). However, in severely vitamin A-deficient Ethiopian children, low levels of T3 were associated with serum iron and low transferrin saturation (Wolde-Gebriel et al., 1993a). A national screening in 2917 children in Iran has reported a highly significant difference in goiter rates by palpation between children with low and normal SF levels (Azizi et al., 2002). Goiter was 3.8 times more prevalent in school children with low SF levels than in children with normal SF concentrations. Moreover, Zimmermann M. et al. (2000c) assessed in 1997 iron status and goiter rate by palpation in 419 children aged 6-15 years in two villages in western Côte d'Ivoire and found a relative risk of 1.9 (confidence interval 1.5-2.3) for goiter for children with IDA. However, the inconsistent data on the relationship between IDD and IDA are probably due to the fact that public health problems in developing countries are multiple. Several factors such as malaria, parasitic infections and other nutritional deficiencies also interfere with iodine and iron metabolism, as well as anemia, and therefore may obscure present interactions between two micronutrients.

Zimmermann M. et al. (2000c) investigated the effect of a 200 mg oral dose of iodine as iodized oil in non-anemic (n=51) and anemic (n=53) children with goiter in western Côte d'Ivoire. At 15 and 30 weeks Tvol was significantly reduced in the non-anemic group compared to the anemic group ($p<0.001$). A clear difference in goiter prevalence was apparent at 15 and 30 weeks, when goiter rates were 62% and 64% in the anemic group and only 31% and 12% in the non-anemic group, respectively. After 30 weeks, TSH and T4 concentrations improved significantly in the non-anemic group compared to the anemic group. Beginning at 30 weeks, the anemic children were given 60 mg oral iron as ferrous sulfate four times/week for 12 weeks (Zimmermann M. et al., 2000b). This resulted in an increase in Hb (±SD) from 97 (±8) g/L at 30 weeks to 122 (±8) g/L at 50 weeks. Change in Tvol, which had reached a plateau at weeks 10 through 30 in the iron

deficient anemic children, began to fall again after iron supplementation. Consequently, goiter prevalence in the anemic group, which had remained at 62% to 64% from weeks 10 through 30, was reduced after iron supplementation to 31% and 20% at 50 and 65 weeks. The findings in these studies suggest 1) that IDA in children may limit the thyroid response to an iodine prophylaxis and 2) that iron supplementation improves the efficacy of oral iodized oil in goitrous children with IDA.

2.3.3 OTHER STUDIES ON HUMAN

IDA also reduces thyroid hormone concentration in humans. Although, (Lukaski et al.1990) observed no differences in thyroid hormone and TSH concentrations between iron depleted and iron repleted women at room temperature, the relative increases in TSH, T4, and T3 after cold exposure were smaller (18, 16, and 18% respectively) when iron balance was negative than when it was positive (23, 23, and 25%, respectively).

Martinez-Torres et al. (1984) reported 10% lower T_3 concentrations in both moderate to severe IDA (Hb 75 g/L) and iron deficiency without anemia compared to control subjects, also this difference was not significant.

In contrast, (Beard et al. 1990) found a highly significant difference in T_3 concentrations between anemic (Hb 110 g/L) and control women. This discrepancy might be due to a smaller within-group variance in the latter study because only women with a certain body fatness and during particular days of their menstruation cycle were included (Beard et al., 1990). In the same study, plasma TSH concentrations of anemic women were within the normal range at baseline and were unaffected by iron status. The subsequent iron supplementation corrected anemia, but only partially normalized thyroid hormone concentrations (Beard et al., 1990).

Studies have also demonstrated a relationship between anemia and hypothyroidism; anemia was found in 25-50% of hypothyroid patients (Das et al., 1975; Horton et al, 1976). Hematological findings were diverse and anemia was due to iron deficiency only in a few cases. However, a recent study found significant differences in SF concentration and total iron binding capacity between 57 hypothyroid patients and 61 euthyroid

controls (Duntas et al., 1999). Moreover, in a group of hypothyroid patients with low serum iron levels, the Hb concentrations increased in response to T4, but the increase was greater in response to T4 and iron (Horton et al., 1976).

Thus, there have been many controversies upon efficacy of iodine and iron supplementation to be better than iodine supplementation only on thyroid analytes. Other way the correlation between iron and iodine deficiencies has been the issue of research by many researchers. However, majority of them have agree upon the statement and the rests could not agree due to same or the other limitations of the studies.

Other than the interrelation at physiological level, an iodine and iron deficiency also shares few causes leading to deficiencies of the micronutrients in the humans. Parasitosis is one of them. As discussed above parasitosis leads to iron deficiency anemia, but it can also lead to iodine deficiency amongst the human.

2.3.4 IODINE, IRON DEFICIENCIES AND PARASITOSIS

A unique study in consenting residents of Miton, Haiti (n=1932) was carried out to evaluate the effectiveness of salt fortified with diethylcarbamazine (DEC) and iodine to eliminate *Bancroftian filariasis* and iodine deficiency. During first year all subjects were supplemented with 0.25% DEC and 25 ppm iodine (Freeman et al 2001). After one year's treatment, the prevalence and intensity of microfilaremia were reduced by more than 95%, while an anemia levels were reduced by 60%. Iodine deficiency, which had been moderate to severe, was eliminated after one year of iodized salt consumption. DEC-fortified salt was well accepted by the community and reduced microfilaremia; its low-level transmission had no reported side-effects.

A study in Malawi by (Furnee et al 1997) amongst school children 8-10 years examined the relation between intestinal parasite treatment and oral iodized oil efficacy. Severely iodine deficient school children with a single parasitic infestation, either A Lumbricoids, hookworm or Entamoeba histolytica were randomly allocated to experimental and control by treatment before taking a 1 ml oral supplement (490 mg iodine) of iodized ethyl esters from poppy seed oil. After supplementation, urinary iodine concentrations were measured

regularly and the study results revealed that, the duration effect of iodine supplement remained 9.2 weeks shorter ($p<0.001$) and their counterparts in experimental group, where the effect was 16.8 weeks. Thus, the study concluded that, by interfering with absorption, intestinal parasitic infestations reduce the efficacy of oral supplementation with iodized ethyl esters.

There were few of the studies which reported remarkable impact of worm infestation on decreased micronutrient status amongst the population rather than their interrelation to each other. A total of 14740 Ethiopian school children were studied for the prevalence of goiter, xerophthelmia and anemia by (Wolde- Gebriel et al 1993) in seven provinces. Goitre, Xerophthelmia and clinical anemia were observed amongst 34.2%, 0.91% and 18.6% children respectively. Mean Hb and corpuscular Hb concentration along with UIE were observed to be lower compared to rest of the parameters to be into normal range. The thyroid hormones and UIE correlated with each other. However, Hb concentration did not correlated significantly with any of the iodine status an assessment, indicating the prevalence of clinical iron deficiency was not of the origin, but was due to effect of intestinal parasite infestation and malaria. This in turn suggests there is a negative impact of worm infestation on iron status of the children other than iodine deficiency.

Thus, these studies on iron, iodine and parasitosis focused another dimension towards the coexistence of infection and deficiencies and there by affecting the efficacy of the supplements. This suggests the importance and need for undertaking deworming as an effective strategy amongst vulnerable populations before incorporating the micronutrient supplement to give effect at its best.

2.4 FOOD FORTIFICATION: AN APPROACH TO COMBAT MICRONUTRIENT DEFICIENCIES

Food industries and nutritionists are striving to provide successful delivery vehicles that would help to bridge the gap between the dietary intakes and combat the occurrence of micronutrient deficiencies- iodine and iron. In **Table 2.11** has been presented on the basis of the review of the studies carried out globally to identify different food vehicles to fortify with iodine and other micronutrients. Some of these have been extensively researched in clinical studies, which included studying the effect of nutritional

intervention with fortified foods on nutritional status or functional outcomes. Within these foods, some are targeted for family consumption, while other are targeted at catering to the iodine requirements of children along with the benefits of the other essential micronutrients. The fortification of iodine has been discussed into different categories in WHO report (2004). Following the similar pattern the fortified foods with iodine have been discussed into mass fortification and targeted fortification in our review as below:

2.4.1 TARGETED FORTIFICATION

As reported by (Mehra R 2009) biscuits and beverages are the two most commonly preferred food items by the children, hence are chosen for fortification. Some of the food products have been used as intervention products in studies with children. There are multiple micronutrients-fortified products shown to have a beneficial effect on nutritional

status, anthropometric measures and cognition in school-aged children (Abrams et al 2003; Lantham et al 2003).

The recommended daily intake for iodine by the WHO (2004) and United States institute of Medicine (IOM), for preschool age (90 µg), school-aged children upto 12 years (120 µg), older children and adults (150 µg) and for pregnant women (250 µg). The fortification levels are determined as a percentage of the daily requirement of iodine. Fortified foods which are consumed as such or require minimum reconstitution, provide an opportunity to include known or measured amount of iodine in the diet.

2.4.2 MASS FORTIFICATION

Mehra R (2009) also reported that, in studies for mass fortification, mostly staple have been studied for stability and acceptability or efficacy in community trials. Seasoning powder for noodles co-fortified with iodine was found to be stable under accelerated storage conditions, with no adverse effects on the sensory quality of the noodles (Chavasit and Tontisirin 1998). However, the iodine content of fortified parboiled milled rice decreased significantly as storage time increased (Tulyathan et al 2007).

Table 2.11: Impact assessment of fortified products with iodine and iron for laboratory* and community† based studies

Author , year and place	Age group (Years)	Intervention	Fortification (content/ serving	Serve Size	Level of iodine per serve or per 100 g or % as RDA	Period
Chavasit and Tonisirin; (1998); Thailand	*	Instant Noodles seasoning powder	Iodine, Iron, Vitamin A	20 mg premix per package noodles	50 µg of iodine/ serve	
Tulyathan et al. (2007); Thailand	*	Parboiled milled brown rice	Iodine	100 gm	96 µg iodine/ 100 gm rice	
NIN (2003-2004); India	*	Sugar	Iodine, Iron	Not mentioned	30 ppm	
Lofti et al (1993); China	*	Brick Tea	Iodine	Not mentioned	Not mentioned	
Chavasit et al; (2003); Thailand	*	Fish Sauce	Iodine, Iron	15 ml	50 µg iodine / serve	
Fisch et al; (2003); Mali, Africa	*	Water	Iodine	Not mentioned	100 µg iodine / Litre	
Capanzana et al; (2005); Philippines	*	Margarine	Iodine, Vitamin A, Vitamin B1, Iron, Omega-3 and 6 fatty acids	30 gms or 2 Table spoons	50 µg iodine / serve	
Stuijvenberg et al;(1999); South Africa	6-11 †	Biscuits	Iodine, Iron, Vitamin A	45 gm (3 biscuits of 15 gm each)	95.4 µg iodine / serve	43 weeks
Stuijvenberg et al;(1999); South Africa	6-11 †	Cold drink	Iodine, Vitamin C	150 ml	39 µg iodine / serve	Not mentioned
Winichagoon et al; (2006); Thailand	9 †	Seasoning powder	Iodine, Iron, Vitamin A, Zinc	50 µg as KI	50 µg iodine / serve	31 weeks
Abraham et al. (2003); Botswana	6-11†	Beverage powder	Iodine, micronutrients	240 ml	60 µg iodine / serve	8 weeks
Lantham et al; (2003); Tanzania	6-11†	Beverage powder	Iodine, micronutrients	25 gm (once a day)	45 µg iodine / serve	6 months
Solon et al; (2003); Philippines	9-10†	Beverage powder	Iodine, micronutrients	25 gm	48 µg iodine / serve	16 weeks
Lantham et al; (2003); Tanzania	Pregnant women; 25 years†	Powdered fruit drink	Iodine, micronutrients	50 gm (2 sachets/day)	90 µg iodine / serve	8 weeks

Multiple micronutrient fortification with fish sauce was done in Thailand (Chavasit et al 2003), the shelf life testing revealed that the product remained stable during a 3-month storage period and was highly acceptable when subjected to sensory home-used tests. In a study by NIN, India (2003-2004), sugar was fortified with potassium iodate (KIO_3) at two concentration at 15 ppm and 30 ppm iodine, either singly or as a co-fortified with iron. Iodine in sugar was found to be stable, in both conditions with no discolouration at the end of 12 months.

Thus, the studies quoted are pointing out towards the food fortification and supplementation of multiple micronutrients together to the community instead of single micronutrient using a same vehicle.

2.4.3 BENEFIT: COST RATIO

The World Bank summarized the benefits of micronutrients in terms of cost per life saved and productivity gained per program (**Table 2.12)**. For saving lives at least cost, targeted supplementation to at-risk groups (pregnant mothers for iron, under-fives for vitamin A) is more cost-effective than fortification, although the latter is a more sustainable solution in the long run as incomes rise and households gain access to higher-quality primary health care.

Table 2.12: Costs and benefits of micronutrient interventions on nutrition investments

Deficiency/Remedy	Cost per life saved (US$)	Discounted Value of productivity gained per program (US$)	Cost per DALY Gained (US$)
Iron deficiency			
Supplementation of pregnant women only	800	25	13
Fortification	2,000	84	4
Iodine deficiency			
Supplementation (repro-aged women only)	1,250	14	19
Supplementation (all people under 60)	4,650	6	37
Fortification	1,000	28	8

(Source: Darnton-Hill I 2005)

These calculations have increased in terms of DALYs with increased economic developments of the countries. From a broader view perspective MI carried out "National damage assessment report" for 80 countries, reported a mean GDP loss to be 1% which ranged from 0.2 to 2.7% in the developing countries including India. These calculations have become history, since they have been estimated before a decade. However, in today's time with the progressing economy, the percentages for loss have also increased gradually in majority of the developing countries.

Another metaanalysis by (Stein et al 2006) reported on the Disability Adjusted Life Years (DALY) and functional outcome. With respect to Iron deficiency anemia, 4 million DALYs are lost and iodine deficiency shares 0.2 million DALYs. These deficiencies aggregated to annual burden of 4.2 million DALYs lost. Later in 2008 same author has reported increased burden with the IDA itself indicated 4 million DALYs annually in India.

Our review on fortified foods has concentrated our thoughts towards one of the most cost effective food ingredient fortified with iodine and iron and provides their supply at a very nominal cost- Double Fortified Salt, has proven its efficiency to be one of the best strategy in many ways in the Indian context.

2.5 DOUBLE FORTIFIED SALT

Double fortified salt (DFS) was first conceptualized in 1969, but it took almost 20 years to solve the technical difficulties and be supplemented in the community (Horton S 2011). There has been many formulas of DFS has been developed by many of the eminent research institutions, to avail the best efficacy on iodine and iron status. Following are the reviews on each characteristics of DFS researched and reviewed,

2.5.1 COST OF DFS *v/s* FORTIFIED FOODS

On calculating benefit: cost ratio, wheat flour fortification (Horton and Ross 2003-2006) and home fortification (Sharieff et al 2006)) are long way ahead than DFS, since the ratio is 9:1, 37:1 and 2-5:1 respectively. This suggests the efficacy of the fortified foods than the ingredient salt. However, the acceptability and cost of production per person per year

puts DFS ahead of home fortification, since the costs are 0.25$ and 1.20$ respectively. While comparison with wheat flour fortification, centralized production and distribution of the same requires more attention and would take time to come into action, where as DFS demands less skills and expertise on the same. Thus, the readiness towards production and consumption may earn more success to DFS.

2.5.2 PRODUCTION AND STORAGE STABILITY OF DFS

There have also been technical problems faced on the stability of iron and iodine together in DFS, since iodine requires alkaline medium for its best bioavailability and iron requires acidic medium for the same. Thus, many research agencies have developed different DFS formulas using best stabilizers according to the demand of the geographical circumstances and climate of the country, since when chemical sources of iodine and iron are brought together in common salt, iodine will be lost as vapour due to oxidation/reduction reaction (NIN 1982 and 1994).

Globally, there have been different formulas developed using a series of stabilizers for best stability of DFS formulas. However, the best stable formula has been proven to be of NIN-DFS, since the institution has run a series of trials since 1974 till 1994 and has developed a stable formula using a poly phosphate, sodium hexametaphosphate (SHMP) (Narsinga Rao 1994), which maintains the stability of iodine in the presence of iron was identified as a stabilizer. Using SHMP as stabilizer the iodine content remained stable at the time of storage for 6 months. **Table 2.13** depicts the comparison of different formulas available globally and stability of iodine content in DFS, majority of the formulas showed stability of iron content, hence not mentioned here.

Table 2.13: Production content and storage stability of DFS

Author and place	Formula	Stabilizer/ encapsulator	Colour developed	Iodized At	Period	Stability
Narsingarao B (1994); India	NIN-DFS	SHMP	No colour	20 ppm	0 month	20 µg/g
					8 months	12 µg/g
Narsingarao B (1994); India	NIN-DFS	SHMP (1%)	No colour	40 ppm	0 months	40 µg/g
					6 months	40 µg/g
					12 months	25 µg/g
Yao Li. (2009); Kenya	MI-DFS	Microencapsulator (Soy stearine)	Not mentioned	100 ppm	0 months	100 µg/g
					6 months	90 µg/g
					12 months	80 µg/g
Zimmerman M.(2003); Morocco	Zurich-DFS	Microencapsulator (Vegetable oil)	Yellow colour	30 ppm	0 months	25.1 µg/g
					6 months	20.1 µg/g
Zimmerman M. (2004); Morocco	Zurich-DFS	Not used	No colour	30 ppm	0 month	24.2 µg/g
					6 months	20.1 µg/g
Anderson M. et al (2008); South India	Zurich/ MI-DFS	Microencapsulation (Soy stearine) in EFF salt	Dark colour	30 ppm	0 months	30 ppm
					6 months	24 ppm
		MGFPP salt	Yellow colour		0 months	30 ppm
					6 months	4.2 ppm (86% loss)
Vinodkumar M. et al. (2007); India	Nutrisalt	Chelator (Malic Acid) + Stabilizer (SHMP). (*KIO3 was encapsulated to prevent decomposition)	Not mentioned	40 ppm	0 months	41.30 ppm
					1 year	38.15 ppm
					2 year	27.70 ppm

2.5.3 BIOAVAILABILITY OF IODINE AND IRON IN DFS

There have been many trials on the bioavailability of iodine and iron content from DFS in vitro and vivo **(Table 2.14)**, since the bioavailability is of the great concern to derive safe limit for the consumption of DFS for the population to achieve expected share of RDA for both the nutrients.

In other words it can be stated that, bioavailability study would help to derive the expected impact on the biochemical indicators for both the micronutrients and calculate the total/ average dietary intake of the population including DFS.

Table 2.14: In vitro or in vivo iron and iodine bioavailability from DFS

Author and Country	Formula	Iron or iodine source	Percent bioavailability/ RBV
Narsingarao B (1994); India	NIN-DFS	$FeSO_4$	6.1% (In vivo)
		KI	90% (In vivo)
Brahmam GNV et al (2000); India	NIN-DFS	$FeSO_4$. $5H_20$	6.1% (In vivo)
		KIO_3	90% (In vivo)
Zimmerman M. et al (2003); Morocco	Zurich-DFS	$FeSO_4$	1-4.3% (In vivo)
		KI	>90% (In vivo)
Wegmuller R. et al (2003); Cote d'Ivoire	Zurich-DFS	Micronized Ground Ferric pytophosphate (MGFePP)	20-70% (In vitro- RBV)
		KIO_3	>90% (In vivo)
Zimmerman et al (2004); Morocco	Zurich-DFS	Micronized Ground Ferric pytophosphate (MGFePP)	2-4% (In vivo) 70% (In vitro-RBV)
		KIO_3	>90% (In vivo)
Vinodkumar M. et al. (2007); India	Nutrisalt	Ferrous Sulphate	Not mentioned
		KIO_3	Not mentioned
Anderson et al (2008); South India	Zurich/MI-DFS	Micronized Ground Ferric pytophosphate (MGFePP)	Not mentioned
		Encapsulated Ferrous Fumarate (EFF)	Not mentioned
		KIO_3	Not mentioned
Yao Li.et al (2009); Kenya	MI-DFS	Micronized Ground ferric pyrophosphate	60-90% (In vitro-RBV)
		Ferrous Fumarate	100%(rats)(In vivo-RBV)
		Ferric pyrophosphate	100% (In vitro-RBV)
		KI	>90% (In vivo)

2.5.4 ACCEPTABILITY OF DFS

Acceptability trial of any food product being introduced, for the consumption by the population by replacing their traditional food item (salt), needs to be assessed to have targeted impact and expected amount consumption by the population. Different researchers had carried out DFS supplementation trial amongst the population and revealed different percentages of acceptability using organoleptic qualities of the DFS or

the food cooked using DFS. Majority of the respondents were specific regarding colour and taste of the cooked food item. **Table 2.15** reveals the comparison between available data on studies carried out by different researchers at community on acceptability of DFS.

Table 2.15: Orgnanoleptic acceptability of DFS in community trials worldwide

Author and place	Formula	Percent acceptability	Colour change while cooking/ storage	Changes in taste
Narsingarao B. (1994); India	NIN-DFS	Not mentioned	No	No
Sivakumar B. et al. (2001); India	NIN-DFS	100%	No change	No change
Asibey Berko E (2007); Ghana	MI-DFS	97.3%	Darker colour	Not mentioned
Sattarzadeh M. and Zlotkin S. (1998); Ghana	MI-DFS	93%	Dark Colour	Not mentioned
Zimmerman (2003); Morocco	Zurich-DFS	86%	Pale/ gray colour	Mild taste
Zimmerman (2004); Morocco	Zurich-DFS	98%	Pale colour	Mild taste
Anderson et al (2008); South India	Zurich/MI-DFS	98.3% 100%	MGFPP- Yellow colour EFF- Dirty/black colour	No change
Vinodkumar M. et al. (2007); India	Nutrisalt	Not mentioned	Not mentioned	Sour taste after 6 hours of storage
Wegmuller R. et al (2003); Cote d'Ivoire	Zurich-DFS	High	Dark colour	No change

Assessments of the formulation and production quality are insufficient to prove any fortified food production or ingredient without its acceptability criteria at community based trial, hence with DFS. The community based studies have also carried out the survey on the level of acceptability of DFS at different organoleptic characteristics. The available data has been revealed in **table 2.15.**

2.5.5 PRODUCTION TECHNIQUES FOR DFS

The reviewed literature has revealed details of the production techniques established for DFS production by different research institutions. Majorly were following two methods-

spray mixing and dry mixing. **Table 2.16** reveals the comparison between both the methods and pro and cones of the methods individually.

Table 2.16: Comparison between two common techniques of DFS production

Variables	**Production technique**	
	Spray mixing	**Dry mixing**
Discolouration of DFS	Pale brown colour	No discolouration
Nature of DFS	Wet and sticky	Free flowing
Cleaning of blender	Frequently	No need
Production schedule	Affected	Smooth
Removal of water	Need for a dryer	No dryer
Iodine loss	95%-98%	No loss
Production cost	More	Less
Suitability	Unsuitable	Ideally suited

(Source : Ranganathan S., Reddy V. and Rammoorthy P. 2005)

2.5.6 IODINE AND IRON CONTENT ESTIMATION FROM NIN-DFS

When the iodine content of NIN-DFS was estimated by the conventional iodometric titration using sulphuric acid (H_2SO_4), problems such as wide variation between duplicate analysis and under/overestimations of iodine content were encountered, which led to inconsistent results on titration.

Hence, (Ranganathan and Karmarkar, 2006) undertook a study to develop a modified method for the estimation of iodine in DFS so as to get reliable iodine content of DFS. A modified method was developed using orthophosphoric acid (H_3PO_4) and the sensitivity of the method was confirmed by estimating the iodine content of potassium iodate (KIO_3) standard at different concentrations of iodine (0 to 100 ppm). The iodine content of DFS and iodized salt (IS) from local market and factory was estimated by the modified method as well as the conventional iodometric titration and the results were compared.

The results showed that the conventional method using H_2SO_4 was not suitable for the estimation of iodine in DFS. The modified method using H_3PO_4 was ideally suited for the estimation of iodine in DFS. Also, iron from DFS did not interfere during estimation of iodine by this method. As both the conventional and the modified methods gave the same

results for the iodine content of IS, it is practically prudent to use the modified method (H_2SO_4) for both DFS and IS instead of following one method (H_3PO_4) for DFS and another (H_2SO_4) for IS. The quantity of KI is also reduced and the order of additions of reagents is changed in the modified procedure.

2.5.7 EFFICACY TRIALS ON DIFFERENT FORMULATIONS OF DFS

Globally there have been a very few studies carried out on the efficacy of double fortified salt amongst population **(Table 2.17)**. This could be due to its issues on acceptability by the governing authorities- looking into the perspective of increased global burden of hypertension, researchers- very few are working towards controlling micronutrient deficiencies using salt as a tool and finally population- who are concerned on the possible change in their traditional dietary food ingredient (salt).

Technology for production of iron fortified salt (Narasinga Rao & Vijayasarathy,1975;Narasinga Rao & Vijayasarathy, 1978; Nadiger et al.1980; Report of the Working Group on Fortification of Salt with Iron, 1982) and iodine and iron fortified salt has been developed in India mainly through the efforts of National Institute of Nutrition (Narasinga Rao 1994; Brahmam et al.1994; Ranganathan et al. 1996; Nair et al. 1998a; Nair et al.1998b).

A formulation of DFS was described by (Suwanik et al. 1978) from Thailand. The salt was found to be stable with good bioavailability of iron. The results of the impact evaluation study have not been published to date.

The efficacy trials have been divided and presented below based on the different formulas developed and used by the researchers/ research institutions.

2.5.7.1 STUDIES ON NIN-DFS/IFS

A history of DFS production was began with the efforts made by (Narasinga Rao and Vijaysarathy 1975), who initiated the formula preparation using Ferric orthophosphate (3500 mg/kg) along with sodium hydrogen sulphate (5000 mg/kg).

Table 2.17: Characteristics of the efficacy trials of double-fortified salt globally

Reference: Location: Type of salt	Study Duration: Type of population	Other comments
Sattarzadeh M. and Zlotkin S. (1998); Ghana	14 days; randomized double-blind trial; 21-53 years	No deworming; tested hemoglobin, serum ferritin, serum transferring and UI.
Rajgopalan and Vinodkumar (2000); India; 'Nutrisalt' (iron plus iodine plus an unspecified stabilizer)	12 months; double-blind randomized controlled trial; male and female tea pickers	Dewormed half of controls and half of experimental group, results on UI and iron indicators; also collected productivity data
Sivakumar et al (2001); India; salt with iron and iodine stabilized with SHMP	2 years (excluding school vacations); double-blind randomized control trial; school children 5-15 years in residential schools	No deworming as a part of study. Results on iodine and iron status indicators were collected
Wegmuller R. et al (2003); Cote d'Ivoire	6 months; randomized-double blind trial; children 6-15 years	No deworming necessary; tested hemoglobin concentration, serum ferritin, serum transferring and UI.
Zimmerman et al (2004); Morocco; micronized ferric pyrophosphate	10 months; double-blind randomized controlled trial; children 6-15 years	No deworming necessary (hookworm prevalence low); tested serum ferritin, serum transferring and UI.
Zimmerman et al (2004); Morocco; encapsulated ferrous sulphate	9 months; double-blind randomized controlled trial; children 6-15 years	No deworming necessary (hookworm prevalence low); tested hemoglobin, serum ferritin, serum transferring and UI.
Wegmuller et al (2006); Cote d'Ivoire; micronized ferric pyrophosphate	10 months; double-blind randomized controlled trial; children 6-15 years	Children dewormed at baseline and after 4 months; iron status improved although not hemoglobin; tested serum ferritin, serum transferring, UI.
Asibey- Berko et al (2007); Rural Ghana; Dextrin coated potassium iodide and ferrous fumarate	8 months; double-blind randomized controlled trial; non-pregnant, non-lactating mothers and their children 1-5 years	Tested UI, serum ferritin, other indicators; no deworming
Vinodkumar M et al. (2007); India; chelated Ferrous sulphate, encapsulated iodine, SHMP	1 year; single-blind trial ; population > 10 years	All the participants deworming at 0 months, 6 months and 12 months; tested hemoglobin and UI.
Anderson et al (2008); India; Encapsulated ferrous fumarate	10 months; double-blind randomized controlled trial; school children 5-18 yrs.	Testing serum ferritin, urinary iodine, other indicators. Children received routine deworming after 1 and 9 months of study.

Further, a supplementation study conducted by (Nadiger et al 1979) amongst population using iron fortified salt provided evidence for the efficacy of iron fortified salt in improving iron status amongst the study population. This study was carried out amongst school children (n=1630) aged between 5-15 years in Hyderabad. The results revealed that there was a significant increase in mean hemoglobin concentration (+1.4 g/dl, $p<0.001$) of boys and girls (+1.1 g/dl, $p<0.001$) after 12 months of supplementation compared to decline amongst boys (-0.39 g/dl, $p<0.001$) and girls (+0.05, $p<0.05$). There was a significant reduction ($p<0.01$) in prevalence of anemia amongst both the genders compared to unchanged status amongst control groups. This revealed a beneficial impact of IFS over the iron status of the school children.

Another efficacy trial was carried out by (The Working group on fortification of salt with iron 1982) using salt (Fortified with $FePO_4$ and $NaHSO_4$). The study was aimed towards the supplementation impact assessment amongst three rural and one urban area of different parts of India after 18 month. The study centers were Hyderabad, Calcutta and New Delhi- rural and Madras-urban, the age groups of the study population ranged from 1- ≥45 years. At baseline the prevalence of iron deficiency anemia ranged upto 99.2% at different age groups, gender and centers. At the end of the intervention period, the improvement in iron deficiency was observed with the reduction in prevalence ranging between 30-60% and improved mean hemoglobin level ranged between 0.25 (≥ 45 years) - 0.79 g/dl (1-5 years). The study population was also dewormed to enhance the effect of IFS. Thus, based on the grouping of the subjects by deworming, the increased hemoglobin concentration ranged from 0.98 – 1.76 g/dl in dewormed group compared to 0.64-1.29 g/dl amongst non-dewormed group. Hence, it was proven that, the IFS could improve iron status of the study subjects and dewormed played a vital role in enhancing the levels of hemoglobin in the community. Thus, can be used as a co-strategy at community level to improve iron status of the population.

A randomized double blind study to assess the impact of double fortified salt supplementation by NIN (Nair M. et al. 1998) was further carried out for a period of two years among children belonging to the backward communities attending a residential school in and around Hyderabad. In total there were four schools (2 Boys and 2 girls)

covered. This study assessed the impact on iron status, urinary iodine levels and calcium-phosphorous homeostasis of the beneficiaries to prove the safety of the consumption of NIN-DFS at long term and daily basis. After 24 months supplementation it was observed that, there was a decline in hemoglobin levels of the girls belonged to both the groups. However, the decline was significantly higher (-1.95 g/dl) in IS group compared to (-0.87 g/dl) in experimental group. The change was non significant amongst boys. However, the mild anemic subjects showed significant increase (0.68 g/dl) ($p<0.05$) compared to IS group. There was also a remarkable increase in urinary iodine levels at different point of time in both the groups. However, there was no change observed in calcium, phosphorous and alkaline phosphatase levels of the children, proving the safety of the consumption of DFS by all age groups.

A two year supplementation study in East Godavari district of Andhra Pradesh was also carried out to assess the efficacy of DFS in tribal population (Brahmam GNV et al. 2000, Sivakumar B. et al. 2001). Four groups of villages with 20-25 villages in each group were selected, with a population of about 5000 were included in the study. Three areas received iodized salt fortified with iodine and iron singly or in combination. The fourth area received no supplementation. The acceptability of the salt was 100% amongst tribal population and there were no allergy or toxic effects were encountered. There was a significant reduction in goitre prevalence in DFS and IS supplemented groups (almost 50%) compared to no change in control group. Overall there was no significant impact on prevalence of anemia. A significant increase in iron status-hemoglobin levels was observed in certain age groups. The increments were significantly higher in lactating women ($p<0.05$) in IFS area and in both 14±17-year-old boys and among lactating women in the DFS area. The non significant increase in rest of the group was reasoned out as higher prevalence of anemia, substantial amount of tamarind consumption and bioavailability of iron from rice based meal in the presence of tamarind.

- **NIN-DFS PROVEN TO BE THE BEST FOR DEVELOPING COUNTIRES**

There have been studies on successful large-scale (Narasinga Rao et al. 1994; Ranganathan S et al. 2005) and local level production (Sivakumar B. et al 2001; Brahmam GNV et al. 2000) have proven that, NIN-DFS is the most cost effective mean

in India, stable in Indian climatic conditions and requires less expertise compared to rest of the formulas.

2.5.7.2 STUDIES ON MI AND ZURICH-DFS

This formula included encapsulation of iron with vegetable oil or other efficient encapsulators to avoid interaction between iodine and iron to enhance efficacy and stability.

Initially a supplementation study was carried out in Brikcha Rural Commune, an area of endemic goiter in the Rif Mountains of northern Morocco (Zimmerman M. et al. 2003; Zimmerman M. et al 2002). The formula included microencapsulated ferrous sulphate. The subjects were 6–15 year-old children from two neighboring primary schools. At baseline there was no significant different in baseline characteristics of the subjects divided into DFS and IS groups. However, at 40 weeks there was a significant ($p<0.02$) increase in hemoglobin levels of the children in DFS group (+1.4 g/dl) compared to IS group (0.5 g/dl). However, there was also a significant reduction in prevalence of iron deficiency anemia ($p<0.001$) compared to IS group ($p<0.05$). The prevalence of goitre also decreased significantly amongst DFS group ($p<0.001$) compared to IS group. The thyroid volume also decreased significantly amongst both the groups. However, it was significantly higher in DFS group ($p<0.01$) compared to baseline and IS group ($p<0.001$). There was no significant difference between both the groups throughout the study period and at 40 weeks it was well above the WHO/ICCIDD cutoffs for at risk ID. There was non significant reduction in TSH levels amongst both the group, since median TSH of the population was within the normal range. Serum T4 increased significantly amongst DFS group ($p<0.01$) compared to IS group ($p<0.05$). The prevalence of hypothyroidism also decreased significantly amongst DFS group ($p<0.001$) compared to IS group.

On continuing the efficacy trials in Moroccon population (Zimmerman M. et al. 2004), another trial was carried out using micronized FePP (Iron pyro phosphate). The school children 6-15 years were included as study population and supplementation was carried out for 10 months in DFS and IS groups. At baseline none of the children were severely anemic and the prevalence of iron deficiency anemia was 46% amongst both the groups.

In comparison with the mean hemoglobin concentrations in the IS group, mean concentrations in the DFS group increased significantly from baseline at 5 and 10 months (P<0.01). All indexes of iron status (SF, TfR, and ZnPP) and body iron stores improved significantly in the DFS group compared with the IS group at 5 and 10 months (P< 0.01).After 10 mo, mean (±SD) total body iron increased in the DFS group from 39.8±74.2 to 145.7± 81.4 mg, whereas mean total body iron decreased in the IS group from 44.7 ±89.2 to 35.1 ±90.7 mg. The prevalence of anemia, IDA, and iron deficiency without anemia were sharply lower in the DFS group than in the IS group at 5 and 10 mo (P <0.001). There were no significant differences in median UI concentration between the 2 groups throughout the study. In both groups, median UI concentrations increased significantly from baseline at 5 and 10 months (P< 0.001). At 10 months, median UI had increased to near the cutoff value (100 μg/L) for risk of iodine deficiency from the WHO/ICCIDD. In both groups, mean Tvol decreased significantly from baseline at 5 and 10 mo (P < 0.01), and there was a significant decrease in goiter prevalence (P <0.01). However, at 10 months, the mean percentage change in Tvol from baseline was significantly higher in the DFS group than in the IS group (P < 0.01), and goiter prevalence was also significantly lower (P< 0.01) in the DFS group at 10 months.

Further trials were carried out by Wegmuller R. et al. (2003) in rural village in the Dabou district of Coˆte d'Ivoire. The subjects were 5- to15-y-old children from 4 primary schools. The study was double-blind randomized, hence two schools in DFS and IS groups each were distributed. At baseline 12% of the children suffered iron deficiency anemia and median urinary iodine excretion was 358 μg/L, indicating excessive intake of iodine. Towards the end, the mean hemoglobin concentration did not change significantly in any of the groups. However, serum ferritin and transferrin receptor concentration were higher in DFS group at 6 months compared to baseline (p<0.05), the change was non significant in IS group. There was significant increase (p<0.001) in total body iron concentration (+1.3 mg/kg) among DFS group and no change in IS group.

A study using Zurich-DFS formula was also carried out by Anderson M. et al. (2008) as a collaborative initiative by Toronto University, Micronutrient Initiative and St John's Medical College, Banglore. Thus, it included one experimental group with MGFePP

(Zurich-DFS) and one with EFF (MI-DFS). It was conducted in 18 rural villages of Karnataka, India. This was a double-blind intervention trial study including school children aged between 5-18 years from 6 schools as the study population. There were no significant between-group differences in any of the baseline characteristics except the SF concentration and the body iron status The hemoglobin concentration improved significantly in all groups over the 10-mo study, but it was significantly ($P<0.01$) higher in the EFF group than in the IS group. Both groups receiving iron significantly improved their SF and body iron status from baseline ($P<0.001$) and as compared with the IS group ($P <0.001$). TfR increased in the control group ($P<0.001$), but did not change in the 2 iron groups. There was no difference in the prevalence of elevated CRP values between the 3 intervention groups at any time point. Compared with baseline, the prevalence of anemia decreased over 5 and 10 mo from 16.8% to 13.2% and 7.7% ($P<0.05$), respectively, in the MGFePP group; from 15.1% to 9.0% ($P< 0.05$) and 5.0% ($P<0.01$), respectively, in the EFF group; and from 19.2% to 13.2% and 14.5% (NS), respectively, in the IS group. The prevalence of iron deficiency dropped over 5 and 10 mo from 56.6% to 39.7% ($P <0.05$) and 32.8% ($P <0.001$), respectively, in the MGFePP group; from 52.4% to 30.8% ($P<0.001$) and 34.6% ($P<0.01$), respectively, in the EFF group; and from 68.2% to 66.4% and 68.0% (NS), respectively, in the IS group. The prevalence of IDA dropped over 5 and 10 mo from 15.2% to 9.9% and 6.4% ($P <0.05$), respectively, in the MGFePP group; from 11.7% to 5.3% ($P<0.05$) and 3.8% ($P<0.001$), respectively, in the EFF group; and from 16.9% to 12.8% and 15.2% (NS), respectively, in the IS group. The median UI was significantly higher in the IS and the EFF groups at 10mo than at baseline ($P<0.001$), but it did not change significantly in the MGFePP group Median UI was significantly lower in the MGFePP ($P <0.001$) and EFF ($P <0.05$) groups at 10 mo than in the IS group. The proportion of children with UI concentrations of <100 µg/L was higher in the MGFePP group at 10 mo than in the IS group ($P <0.01$) or the EFF group ($P <0.05$). thus, both the formulas were proven to be efficient to improve iron and iodine status of the children within a period of 10 months.

A clinical trial using MI-DFS formula was carried out firstly by Sattarzadeh M. and Zlotkin S (1998) in adult subjects aged 27-44 years. The study subjects were provided

with high bioavailability iron compared to low-bioavailable in control group. The mean hemoglobin concentration before (135 6 5.3 g/L) and after the completion of the study (134 6 5.2 g/L) was not significantly different. Serum ferritin and hemoglobin concentrations were within the normal range for 14 of the 16 subjects included in the analysis. Two female subjects with the lowest hemoglobin and serum ferritin values (hemoglobin, 102.4 and 90.4 g/L; serum ferritin concentrations; 5.5 and 3.0 mg/L) had the highest rates of iron absorption from each meal. Mean "uncorrected" absorption from the Fe-enhancing meal was significantly higher than that from the Fe-inhibitory meal. Similarly, mean absorption, after correction based on individual absorption of a reference dose of inorganic iron, was also significantly higher with the Fe-enhancing meal. There was a significant negative correlation between log serum ferritin and the absorption from the reference dose of iron (r-0.35, $P < 0.0003$). There were no significant differences in excretion after each of the two test meals. Urinary excretion of iodine from baseline and post ingestion was not significantly different and was within the normal range. Thus, the study concluded that, iron is well absorbed by healthy adults after ingestion of double-fortified (iron and dextran-coated iodine) table salt and urinary iodine excretion is unaffected.

Another study was conducted by Asibey-berko E. (2007) using MI-DFS formula among the agrarian region of Sekyere District of Ghana. The study period was of 9 months and 17 villages using cluster sampling were chosen as study area. Non-pregnant/ non-lactating women (aged 15-45 years) (preparing family meals by herself) and their 1-5 years old children made the study population (n=390). At baseline there was non significant difference between study subjects. Then random allocation to DFS + Placebo, IS + Iron supplement and IS Placebo group was carried out. At the end of the study, prevalence of anemia increased significantly in women from IS group (19% to 34.5%-$p<0.05$), non significant change in DFS group (34.4% to 37.7%) and IS+Fe group (35.4% to 36.9%). However, the prevalence of anemia decreased significantly amongst children (56.5% to 34.8%) ($p<0.02$) compared to non significant difference in rest of the two groups. There was a significant reduction in prevalence of iodine deficiency ($p<0.001$) in all the three groups. Thus, it was concluded that, DFS and IS+Fe both prevented non-

anemic women and children to become anemic compared to IS group. However, it also improved iodine status comparable to IS groups. Hence, DFS is an excellent source of supplying micronutrients to all age groups.

2.5.7.3 STUDIES ON NUTRI-SALT

A study was conducted jointly by Sundar Chemicals, Parry Agro Group, and United Planters Association of South India (Rajgopalan S. and Vinodkumar M. 2000). The CWS tea estate of the Parry Agro Group, in the hilly region of Valparai in South India. By using a map of the entire plantation, the area was divided into 20 clusters. The study was a double-blind, randomized, placebo-controlled trial. The subjects (n=793) were divided randomly in the experimental (DFS) and control groups (IS) and half population of both the groups were dewormed to assess the effect of multiple intervention after one year. At baseline the mean Hb of control group was higher than the experimental group. Towards the end there was a significant increase in mean hemoglobin concentration in experimental group females (+1.55 g/dl) compared to (+0.83 g.dl). However, amongst males the difference was non significant between both the groups (Experimental group: +0.85 g/dl) and (Control group: +0.59 g/dl). At the end of the year,the average haemoglobin level of the population had increased from 8.9 to 10.2 g/dl and their corresponding picking average had increased from 24.8 kg to 26.2 kg, which could increase annual tea production by 330 tonnes.

A multicentric community study by Vinodkumar M et al. (2007) was also carried out to assess stability and efficacy of Nutrisalt- DFS. The multicenter single-blind study was carried out in three states of the country: Karnataka (three clusters—Tumkur, Uttar Kanada, and Dharwad), Gujarat (two clusters—Surat and Bharuch), and Uttar Pradesh (two clusters—Pratapgad and Gonda). Sample sizes of 63 persons per cluster in the experimental group and 63 persons per cluster in the control group were calculated for measurement of hemoglobin. Two to three members (preferably adults; children less than 10 years of age were excluded. Each of the 30 families in the experimental group to constitute a sample size of 63 to 90 per cluster, and similarly 30 families with two or three members per family were included to constitute a sample size of 60 to 90 per cluster

for the control. A total of seven clusters were selected. The results revealed that, the iron and iodine in the DFS were stable during storage for 2 years. Over a period of 1 year, there was an increase of 1.98 g/dL of hemoglobin in the experimental group and 0.77 g/dL of hemoglobin in the control group; the latter increase may have been due to deworming. The median urinary iodine changed from 200 μg/dL at baseline to 205 μg/dL at the end of the study in the experimental group and from 225 μg/dL to 220 μg/dL in the control group. There was a statistically significant ($p < .05$) improvement in the median urinary iodine status of subjects who were iodine deficient (urinary iodine < 100 μg/L) in both the experimental and the control groups, a result showing that DFS was as efficient as iodized salt in increasing urinary iodine from a deficient to sufficient status. There was a statistically significant increase ($p < .05$) in hemoglobin in all seven clusters in the experimental group compared with the control.

Nutrisalt has also carried out studies on the effect of multiple micronutrient fortified salt and its impact on iron status and cognition of school children. The studies are depicted in the **Table 2.18.** These studies prove that, salt can be used as an effective vehicle to fortify essential micronutrients to meet the requirements in minor quantity. This also concludes that, the consumption of the fortified salt for at least one year could bring remarkable improvement in micornutrient status and cognition of the children. However, the stability and cost of multiple micronutrient fortified salt is an area of concern.

In view of that, DFS production demands less expertise and cost of production (NIN 2008), which would fit in to the monthly budget of a common man. It is also efficacious to improve iodine and iron status of all age groups, which has also been researched and proven. Hence, DFS is the only sustained tool to combat both the micronutrient deficiencies in current times.

Table 2.18: Impact of multiple micronutrient salt (Nutri-salt) on micronutrient status of school children

Nutri-salt Content	Age group; Location; year	Duration and Impact
A salt fortified with multiple micronutrients was developed containing chelated ferrous sulphate, microencapsulated vitamin A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate and iodine	**7-11 years; residential schools; Chennai, India; 2007**	**1 year:** There was a significant (p<0.05) improvement in the experimental group in hemoglobin, red cell count, urinary iodine and serum vitamin A whereas in the control group there was a statistically significant decline (p<0.05) in hemoglobin, hematocrit, red cell count and urinary iodine. Angular stomatitis was eliminated from baseline 30.4% in the experimental group whereas it increased from 3.25% to 25.5% in the control group. In 4 tests out of the 7 memory tests and in the letter cancellation test for attention, the mean increment in scores in the experimental group is significantly more (p<0.05) than the control group. There was no significant improvement in overall intelligence as seen in the Ravens progressive matrices between the experimental and control groups.
A salt fortified with multiple micronutrients was developed containing chelated ferrous sulphate, microencapsulated vitamin A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate and iodine	**5-15 years; Residential schools; Chennai, India; 2007**	**1 year:** There was a significant improvement (P<0.05) in hemoglobin, red cell count, urinary iodine and serum vitamin A in the experimental group, while there was a significant drop (P<0.05) in hemoglobin, hematocrit, red cell count and urinary iodine in the control group. In the experimental group, there was a mean increase of 0.55 g/dl in hemoglobin, 0.001 l/l in hematocrit, 0.470 million/mm3 in red cell count, 212 mg/l in urinary iodine and 5.6 mg/dl in serum vitamin A.
A multiple micronutrient-fortified salt containing vitamins A, B1, B2, B6, B12, as well as folic acid, niacin, iron, iodine, and zinc	**5-18 years; Residential schools; Chennai, India; 2009``**	**9 months:** There was a significant improvement (p<0.05) in all the biochemical measurements and memory tests in the experimental group when compared with the control group. Post-intervention in the experimental group, the increase in hemoglobin was 0.67 g/dL (p<0.05). Iron status and body iron stores increased significantly (p<0.05) in the experimental group compared to the control group, while serum zinc increased by 50 mg/dL (p<0.05), and the prevalence of retinol deficiency decreased from 57.1 % at baseline to 16% post-intervention (p<0.05)

2.6 COMMUNITY BASED NON FOOD BASED APPROACHES TO PREVENT MICRONUTRIENT MALNUTRITION

The most simple and cost effective interventions in reducing malnutrition in children are micronutrient supplementation and deworming amongst the school age children. Thus, giving high priority to several of these interventions in order combat malnutrition was emphasized by the Copenhagen Consensus panel (2008). We initiated our study parallely during the same period and hence results are considerably relevant to the world's current context on combating malnutrition.

School- age children typically have the highest intensity of worm infection of any age group. In addition, the chances of getting infected are very higher at school levels, since the rate of exposure to the majority of the contaminants is very high. A high prevalence of intestinal parasitic infections is closely correlated to poverty and poor environmental hygiene also. In the long run, worm infections increase susceptibility to other infections and diminish learning ability and growth. Hence, the most cost effective way to deliver deworming pills regularly to children is through school because schools offer a readily available, extensive and sustained infrastructure with a skilled workforce that is in close contact with the community (Stoltzfus et al 2004).

School-children harbor the most intense infections with roundworm (*Ascarislumbricoides*), hookworm (*Ancylostoma duodenale* and *Necator americanus*) and Whipworm (*Trichuris trichuria*). Therefore, treatment of this age group- which is easily accessible through the school system- achieves optimal improvements in health status and educational performance.

In Zanzibar, school children living in high worm transmission areas were treated with Mebendazole thrice a year. The study reported that a quarter of a litre of blood can be saved per child per year for as little as 1 US cent per child per year. It has also been depicted that deworming of the children results in remarkable growth spurts. In one of the trial in Kenya, treated school-age children gained one centimeter more in height in the four months following treatment than did children who received a placebo (Stoltzfus R. et al 2004).

A randomized control trial study among children belonged to urban slums conducted in India using periodic deworming as a treatment by Sur D. et al (2005) revealed significant weight gain ($p<0.01$) amongst at 3 months, 6 months and 9 months compared to controlled group. Further, the albendazole group also experienced fewer episodes of diarrhoea than their control counterparts (relative risk 1.3, 95% CI 1.07-1.53) with a 28% reduction. Thus, concluding that, periodic mass deworming with albendazole would seem to be a safe and effective method that could be adopted at the community level or as an integral part of school health services and could be expected to improve growth and reduce the incidence of diarrhoea in children.

Another study by Awasthi S. et al (2001), a cluster randomized trail in North Indian urban slums revealed that, the albendazole-treated arm exhibited a similar height gain but a 35 (SE 5) % greater weight gain, equivalent to an extra 1 (SE 0.15) kg over 2 years (99% CI 0.6–1.4 kg), indicating the beneficial impact of deworming on the growth of the children.

Deworming at a regular interval contributes to good health and nutrition for children of school age, which in turn leads to increased enrollment and attendance, reduced class repetition and increased scholastic performance. (World Bank Report 2003). Inexpensiveness, wider availability, effectiveness, no side effects and easy to administer qualities of deworming pills, makes this intervention as one of the most cost effective strategies.

2.7 OTHER ESSENTIAL SUPPORTIVE STRATEGIES

2.7.1 ADDRESSING DIETARY DIVERSIFICATION APPROACHES

An intervention strategy that is sustainable without external support and combats multiple micronutrient deficiencies simultaneously is a food based strategy. Change in dietary pattern e.g. increase in consumption of vegetables and fruits, dairy products and animal foods, and modification in food processing methods help in improving diet and micronutrient status.

Asian diets are mainly cereal-based and bioavailability of certain nutrients from such diets is limited. Iron from animal sources is more available, but consumption of such

foods of restricted due to poverty. Means of improving bioavailability from vegetarian foods such as consumption of Vitamin C rich foods, avoidance of iron absorption inhibitors and fermentation of cereals and legumes should be encouraged. Intervention strategies to improve inter and intra- household food securities are also important.

2.7.2 BEHAVIOR CHANGE COMMUNICATION (BCC) AND SOCIAL MARKETING

To combat nutritional insufficiencies, sometimes not the lack of availability but the lack of awareness plays a vital role. Moreover, the resources are available within the surroundings but the ability to utilize that economical resource is not prevalent amongst the community. Hence, BCC is used as a multi-level tool for promoting and sustaining the desired behavior in individuals and communities by using a variety of communication channels and creating demand for information and service (WHO 1998).

An intensive social marketing strategy is an essential behavior change. Social marketing has been shown to be effective in promoting consumption foods rich in various vital nutrients in the form of fortified foods or supplements. Thus, each strategy of the intervention are interlinked and when they are used as multiple approaches amongst targeted population, they can give better impact compared to the singleton approach.

2.7.3 PUBLIC EDUCATION AND SOCIAL MOBILIZATION AS STRATEGIES TO COMBAT MICRONUTRIENT MALNUTRITION

Goiter and cretinism provided the visual picture of iodine deficiency that gave it easily identifiable reference. As IDD elimination progressed, these physical manifestations became far and fewer between, giving the impression that IDD had been solved. Yet iodine deficiency persists, in its more common form – brain damage, to which the unborn fetus is especially vulnerable. In effect, IDD elimination programs are threatened to be victims of their own success yet a deficiency must be continuously addressed or it will re-emerge. Thus on-going communication efforts are necessary.

Although there has been no exhaustive study undertaken on effective communication and public education strategies on IDD/ IDA that would provide defined indicators of achievement, a number of practices have been noted for their effectiveness:

- Relating IDD to brain damage, thereby creating an understanding of the functional outcomes –beyond goiter and cretinism - that result from iodine deficiency. These include mental impairment, the loss of IQ points, the impact on educational achievement and ultimately productivity. This was the critical information that influenced the Chinese Vice-Premier to commit to IDD elimination (Yip, Chen, & Ling, 2004). This is also applicable to IDA amongst community.
- Tailoring messages to the audience with a specific call of action they can take. The audience to be influenced ranges from top levels of government to the public health community to salt industry to the household (Ling, 2007).
- Understanding the "common wisdoms" that exist in a community and correcting misinformation. Religious leaders and community leaders have been engaged to address culturally entrenched practices (ie. washing of salt before use) which are obstacles to salt fortification (Ling, 2007).
- Using multi-media to get awareness messages into popular culture (Akunyili, 2007)
- Integrating up-dated information about micronutrient deficiencies into technical and educational materials of food inspection and control bodies, health care training and academic curriculums (Sharmanov, et al., 2008).

Ultimately, public education serves to solidify support for elimination of micronutrient deficiencies at all levels of society and thereby creates a demand for DFS, a necessary component for the success of a salt fortification strategy.

2.8 CONCLUDING NOTES

Salt iodization is the preferred strategy in eliminating IDD as a public health problem, and universal iodization is the target for the beginning of the twenty- first century. The addition of iron to iodized salt improves the effectiveness of iodine in the target population where the prevalence of anemia is high. School health programs that include deworming, feeding, giving an adequate supply of iron and iodine supplements, as well as health education, all have potentially beneficial effects on the health and education of school children. However, industry based interventions in the form of advocacy, monitoring and quality product sustenance are essential till DFS comes into the picture.

Thus, to provide at least one of the essential micronutrient where both are deficient amongst the population.

The Tenth Five Year Plan (Planning Commission GOI 2007) suggested multipronged strategies for the control of anaemia in pregnancy. The program has included ***fortification of common food items like salt with iron to increase the dietary intake of iron and improve the haemoglobin status of the entire population, including girls and women prior to pregnancy; nutrition education for dietary diversification to improve the iron and folate intake.***

Focusing all these strategies, our research work has been planned and undertaken to combat iodine and iron deficiencies amongst the most vulnerable groups of the community- pregnant women and school age children- using Double Fortified Salt as a tool.

2.9 MILESTONES FOR ACCEPTANCE OF NIN- DFS AS AN EFFECTIVE MEASURE FOR THE COMMUNITY

2.9.1 THE INITIATION OF THE REVOLUTION

As reported by newspaper ***The Asian Age*** Jan 18, 2011 announced the preface of the meet on implementing DFS as a strategy to combat iodine and iron deficiencies. It announced that, in a bid to address Prime Minister Manmohan Singh's concern on "unacceptably high" levels of malnutrition in India, a meeting has been called at the PMO to examine the possibility of promoting double fortification of salt with iron (it is already fortified with iodine). To be chaired by the Prime Minister's principal secretary T.K.A. Nair, it will be attended by senior officials of the ministries of health, women and child development, industries, as well as the Hyderabad-based National Institute of Nutrition. The matter assumes significance given that nearly 46 per cent of Indian women in the 5-to-54 age group as well as 70 per cent of children suffer from anaemia, which is caused by iron deficiency. The meeting is expected to discuss how malnutrition in such categories of the population can be tackled. NIN Hyderabad, which has already developed technology for salt's double fortification to check anaemia, has estimated that nearly 70-80 per cent of India's population suffers from an iron deficiency.

2.9.2 THE RESULT OF THE VIGOROUS EFFORTS MADE BY ALL THE RESEARCHERS FAVORING DFS

As reported by media including ***Indian Health News, The Hindu and IBN live,*** a meeting conducted at PM's of office by different Ministries at Delhi on 20 April, 2011 has decided that consumption of iron fortified salt would be actively promoted. The Integrated Child Development Scheme and Midday Meal Programme will use iron fortified iodized salt (double fortified salt - DFS). The Department of Food and Public Distribution and the Ministries of Women and Child Development and Health & Family Welfare will be involved in campaigning to promote and supply DFS. The Department of Industrial Policy & Promotion will work with in actively promoting the manufacture of iron fortified iodized salt, in conjunction with private industry and cooperatives. The Departments of Health and Family Welfare and Health Research under the Ministry of Health and Family Welfare will undertake the responsibility of advising the Ministries of Women and Child Development, Human Resource Development and Consumer Affairs, Food and Public Distribution on use of DFS. Technologies for the double fortification of salt with iodine and iron have been developed by the National Institute of Nutrition (NIN) in Hyderabad. The daily diet, especially among Indian women, girls and children, has very little amount of iron, which can be dealt with through building an awareness of iron-rich nutrition and supplements and through inexpensive ways as in DFS.

2.9.3 NIN SPEAKS ON THE SUCCESS AT DELHI MEET

As reported by ***Times of India***, May 5,2011, Double fortified salt, which is iron fortified iodized salt, will soon be available in the market with seven big manufacturers from all over the country being transferred the technology by the NIN."Lack of iron in the body leads to anaemia. The double fortified salt will meet the need and supply the body with at least 50-60 per cent of the needed iron. The rest of the iron needed is anyway got through other dietary sources," NIN director B Sesikeran said. When the body gets the needed iron, the haemoglobin levels become better only to make the person more active. The double fortified salt is already available in Orissa, Chattisgarh, and Karnataka in public health programmes.

The new double fortified salt will be available commercially soon with the mandatory green signal from food safety authorities having already come. Recently, at a meeting with the Prime Minister, it was decided to use the DFS technology in all ICDS and PDS schemes.

Out of the seven companies that have been transferred the DFS technology free of cost, two belong to Andhra Pradesh, including A P Foods of the state government. For those who would not like to compromise on the taste of their salt, even if it were to be a double fortified salt, here's an assurance: "Put it on the tongue, one might feel a slight change in the taste but when mixed with food, there will be no difference," said scientist Madhavan Nair.

Supplementation studies using salt as a vehicle to deliver micronutrients are being conducted from last five decades but still there have been certain blocks or resistance at different levels towards the acceptance of the truth. However, has been proven that the salt is the best media to indulge micronutrient supplementation at complete supply (iodine) or a partial supply (iron) of the RDA of the population at very cost effective and easy way. Hence, to contribute to the concept of DFS, we have conducted our study to join hands with the researchers striving to make world to accept DFS as a fundamental and most required strategy.

CHAPTER 3
METHODS AND MATERIALS

Any new research study planned is initiated as a trial and error based approach. This approach provides a margin to rectify the gaps which arise during the trials. Thus this study aimed in conducting research trials on efficacy of double fortified salt (DFS) supplementation in improving iodine and iron profile amongst pregnant women and school children. The study design was an experimental-control (intervention) based longitudinal approach.

3.1 OVERVIEW OF THE STUDY PHASES

The study was comprised of three phases:

3.1.1 Phase I: Impact Assessment of Double Fortified Salt Supplementation amongst Pregnant women

Study population: Pregnant women (n=256) (during first trimester, <12 weeks) with singleton pregnancy and without known thyroid dysfunction. Further subsampled to n=150 due to various reasons for dropouts (abortion, miscarriages, missing follow-ups, unwillingness to participate and non-reachability). N=121 could be followed up till the end of gestation.

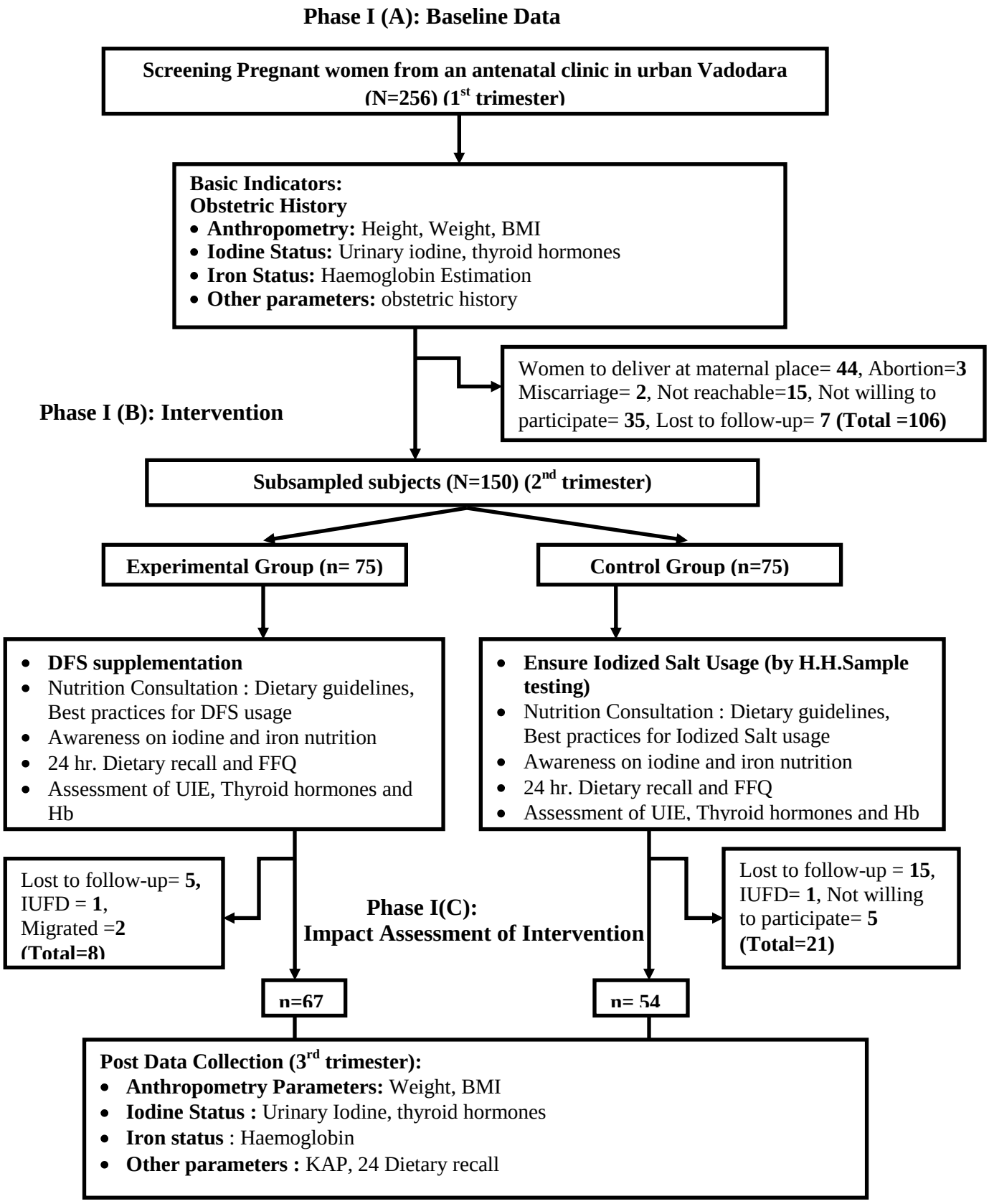
Study area: An antenatal clinic of Jamnabai Hospital, urban Vadodara. It is a semi-government hospital including second highest number of deliveries per month in the state.

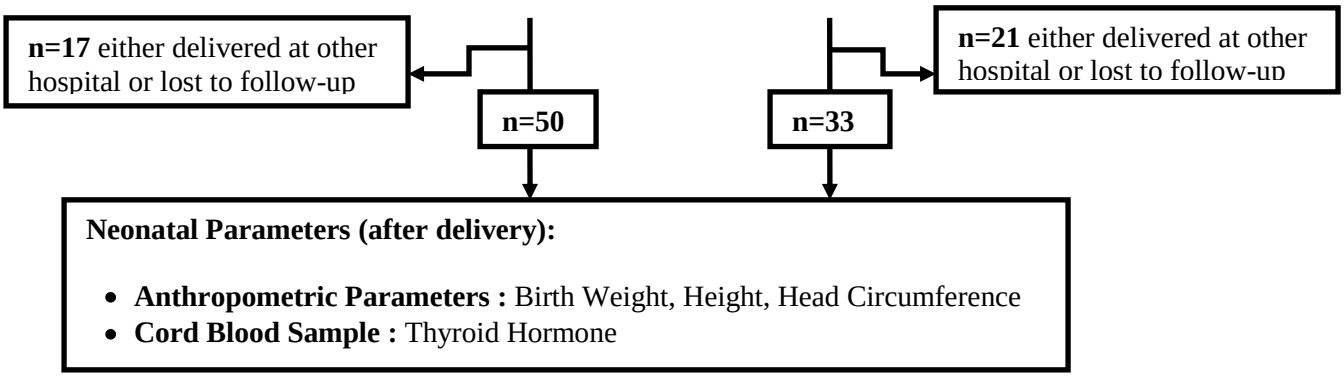
Sampling technique: Random sampling during screening. Further, stratified sampling was done to distribute subjects into experimental (n=75) and control (n=75) groups.

Sample size: Using OpenEpi calculator: Open Source Epidemiologic Statistics for Public Health, Version 2.3.1., calculated sample size for screening was N=246 required at 95% CI, assuming 80% prevalence of anemia during pregnancy.

Experimental design of phase I has been depicted in **Figure 3.1**, indicating an overview of the work conducted.

Figure 3.1: EXPERIMENTAL DESIGN – PHASE I





3.1.2 Phase II: Impact Assessment of Double Fortified Salt Supplementation amongst School children

Study population: School aged children (6-15 yrs.) studying in 1st to 6th standard were selected. N=1184 subjects made the baseline sample size. However, n=947 subjects could complete the study due to various reasons

Study area: This phase was carried out in rural area- Waghodia of Vadodara district.

Sampling technique: Rural area was divided into 4 demographic regions based on population and number of schools available. This area comprises of 172 primary government schools. Four schools on a same belt were selected randomly. Out of these four schools, two schools were chosen as control group, where there was better availability of iodized salt than rest two schools (experimental group).

Sample size: The sample size formula for determining the sample size is as follows:

$N = 16p (100-p)/ w^2$ where, p= estimated prevalence based on earlier study or pilot trial

w= width of CI eg. if the interval is 95% the width will be 10 (±5)

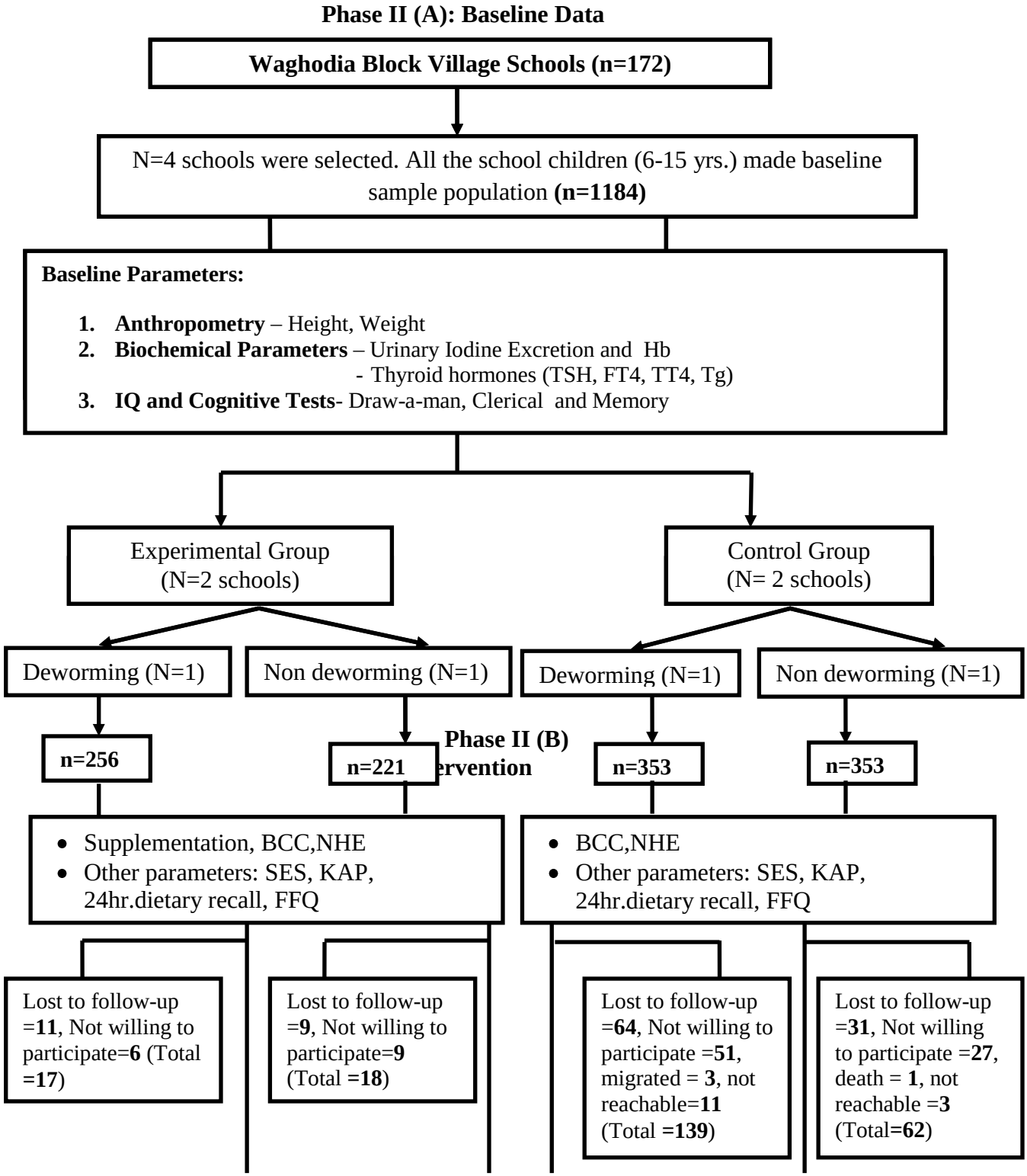
Now for this phase, considering the prevalence of anemia to be 60% the sample size was estimated as, $N= 16 X 60 (100-60)/ 10^2$

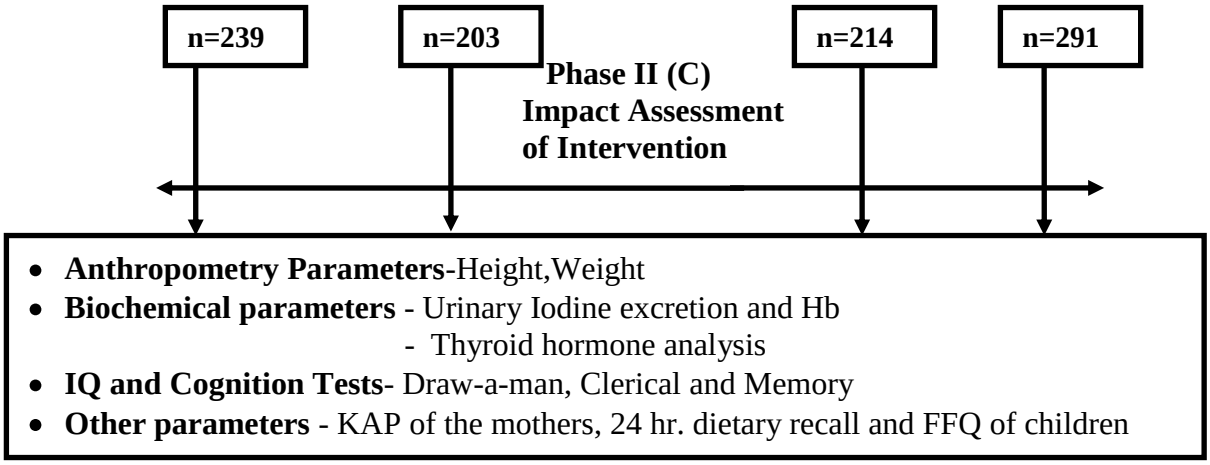
= 384

Thus, we screened 1184 children and 947 completed the study.

Experimental design of phase II has been depicted in **Figure 3.2**, indicating an overview of the work conducted.

Figure 3.2: EXPERIMENTAL DESIGN- PHASE II





3.1.3 Phase III: Upgrading salt iodization at local level and feasibility for Double Fortified Salt Production at local level

This phase for DFS production at local level was a first step towards future research- as an approach and a feasible method to combat 2 micronutrient deficiencies.

Study population and area: Based on the availability of **local salt producers** within and around **Anand, Kheda, Nadiyad and Baroda districts**; and also due to higher prevalence of still birth in the region, these local salt producers were selected as study participants. Timely monitoring and improved salt iodization were the short term goals to attain quality production.

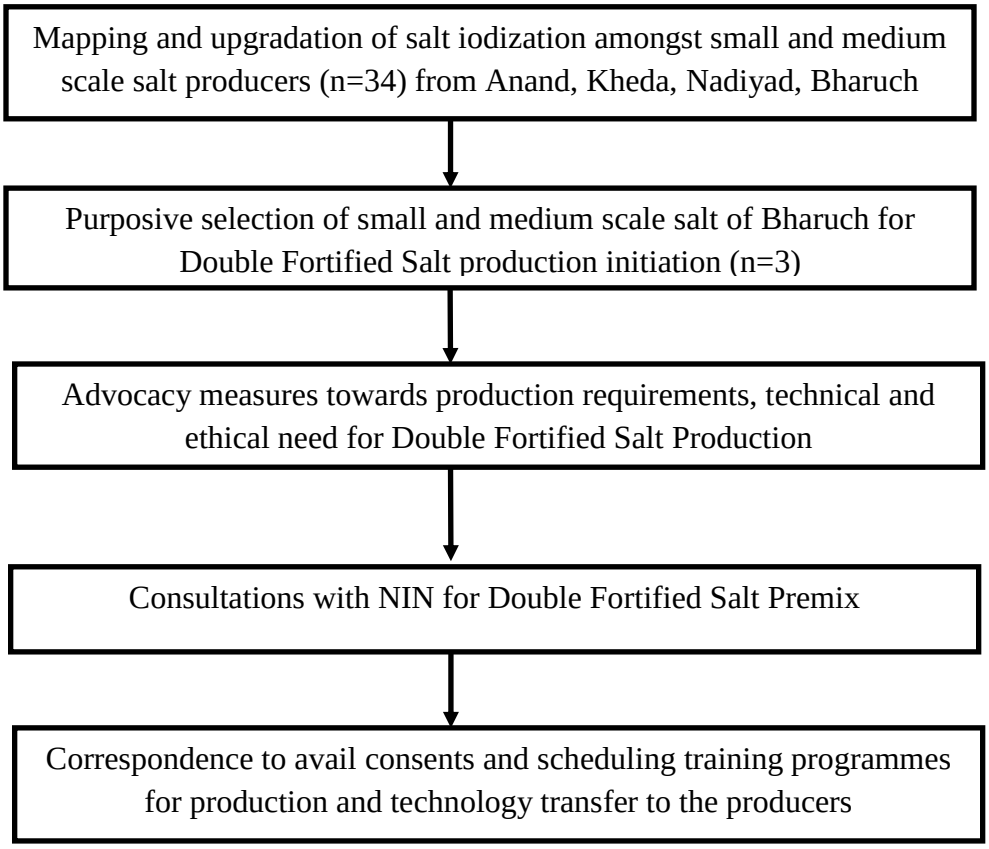
Sampling technique: Purposive sampling

The long run target of DFS production was carried out by motivating the producers for further entrepreneurship. The producers were provided with information on availing technology transfer from National Institute of Nutrition-NIN Hyderabad. The producers were made to meet the officials who were responsible for the needful support. The crux of business, trade etc was explained to them. Their role as a societal contribution was explained and was well received by the producers.

It is a known fact that, any new venture takes time to establish its roots. This venture follows the same pattern. Here, the setup is in slow progress due to financial limitations of the producers. Laisoning is being carried out to provide further directions; work is in progress towards achievement of the same.

Experimental design of phase III has been depicted in **Figure 3.3**, indicating an overview of the work conducted.

Figure 3.3: EXPERIMENTAL DESIGN- PHASE III



STUDY DURATION

Phase I: Study duration - October 2009 to November 2010

Report writing - December 2010 to March 2011

Phase II: Study Duration- March 2010 to June 2011

Report writing- July 2011 to December 2011

Phase III: Iodization upgradation and monitoring - January 2008 to May 2009

DFS production discussion and correspondence- May 2009 to May 2011

Report writing- January 2012 to February 2012

Ethical Issues

Approval for the study was obtained from the ethical committee of the home institution ethical board in compliance with the guidelines issued by Indian council of Medical research (No. F. C. Sc FN ME70).

Phase I: Written permission from the hospital authorities and concerned doctors was availed before the commencement of the study. All the pregnant women were explained the purpose of the study and signed written consent were collected (in local language).

Phase II: Permissions from all the schools and District education officer were availed to carry out the work. All the children from 1^{st} to 6^{th} standard were enrolled for the study. Written consent from the parents of the children (in local language) and oral consent from the children was also availed.

Phase III: Oral consent from the salt producers was availed after explaining the purpose of the study.

3.2 INDICATORS FOR DATA COLLECTION

Study indicators used for all the three phases have been mentioned in **Table 3.1** along with the required sample size and methods references details.

Table 3.1: Indicators used for the study

Sr. No.	Indicators	Tools	Phases	Samples Size
1.	Socio-Economic Status	Structured Questionnaire	Phase I	256
			Phase II	212
2.	Anthropometry	Standard methods and tools	Phase I	256(Baseline) 121(Final)
			Phase II	1184(Baseline) 947(Final)
3.	Hemoglobin Estimation	Cynmet-hemoglobin method	Phase I	256 (Baseline) 121 (Final)
			Phase II	972 (Baseline) 947 (Final)
4.	Urinary iodine excretion	Sandell-kolthoff reaction (Modified microplate technique)	Phase I	256 (Baseline) 121 (Final)
			Phase II	1034(Baseline) 947 (Final)
5.	DFS content estimation	BIS standards		3
6.	Thyroid hormones-TSH, FT_4,TT_4,Tg	RIA technique	Phase I	256(Baseline) 121 (Final)
			Phase II	212 (Baseline) 189 (Final)
7.	Cord blood Analysis- TSH, FT_4,TT_4,Tg	RIA technique	Phase I	64
8.	Dietary intake- 24 hr. dietary recall and FFQ	Structured questionnaire	Phase I	121 (Baseline and Final)
			Phase II	212(Baseline and Final)
9.	Knowledge, attitude and practices	Semi-structured questionnaire	Phase I	121 (Baseline and Final)
			Phase II	212(Baseline and Final)
10.	Household salt samples	Spot testing kit	Phase I	80
			Phase I	302
11.	Salt samples from salt units	Titrimetric method	Phase III	250
12.	IQ and Cognition Tests- Draw-a-man, Visual Memory test, Clerical Test	Standardized methods	Phase II	823-864 (Baseline) 700 (Final)

3.3 METHODS OF DATA COLLECTION

3.3.1 NUTRITIONAL STATUS ASSESSMENT

3.3.1.1 Weight

It was a key anthropometric measurement of body mass. Weight deficiency is a best indicator for the detection of protein energy malnutrition (Gibson 1989).

Procedure: Subjects were asked to stand bare foot on the Standardized bathroom scales. They were used for weighing with the least count of 0.5 kg. The subjects were asked to stand straight on the scale without touching anything and look straight ahead. Then the weights were recorded. Weight for age was calculated using CDC standards in the case of school children

3.3.1.2 Height

Height is a linear measurement of body. Deficit in the height is associated with chronic insufficient food intake and frequent infections.

Procedure: Height was measured by a wall mounted fiber glass tape with the least count of 0.5 cm. The tape was mounted accurately on the wall perpendicular to the floor and the subjects were asked to stand against it. The head, shoulders and heels touched the wall and the subjects were asked to look straight. The flat surface of the floor and the wall was taken into consideration for even measurements. A thin scale was kept straight on the head, perpendicular to the wall, so as to slightly press the hair. This was the feasible method in the rural field level and a government hospital setting. Height for age was calculated using CDC standards in the case of school children.

3.3.1.3 Body mass index (BMI)

BMI has been recommended by WHO (1995) as an indicator of choice for measuring undernutrition. It is calculated as follows:

Z score was used for defining underweight, stunted and wasted based on CDC growth standards (2005).

>-2SD – marginally malnourished.

-2SD to -3SD – moderately malnourished

<-3SD – severely malnourished

WHO growth standards (2007) have now been recommended as the reference standard for children. However, weight-for-age norms for children above 10 years have not been available. Hence, to use all the three Z scores using same standards would give better homogeneity and avoid complications in concluding the results on the anthropometry. Hence, CDC growth standards 2005 have been used for comparing anthropometric indices.

3.3.2 BIOCHEMICAL PARAMETERS

3.3.2.1 Determination of Urinary Iodine Excretion

Urinary iodine excretion is a good marker of the recent dietary intake of iodine and therefore used as an index as an index for evaluating the degree of iodine deficiency.

Principle

Iodine in urine occurs as the iodine ion (I^-). Most of the popular methods for urinary iodine concentration determination are based on Sandell-Kolthoff reaction. Iodide is measured by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerous ion (Ce^{3+}) coupled to the oxidation of arsenous to arsenic, As^{3+} to As^{5+}.

$$2Ce^{4+} + 2I^- \longrightarrow 2Ce^{3+} + I_2$$

$$I_2 + As^{3+} \longrightarrow As^{5+} + 2I^-$$

The Ceric ion (Ce4+) has a yellow colour, while the Cerous (Ce3+) is colourless. The course of reaction can be followed by the disappearance of yellow colour as the Ceric ion is reduced and can be measured colorimetrically. With other reactants held stable, the speed of this colour disappearance is directly proportional to the amount of catalyzing

iodide. Because of its specificity and high sensitivity, this reaction has been the basis for almost all chemical methods for the detection of iodine in urine.

However, many studies have indicated that there are interfering substances such as nitrite, thiocynate or ferrous ion in the urine that might interfere by reducing or oxidizing the Ceric or arsenic reactants and thus needs to be removed initially. Different methods are used such as dry ashing, dialysis or digestion with strong acid. At present the safest course is to include digestion step prior to colorimetric determination for urinary iodine.

Chloric acid digestion is the most commonly used method. Although it provides an accurate measurement, the method also has following disadvantages:

- Production of toxic wastes (>5ml/test) from arsenic trioxide in Sandell-Kolthoff reaction.
- Leakage of gas during sample digestion, requiring a special fumehood
- Difficulty in locating Chloric acid from chemical vendors because of its instability

On the other hand, an alternative method that uses ammonium persulphate digestion has been reported recently as a non-hazardous, non-explosive and easy to use method. The persulphate digestion makes possible a comparatively non-hazardous (no chlorine gas) measurement. However, this method is still not completely suitable for testing because it is time consuming and produces a non-negligible amount of toxic waste. To further minimize the amount of toxic wastes as well as simplify and speed up the procedure simple microplate method using ammonium persulphate digestion is used. (Ohashi, et al.2000)

Reagents

1. **Standards:** Measure 168.5 mg of KIO_3 and put in 100ml volumetric flask. Make up the volume to 100 ml by deionized water. Mix it well. This is the stock solution 1.
   Take out 100 µL of this stick solution1 in 2^{nd} 100 ml volumetric flask and make up the volume to 100 ml by deionized water. This is stock solution 2.

Table 3.2: Preparation of working standards

Standard (µg/L)	Stock Solution 2 (µL)	Deionized water (ml)
10	100	9.9
20	200	9.8
100	1000	9
150	1500	8.5
200	2000	8
300	3000	7
400	4000	6

2. ***Ammounium persulphate solution:*** Measure 5 mg of ammonium persulphate and add in 15 ml of deionized water. Mix it properly. It should be prepared fresh.
3. ***Aresenous acid solution (500 ml):*** Arsenic trioxide (5 g) was dissolved in 100 ml NaOH solution (3.5 w/v). Keep it in ice bath and add 16 ml of conc. H_2SO_4 slowly. After cooling add 12.5 g NaCl and dilute upto 500 ml with water. Filter and store in amber colored bottle.
4. ***Ceric ammonium sulphate solution (250 ml :*** Weigh 3 gm of ceric ammonium sulphate and add 250 ml of 3.5 N H_2SO_4. Store the solution in amber color bottle.

Procedure:

- 50 µl of each calibrators (with known concentration of 0, 10, 20, 100, 150, 200, 300 and 400 µg/L) and urine **s**amples are pipetted into the wells of a polypropylene plate (PP).
- Into the sample wells 100µl of 3% ammonium per sulphate is added. The plate is then set in a cassette.
- The cassette is tightly closed and kept for 60 minutes in an oven adjusted at 110º C.
- After digestion, the bottom of the cassette is cooled to room temperature with tap water to avoid condensation of vapour on the top of wells and to stop the digestion.

- The cassette is opened and 50 µl aliquots of the resulting digests are transferred to the corresponding wells of a polystyrene 96 wells microtiter plate.
- Arsenious acid solution (100µl) is added to the wells and mixed; 50µl of ceric ammonium sulphate solution is then added quickly (within 1 minute), using a multichannel pipette. The reaction mixture is allowed to sit for 30 mins.
- The absorbance is measured at 405 nm with an ELIZA reader.

Table 3.3: Epidemiological criteria for assessing iodine nutrition based on the median or range in urinary iodine concentrations of pregnant women and children [a](WHO/UNICEF/ ICCIDD 2007)

Population Group	Median Urinary iodine excretion (µg/L)	Iodine concentration
Pregnant Women	<150	Insufficient
	150-249	Adequate
	250-499	Above requirements
	≥ 500	Excessive [b]
Children	<20	Severely deficient
	20-49	Moderately deficient
	50-99	Mildly deficient
	≥ 100	Normal

[a] For lactating women and children < 2 years of age median urinary iodine concentration of 100 µg/L can be used to define adequate iodine intake, but no other categories of iodine intake are defined. Although lactating women have the same requirements as pregnant women, the median urinary iodine is lower because iodine is excreted in breast milk. [b] the term "excessive" means in excess of the amount required to prevent and control iodine deficiency.

3.3.2.2 Determination of Haemoglobin Concentration

Estimation of Haemoglobin was carried out by,

(A) Sahli's haemoglobinometer (Phase I)

(B) Finger prick blood Spot – Cyanmethhemoglobin (Phase II)

(A) Sahli's haemoglobinometer

Blood collection was done on the spot using Sahli's method. Blood was collected using disposable lancets.

Principle - Haemeoglobin (Hb) is converted to acid haemeatin by addition of 0.1 N Hydrochloric acid and the resulting brown colour is compared with standard brown glass reference blocks.

Procedure

- 0.1 N HCL is placed in the graduated tube to the mark 10.
- Blood is drawn upto the 20 mark in the Sahli's pipette, added to the acid in the tube and the pipette rinsed thoroughly.
- The mixture is allowed to stand for 10 min. for complete conversion to acid haemeatin. (Maximum colour develops in 1 hr.).
- The solution is diluted with a few drops of distilled water at a time until the colour matches with the standard glass reference block.
- The height of the solution corresponds to the Hb content.

(B) Finger prick blood Spot- Cyanmethemoglobin

Haemogloin was assessed using finger prick blood sample by cyanmethaemoglobin method using filter paper technique.

Procedure:

- Label the Whatman No.1 filter paper strip appropriately (with identification particulars) before collection of blood sample.
- Clean the middle finger of left hand with spirit and cotton and allow it to dry. Squeeze the finger slightly and hold it firmly with left hand.
- Using a lancet, puncture the skin at right angles to the tip of the finger. Discard the lancet after folding it. Wipe off the first drop of the blood with tissue paper and press the finger gently so as to form a drop of blood. Collect the blood into clean hemoglobin pipette.
- Using a clean and dry hemoglobin pipette draw the blood slowly up to a little above the 20µl mark on the pipette. Care should be taken to avoid any air bubbles in the blood column. Clean the tip of the pipette using a tissue paper. Adjust the volume to 0.02 ml (up to the mark on the pipette) by touching the tip of pipette with a wet tissue paper.

- Transfer the blood from the pipette by slowly blowing it out on to the coded filter paper, in the form of a circular spot of about 1 cm diameter, by keeping the pipette perpendicular with its tip touching the filter paper and moving in a circle. Take care not to splash the blood, by blowing very slowly. No trace of blood should remain, either in the pipette or on its tip.
- As soon as the sample blood collection is over, wipe the finger with dry cotton. Press the site of puncture with spirit swab. Avoid direct contact with blood.
- Discard the sample in case the blood sticks to the inner surface of the pipette. Pipette the blood once again with a fresh pipette.
- Fold the filter paper diagonally and place it in a breadbox and allow it to dry in shade. Protect the sample from sunlight, flies and dust. Pack the dried samples in polythene cover.

Haemoglobin estimation

Principle: When blood is mixed with drabkin's reagent containing potassium cynide and potassium ferricynide, haemoglobin reacts with ferricynide to form methemoglobin which is converted to stable Cynmethemoglobin by the cynide. The intensity of the color is proportional to haemoglobin concentration and it is compared with a known cynmethemoglobin standard at 540 nm (green filter).

Requirement

1. Drabkin's reagent : It contains in 1000 ml of distilled water.
   a. Potassium ferricynide : 400 mg
   b. Potassium dihydrogen phosphate : 280 mg
   c. Potassium cynide : 100 mg
   d. Ninidet : 1 ml
2. Cyanmethemoglobin standard : It is commercially available. This standard is dierectly pipetted in a cuvette and optical density is measured at 540 nm. The reading obtained, corresponds to _____g/dl haemoglobin.
3. Hb-pipette (20 µl calibrated)

4. Test tubes (15 X 125 mm)
5. Spectrophotometer (Spectronic 20D)

Procedure

- Exactly 5 ml of the diluted Drabkin's reagent was pippetted out in test tubes.
- The portion of filter paper with blood spot was cut carefully using scissors and the same was transferred in to a pre-coded test tube having 5 ml of Drabkin's solution.
- These test tubes with soaked blood spot on filter paper, was kept for sufficient length of time (overnight/6-8 hrs) in Drabkin's reagent for complete extraction of the blood. On complete extraction, the filter paper appeared white.
- On complete extraction, content was mixed thoroughly before reading.
- Absorbance of test was read at 540 nm by setting blank to 100% T
- Absorbance of standard and samples were read by pipetting directly in a cuvette.

Calculation Formula

$$\text{Hb in g/dl} = \frac{\text{OD of sample} \times \text{Concentration of Hb standard} \times \text{Dilution factor (251)}}{\text{OD of standard} \times 100}$$

Where concentration of Hb standard is 60mg/dl, and OD stands for optical density.

Table 3.4: Hemoglobin cutoffs used to define anemia in people living at sea level

Age or Sex group	Hemoglobin below (g/dl)
Children 6 months to 59 months	11.0
Children 5 – 11 years	11.5
Children 12- 14 years	12.0
Nonpregnant women (above 15 years of age)	12.0
Pregnant women	11.0
Men (above 15 years of age)	13.0

Source: (WHO/UNICEF/UNU 2001)

3.3.2.3 DETERMINATION OF THYROID ANALYTES

Collection of blood and separating serum

Venous blood was collected using a plain Vaccutainer (i.e. not containing any anticoagulant) for all subjects. Blood collection was carried out by a technician. These vaccutainers were centrifuged at 3000 RPM for 5 minutes. Serum is separated as supernatant. Carefully serum is transferred to other screw capped tube and deep freezed (-20^{o}C) till it is used. From these samples Tg, T_4, $FT_4$and TSH were analyzed.

(A) Measurement of serum TSH

Method of analysis:

TSH was measured from serum by Immuno-radiometric assay (IRMA) using kit. The kit was IRMAK-9, procured from The Board of Radiation and Isotope Technology (BRIT) Government of India, Navi Mumbai.

Principle

In immuno-radiometric assay (IRMA), two antibodies generated against different epitopes of the same antigen are used. One antibody is bound to a solid-phase, usually a tube, while the other antibody is labelled with ^{125}I. Thus, when an antigen is present, it simultaneously binds both antibodies in a "bridge" fashion (i.e. it gets sandwiched between two antibodies. This entire complex remains bound to the tube. The radioactivity in the bound fraction may then be quantified using a Gamma Counter. The radioactivity measured, is directly proportional to the concentration of antigen. A standard curve is constructed from which unknown concentration of human thyroid stimulating hormone can be extrapolated.

Reagents

Reagents were provided with kit.

1. hTSH monoclonal antibody tubes: 100
2. ^{125}I-Anti-hTSH: 1 vial 10 ml
3. hTSH standards: 8 vials (10 ml each)(concentration- 0, 0.15, 0.5, 1.5, 5.0, 15, 50 µIU/ml)

4. Wash diluent: 1 vial (50 ml)
5. Control: 2 vials (10 ml each)

Reagent preparation

1. hTSH standards: ready to use
2. ^{125}I-Anti-hTSH: ready to use
3. Wash diluent: Dilute with 950 ml double distilled water
4. Control: ready to use.

Protocol

Table 3.5: Defined dose for standard graph (TSH estimation)

Serial number	Tube number	Defined dose (µIU/ml)	Percentage bound (%B)
1	S_A	0.00	0.1
2	S_B	0.15	0.3
3	S_C	0.50	1.0
4	S_D	1.50	2.8
5	S_E	5.00	8.2
6	S_F	15.00	23.4
7	S_G	50.00	68.3
8	S_H	100.00	100.0

Figure 3.4: Standard graph for TSH

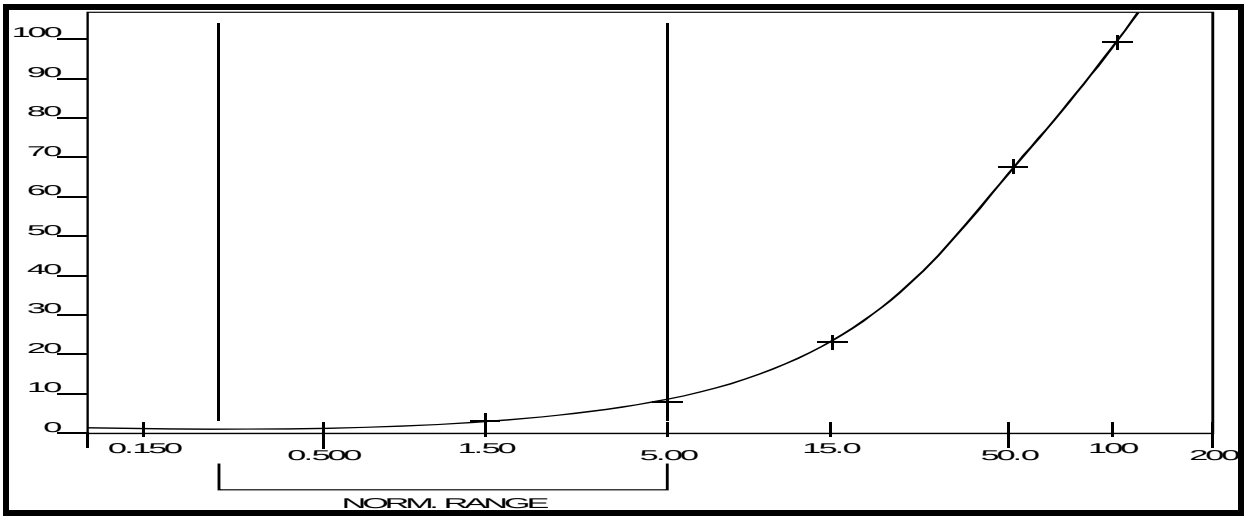


Table 3.6: Steps in measurement of TSH

Serial number	Tube number	Standard/ control/ Sample(µl)	Tracer ^{125}I -Anti-hTSH (µl)		
1.	Standard A	100 µl	100 µl	Incubate for 60 min. at Romm Temp. with gentle shaking. Add 2ml diluted wash solution to each tube . Mix. Decant the solution . Repeat wash step once more.	Count the tubes in Gamma Counter set for ^{125}I for 2 min.
2.	Standard B	100 µl	100 µl		
3.	Standard C	100 µl	100 µl		
4.	Standard D	100 µl	100 µl		
5.	Standard E	100 µl	100 µl		
6.	Standard F	100 µl	100 µl		
7.	Standard G	100 µl	100 µl		
8.	Standard H	100 µl	100 µl		
9.	Control 1	100 µl	100 µl		
10.	Control2	100 µl	100 µl		
11.	Control 3	100 µl	100 µl		
12.	Test sample	100 µl	100 µl		

(B) Measurement of serum FT_4 and TT_4

Method of analysis

T_4 from serum was measured by radioimmunoassay.

Principle

This assay is based on the competition between unlabeled T_4 and fixed quantity of ^{125}I-labeled T_4 (tracer) for limited number of binding sites on T_4 specific antibody. These T_4 antibodies are coupled to magnetic particles. Allowing to react a fixed amount of tracer and antibodies (bound to magnetic particles) with different amount of unlabeled antigen,

the amount of tracer bound by antibody will be proportional to the concentration of unlabeled antigen.

On providing a magnetic field, (using magnetic racks) all antigen-antibody immune complexes settle at the bottom of the tube. In presence of magnetic field, the reaction mixture is discarded and tubes are dried. Then, radioactivity is measured in Gamma Counter.

The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against known amount of T_4, a standard curve is constructed, from which the unknown concentration of T_4 in patient's sample can be determined.

Reagents

Reagents were supplied with kit.

- Standard T4: 5 vials 10 ml each (concentration- 0, 2.5, 5, 10, 20 µg/dl)
- Tracer: 1 vial of 10 ml
- Antibody-magnetic particles: 1 vial of 20 ml
- Control: 2 vials (10 ml each)

Reagent preparation

All the reagents were ready to use. Hence, they were directly used without reconstitution.

Protocol

Table 3.7: Defined dose for standard graph (FT_4 & TT_4)

Serial number	Tube number	Defined dose (µg/dL)	Percentage bound (%B)
1	S_A	0.0	100.0
2	S_B	2.5	67.5
3	S_c	5.0	49.8
4	S_D	10.0	33.6
5	S_E	20.0	20.9

Figure 3.5: Standard graph for T_4

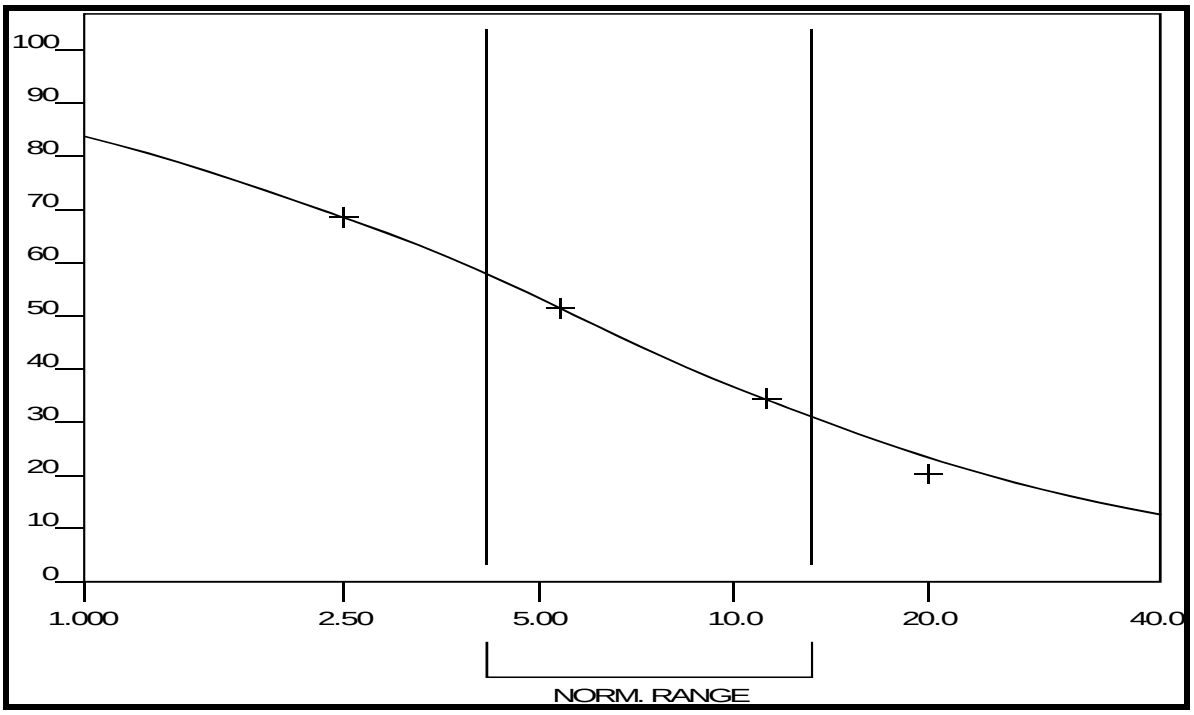


Table 3.8: Steps in measurement of FT4

Tube Reagents	Std/Control/Sample (µl)	Tracer (µl)	Ligand (µl)	Vortex. Incubate for 60 min. at 18-25°C, with shaking.	Aspirate carefully the contents. Count.
STD A	25	400	100		
STD B	25	400	100		
STD C	25	400	100		
STD D	25	400	100		
STD E	25	400	100		
QC1(kit)	25	400	100		
QC1 (in-house)	25	400	100		
QC2 (in-house)	25	400	100		
Samples	25	400	100		

Table 3.9: Steps in measurement of TT_4

Tube Reagents	Std/Control/Sample (µl)	Tracer (^{125}I-T4) (µl)	T4 – Ab magnetic particles (µl)	Vortex. Incubate for 90 min./water bath (37°C), shaking.	Place the tubes for 20 mins on magnetic rack. Decant, wipe the rim of the tubes with tissue paper and count.
STD A	10	400	500		
STD B	10	400	500		
STD C	10	400	500		
STD D	10	400	500		
STD E	10	400	500		
P1	10	400	500		
P2	10	400	500		
P3	10	400	500		
Samples	10	400	500		

(C) Measurement of serum Tg

Thyroglobulin from serum was measured by radioimmunoassay.

Principle

In radioimmunoassay (RIA), a fixed concentration of labeled tracer (antigen) is incubated with a constant amount of antiserum such that the number of antigen binding sites on the antibody is limiting. If unlabeled antigen is added to this system, there is a competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody and thus the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody bound from free tracer and counting the bound fraction in Gamma Counter. The radioactivity measured is inversely proportional to concentration of unlabeled antigen. A calibration or standard curve is set up with increasing amounts of known antigen and from this curve the amount of antigen in the unknown samples can be calculated.

Reagents:

Reagents were provided with kit.

1. Standard Tg- 8 vials containing 10 ml each (concentration 0, 12.5, 25, 50, 100, 200, 400, 800 ng/ml)
2. Tg free serum: 1 vial (10 ml)
3. r-antiTg-antibodies: 1 vial (10 ml)
4. Labeled Tg: 1 vial (10 ml)
5. Control: 2 vials (10 ml each)
6. DAB-magnetic particles: 1 vial (10ml)

Reagent Preparation: (Carried out by BARC Laboratory, RIA Unit, Mumbai)

Protocol for serum thyroglobulin

Table 3.10: Defined dose for standard graph (Tg)

Serial number	Tube number	Defined dose (ng/ml)	Percentage bound (%B)
1	B_0	0.61	18.76
2	S_1	12.5	16.04
3	S_2	25.0	12.98
4	S_3	50.0	10.24
5	S_4	100.0	7.38
6	S_5	200.0	5.59
7	S_6	400.0	4.22
8	S_7	800.0	3.33

Figure 3.6: Standard graph for serum thyroglobulin

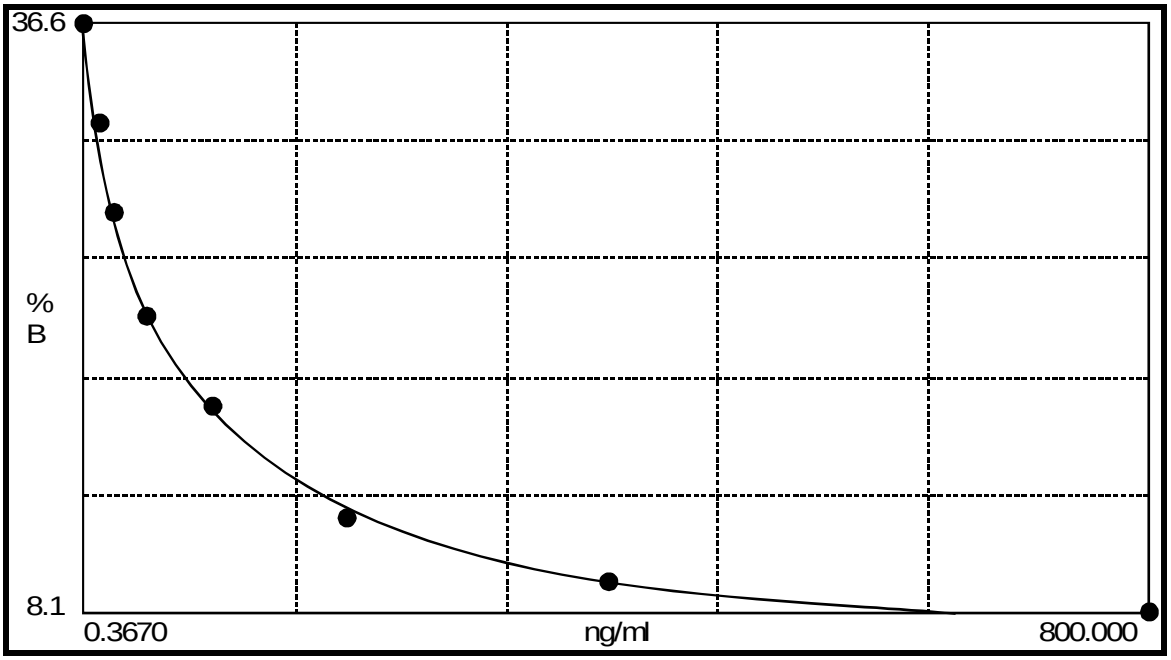


Table 3.11: Steps for measurement of Serum Tg

Tube	Buffer (µl)	Free serum (µl)	125I (tracer) (µl)	Standard/ Sample/ control (µl)	Antiserum (µl)	Incubate at 4°C for 3 overnights	DAB-magnetic particles (µl)	Keep for 2 hours on shaker. Add 0.4 ml assay buffer and leave it for 20 minutes on magnetic racks, decant and count.
Total counts	---	----	100	---	-----		---	
B_0	100	100	100	___	100		100	
S_1	___	100	100	100	100		100	
S_2	___	100	100	100	100		100	
S_3	___	100	100	100	100		100	
S_4	___	100	100	100	100		100	
S_5	___	100	100	100	100		100	
S_6	___	100	100	100	100		100	
S_7	___	100	100	100	100		100	
Control	100	___	100	100	100		100	
samples	100	___	100	100	100		100	

3.3.3 QUALITY ESTIMATIONS

3.3.3.1 Determination of iodine from iodized salt

(A) Titrimetric Method

Description of reaction: The iodine content of salt iodated samples is measured using an iodometric titration, as described by Dc. Macyer, Lowenstein and Thilly (1979). The reaction mechanism can be considered in two steps:

Reaction 1: Liberation of free iodine from salt.

- Addition of H_2SO_4 liberates free iodine from the iodate in the salt sample.
- Excess KI is added to help solubilize the free iodine, which is quite insoluble in pure water under normal conditions.

Reaction 2: Titration of free iodine with Sodium thiosulfate.

- Free iodine is consumed by sodium thiosulfate in the titration step. The amount of thiosulfate used is proportional to the amount of free iodine liberated from the salt.
- Starch is added as an external (indirect) indicator of this reaction. It reacts with free iodine to produce a blue colour. When added towards the end of the titration (that is, when only a trace amount of free iodine is left) the loss of blue colour or endpoint, which occurs with further titration, indicates that all remaining free iodine has been consumed by thiosulfate.
- Reaction steps for Iodometric Titration of iodate :

1. $IO_3 + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$.

2. $2Na_2S_2O_3 + I_2 \longrightarrow 2NaI + 2Na_2S_4O_6$.

(Sodium Thiosulfate) (Iodine) (Sodium Iodide) (Sodium Tetrathionate)

Reagent Preparation:

- **Water requirements for Reagent Preparation :**

Water required for this method should be boiled distilled Water, which requires provision of a distillation unit as a simpler alternative.

- **0.05 N Sodium thiosulfate ($Na_2S_2O_3$):** Dissolve 1.24 gm $Na_2S_2O_3.5H_2O$ in 1000 ml water. Store in a cool dark place. This volume is sufficient for 100-200 samples, depending on the iodine content of samples. The solution is stable at least for 1 month, if stored properly.

- **2N Sulfuric Acid (H_2SO_4) :** slowly add 6ml concentrated H_2SO_4 to 90 ml distilled water. Make up the volume to 100 ml with distilled water.

- **10% Potassium iodide (KI) :** Dissolve 100 gm KI in 1000 ml water. Store in a cool dark place. This volume is sufficient for 200 samples. Properly stored solution is stable for 6 months.

- **Starch indicator solution:** Make 100ml of a saturated solution of NaCl. By adding NaCl to approximately 80 ml distilled water in a beaker, with stirring and/or heating until no further solid will dissolve. This solution is stable for at least one year. Weigh 1 gm soluble starch into a 100ml beaker; add 10ml water and heat to dissolve. Add saturated NaCl solution to the hot starch solution to make up to 100 ml. Store in a cool dark place. This volume is sufficient for 50 samples.

Procedural Steps:

- Weigh 10g of the salt sample into a 250 ml Erlenmeyer flask with a stopper.
- Add approximately 30 ml water, swirl to dissolve salt sample.
- Add distilled water to make the volume upto 50 ml.
- Add 1 ml 2N H_2SO_4.
- Add 5 ml 10% KI. The solution should turn yellow if iodine is present.
- Stopper the flask and put in the cupboard or drawer (dark) for 10 minutes.
- Rinse and fill burette with 0.005 M $Na_2S_2O_3$ and adjust level to zero.

- Remove flask from drawer, and add some $Na_2S_2O_3$ from the titration burette until the solution turns pale yellow.
- Add approximately 2ml of starch indicator solution (the solution should turn dark purple) and continue titrating until the solution becomes pink, and finally colourless.
- Record the level of thiosulfate in the burette and convert to parts per million (ppm) using the conversion table in **(Annexure V).**

Precautions:

- The reaction mixture should be kept in the dark before titration because a side reaction can occur when the solution is exposed to light that causes iodide ions to be oxidized to iodine.
- Inaccurate results may occur if starch solution is used while still warm.
- If starch indicator is added too early, a strong iodine starch complex is formed which reacts slowly, and gives falsely elevated results.
- The reaction should be performed at mild room temperature ($>30^{o}$ C) since the iodine is volatile, and the indicator solution loses sensitivity when exposed to high temperature.

(B) Spot Test Kit

It is used for semi quantitative test of iodine in salt. This kit consists of two ampules of test solutions and one ampule of recheck solution.

- **The test solution** contains starch solution in acidic medium, a drop of which when added to iodized salt, it gives blue colour. The colour developed is compared with the reference colour chart from assessment of the ppm (parts per million) of iodine.
- **The recheck solution** contains 50% Phosphoric acid. It is used when salt contain excess of alkaline materials, impurities or additives which interfere with the reaction of iodates with starch. When a drop of test solution added to iodized salt doesn't give blues colour, a drop of recheck solution is added to the salt.

Principle : The reaction mechanism for iodate spot test is that iodate from salt, in the presence of free hydrogen ion, oxidizes added iodine to give free iodine; this then turns starch to give a blue colour :-

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O.$$

$$I_2 + \text{Starch} \longrightarrow \text{Blue colour.}$$

Procedure:

- One drop of test solution is added to a spoonful of salt sample. The change in colour of salt sample ranges from white to light blue to dark violet depending on the iodine content of salt, i.e. Nil, 7 ppm, 15 ppm, 30 ppm. This is compared to the standard colour chart provided with the kit.

- If on addition of test solution, no change in colour of salt is observed, the recheck solution is added. This is done to make the salt medium acidic, (in case the salt has alkaline constituents), and then the test solution is added once again. The intensity of blue colour is directly proportional to the iodine content of the salt.

Precautions:

- Contamination may occur if measuring spoon and plate are not washed.

- The kit should show an expected shelf life, usually 12-18 months. Thus kits, which have out-lasted their shelf life, should not be used.

- In addition to the date of expiration of the kit as a whole the test solution also has a limited shelf life, once the dropper bottle has been opened and used.

3.3.3.2 Determination of Iodine and iron content from DFS was carried out by a standardized method established by (Ranganathan et al 2007). The estimations were carried out by one of the QC laboratory at DFS production unit, Gujarat.

3.4 QUESTIONNAIRES

3.4.1 STRUCTURED QUESTIONNAIRES

Background information was obtained from the study subjects on socio-economic status availing details on names of the subjects, names of spouse (Phase I)/ parents (Phase II) of

the subjects, age, sex, education, occupation and other relevant information. For pregnancy obstetric history and previous adverse experiences were also reported **(Annexure VI and VII).**

3.4.2 SEMI STRUCTURED QUESTIONNAIRES

Background and post interventional effect of NHE on KAP was assessed using this tool. The information on iodine nutrition, iron nutrition, dietary, cooking and behavioral practices related to different aspects were collected, which were addressed during the education sessions and covered based on their baseline levels of information **(Annexure II to VI)**.

3.5 DIETARY INTAKE ASSESSMENTS

Food and nutrient intake is an important component of nutritional status assessment. The 24 hour dietary recall and food frequency methods were used to record the dietary intake of the subjects. Both baseline and end intervention data regarding dietary intake was collected using these tools.

3.5.1 DIETARY RECALL- 24 HOURS

This method is based on the process of recall of food consumption over a specified period of time (24 hour), prior to the survey. The ingredients recalled by the respondents are measured using standard cups and spoons. From the cooked amount, the raw ingredients as well as their nutritive values are calculated using recipes of the conventional cooked foods. In certain cases on the day of survey, if the subject had not consumed sufficient diet than their routine life due to some constraint, then they were motivated to recall the previous days diet/ their regular dietary practice. Thus, it helped to avoid the chances of under estimation of dietary intake.

Procedure: The food intake of subjects was noted for the previous day through individual interviews. Amount of cooked food eaten in each meal was recorded using standard measures. Food consumed by the subjects outside home was also recorded. The nutrient content of food consumed was calculated using the food composition tables (NIN 2009). **(Annexure VII).**

3.5.2 FOOD FREQUENCY PATTERN

Food frequency method is usually used to assess the habitual food intake of the subjects and their families qualitatively. The subjects were asked to respond to frequencies of consumption of each food from a list of foods, rich in iron, vitamin C, iodine, protein rich and green leafy vegetable **(Annexure VIII)**. The frequencies were listed from daily to never into 9 different categories. It is an accepted method to estimate usual dietary intake (Thompson and Byers 1994).

3.6 IQ AND COGNITIVE FUNCTION TESTS

Iodine and iron deficiencies adversely affect the ability of the school children to learn. The IQ and cognitive functions of the school children were assessed using selected tests from the Gujarati version of Wechsler Intelligence Scale for children (WISC) (Bhatt 1973 and Phatak P 2002), which has been pretested on different groups of population and were modified for the present study groups. The WISC is a battery of tests for 6-17 years olds, which assesses intellectual abilities. The various tests used were Draw-a-man, Visual memory test and Clerical test.

3.6.1 DRAW-A-MAN TEST

This test is used to assess the intelligent quotient of the children and their brain development. Hence, this test was selected considering the effect of iodine deficiency on IQ of the children

Procedure: A child is asked to draw a picture of a full human being in the space on a blank sheet of paper and score were analyzed further using specific scales mentioned in the manual **"Draw a Man Test for Indian Children" (Pramila Pathak, 2002)**. The use of human figure to measure maturity (intelligence) is based upon the development of concept of human body. The scale includes scoring of major body parts and their crude proportions for 25 different scoring points**.**

Scoring: The scoring was carried out for the quality of each body part drawings, matched with the age wise standard drawings provided in the manual. The scores ranged from 1-5 for each body part. Then the total scores for all the 25 parts made the final score (leads to

mental age calculation using table 8 in the manual), which in turn made the final IQ score using a formula as below:

IQ = Mental Age / Chronological age × 100

3.6.2 VISUAL MEMORY TEST

Visual memory test is used to assess the short term memory of the school children.

Procedure: Twenty commonly used items (pictures), which they observed in their daily life which were a part of their routine activity was selected. These pictures were presented in a coloured picture form on a visible chart form a 10 feet distance. The children were allowed to observe the objects for one minute, and then the chart was removed from the display. The children were asked to recall and list down the items which they had observed within 2 minutes. A single score point was rewarded for each item listed correctly**.**

Scoring: The score was calculated as the ratio of number of items correctly identified against the total number of items, i.e. 20. The highest score was one.

3.6.3 CLERICAL TEST

Clerical test helps to assess the ability of the children to concentrate and discriminate

Procedure: The children were given a typed sheet of Gujarati alphabets pre-tested in a different school who belonged to the same group (Kuruvilla. A, Nair S and Patel A 2005; Nair S and Dutta S 2009). They were required to encircle a particular letter assigned by the investigator in Gujarati within 5 minutes. The children were instructed not to encircle any other letters at all. The investigator motivated the children to encircle as many as they could in 5 minutes.

Scoring: Score points were rewarded by providing the maximum points for the maximum letters encircled. All the particular letters in sheet was repeated 32 times in the given section. The children had to find and circle them. The final score was calculated as the ratio of the number of letters encircled by the children against the total number of letter repeated in the section i.e. 32. The highest score was one.

3.7 DATA ANALYSIS

All the data were processed, entered and analyzed in the Statistical Package for Social Sciences for windows version 15.00 (SPSS 15.0) and growth indices were analyzed in Epi-Info, Version 3.5.3 (2011), Microsoft Excel 2007,Windows XP and WHO Anthroplus (Neonatal anthropometric indices- phase I).

Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square (χ^2) when appropriate for categorical data.

Normality of the data was assessed by the Kolmogorov-Smirnov test. Where indicated, the data was normalized using log transformation to facilitate the use of normal-theory analytic methods.

Nonparametric (Mann-Whitney U test, Kruskal-Wallis test and Wilcoxan test) or parametric (Student's t-test, paired t test and ANOVA) statistical tests, depending on the normality of the data, were used to detect within-group and between group differences. Further post-hoc bonferroni analysis was done. To determine associations between analytes Pearson's correlation or Spearman's rank correlation were calculated. 95% CI was calculated to reveal ±2SD from the mean values or percent prevalence. A two-tailed p values <0.05 was considered statistically significant.

3.8 Conduction of research work

This section contains pictures captured in the field and laboratory during the work was being conducted.


(A) Blood collection in the field

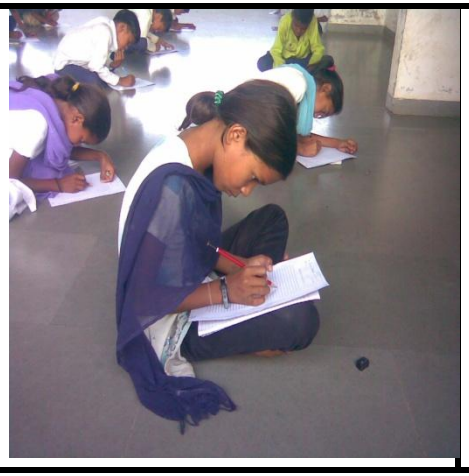

(B) Anthropometry Assessment


(C) Salt testing for iodine content in the field


(D) NHE and BCC to the parents of the children

(E) KAP data collection of the parents of the children

(F) NHE and BCC to the pregnant women



(G) Children participating in congitive tests

(H) Lab estimations

(I) DFS distribution

(J) Acceptability of DFS by the children

CHAPTER 4
RESULTS AND DISCUSSION

PHASE I

EFFICACY OF DOUBLE FORTIFIED SALT SUPPLEMENTATION AMONGST PREGNANT WOMEN

4.1 BASELINE SURVEY

The phase includes pregnant women availing antenatal services at a semi govt Hospital. It has got accountable delivery rate (250-300/month). A mixed population including all ethnic groups availed the services like antenatal care, vaccination, immunization. Hence, this was the convenient place to collect samples of the subjects representing the entire population from lower strata.

All the subjects were enrolled for the study during their first trimester (<12 weeks). The random recruitment was carried out to overcome regional, nutritional, socioeconomic diversifications.

4.1.1 GENERAL PROFILE OF THE POPULATION

Total two hundred and fifty six pregnant women in the first trimester aged between 18-36 years were enrolled.

The subjects were initially informed about the purpose of the study, a written consent was availed and basic proforma was filled up which included their number of parity, number of abortions (If any), number of Still birth (if any), their addresses and contact details, occupation, education etc.

There were 69.53% Hindus and 29.27% Muslims. Data on their family type revealed that, 80% of the subjects were living in joint families; and 20% had nuclear families. Majority of the subjects enrolled were residing in the urban or periurban LIG areas (94.53%) and 5.47% belonged to rural outskirts. It was observed that almost half of the subjects were educated up to primary school level (42.96%) and 38.28% with secondary education. Approximately 9% of the subjects could reach up to higher secondary education and 9% of the subjects did not attend schools ever.

Most of them were homemakers except four (1%) subjects. Monthly family income of majority subjects was ≥ 2000-5000/- per month **(Table 4.1).**

Table 4.1 General Profile of the subjects

Details	Subjects (N=256)	95% CI
Area of Residence		
Urban	242 (94.53)	93.11 - 95.95
Rural	14 (5.46)	2.62- 8.3
Religion		
Hindu	178 (69.53)	63.78- 75.28
Muslim	78 (29.47)	23.78- 35.16
Other	-	-
Education		
Illiterate	23 (8.98)	5.41- 12.55
Primary	110 (42.96)	36.78- 49.14
Secondary	98 (38.28)	32.21- 44.35
Higher Secondary	23 (8.98)	5.41-12.55
Graduate	2 (0.78)	-0.3- 1.86
Occupation		
Housewife	254 (99.2)	98.08- 100.32
Working	2 (0.8)	-0.32- 1.92

Values in parenthesis depicts percentage

Mean age of the subjects was 23.24 ± 3.6 years (Range: 22.79-23.67). Mean height was 151 ± 5.36 cms (Range: 150-152) and mean weight was 45.3 ± 7.86 kg (Range: 44.4 – 46.3). Of the total population, 15.23% subjects, had history of abortions/miscarriages and 5.85% had experienced still birth or neonatal mortality during their previous deliveries **(Table 4.2).**

Of the enrolled subjects, 44.53% were primiparous and rest were (55.56%) multiparous. Enrollment of the subjects was carried out during their first, second or third month of gestation. Majority of the subjects were enrolled during 3rd month of the gestation

(68.53%), which is normally a confirmation time for pregnancy, amongst lower strata of the community **(Table 4.2).**

Table 4.2 Baseline Characteristics of the subjects

Characteristics	Subjects N=256			
	Mean	**SD**	**Median**	**95% CI**
Age (Yrs.)	23.24	3.6	22	22.79 - 23.67
Height (Cms)	151	5.36	150	150 - 152
Weight (Kg)	45.3	7.86	45	44.4 - 46.3
Parity	0.66	0.76	1	0 - 1.32
Abortion	0.17	0.43	0	- 0.5 - 0.83
Still Birth/ Neonatal Death	0.1	0.2	0	- 1 - 0.7

4.1.2 NUTRITIONAL STATUS ASSESSMENT

Pregnancy is a physiological condition when there is an increased requirement of essential macro and micronutrients due to metabolic changes in the maternal physiology. If the standard cutoffs are not met, then it may restrict the growth of the developing fetus and many detrimental consequences to fetus may occur over a period of time. Hence, their nutritional status during pregnancy drags attention.

Table 4.3 Distribution of subjects with respect to BMI

Gestational Age	N (%)	Level of BMI (Kg/m^2)			Mean ± SD	95% CI
		<18.5	**18.5 – 25**	**>25**		
1st Month	4 (1.56)	2 (50)	2(50)	-	20.03± 3.91	16.2 – 23.86
2nd Month	77(30.07)	29 (37.66)	41(53.24)	7(9.09)	19.98± 3.17	19.28 – 20.68
3rd month	175(68.35)	59 (33.71)	102(58.28)	14(8)	19.97± 3.33	19.47 – 20.47
Total	256(100)	90(35.15)	145(56.64)	21(8.2)	19.98± 3.28	19.58 – 20.38

Values in parenthesis depicts percentage

According to **NFHS III** (2005-2006) survey, 36% of the women in their reproductive age (15-49 years) have BMI < 18.5 kg/m^2. Our data on nutritional status of the population also revealed that, overall 35.15% pregnant women were undernourished (<18.5 kg/m^2)

based on Basal Metabolic Index classification (BMI), which in turn reveals same picture after five years as per NFHS-III. It was also observed that, 56.64% of the subjects were having normal BMI (18.5 – 25 kg/m^2) **(Table 4.3).** Prevalence of under nutrition was comparatively higher than the other stratas of community, since pregnant women and children are the most vulnerable groups for compromised nutritional needs in lower income groups in developing countries.

4.1.3 MICRONUTRIENT STATUS OF THE POPULATION

This includes assessment of iron and iodine as major micronutrients, which may be deficient during pregnancy.

At the time of enrollment, spot urine sample and 3 ml whole blood were collected to assess their iron and iodine status for biochemical estimations.

4.1.3.1. Iron status assessment

Hemoglobin from whole blood samples of the subjects was subjected to assess circulating iron status. The results revealed that mean hemoglobin level of the subjects was 9.26 ± 1.08 g/dl. This was similar to Hb levels of the subjects- enrolled during 3rd month of gestation **(Table 4.4).**

Table 4.4 Iron status of the subjects using hemoglobin as an indicator

Gestational Age	Subjects N (%)	Mean	SD	Median	95th Percentile	5th Percentile
1st Month	4 (1.56)	10.12	0.94	9.75	11.27	9.5
2nd Month	77(30.07)	9.20	1.14	9.5	11.0	6.92
3rd Month	175(68.35)	9.27	1.07	9.0	11.0	7.5
Total	256(100)	9.26	1.08	9.0	11.0	7.5

Values in parenthesis depicts percentage

It was observed that, there were 4.3% of the subjects with hemoglobin levels < 5th percentile (< -2SD) and 1.56% of the subjects with hemoglobin levels > 95th percentile (> 2SD) of the population. This indicates that, majority of the subjects hemoglobin levels were falling between the range of 2SD (7.5-11.0 g/dl).

Table 4.5: Classification of Iron deficiency anaemia (Based on Haemoglobin) (g/dl)

Severity	Cutoffs	Subjects N (%)
Normal	≥ 11	21(8.2)
Mild	10 – 10.99	65 (25.4)
Moderate	7 – 9.99	159 (62.1)
Severe	< 7	11 (4.3)

Values in parenthesis depicts percentage

Data on mean ± SD of the population revealed that, the population is in mild category of anemia **(Table 4.4).** Based on the WHO 2001 classification for hemoglobin cutoffs for anemia, only 8.2% of the subjects were observed having normal levels of hemoglobin. Mild iron deficiency was observed amongst 25.4% of the subjects and 62.1% were moderately anemic, where as severity was also observed in approximately 4% of the subjects **(Table 4.5).**

Thus it was necessary to keep close monitoring throughout gestation along with the supplementation of iron folic acid.

4.1.3.2 Iodine Status and Thyroid Hormone Profile Assessment

In population studies, iodine status is assessed by estimating urinary iodine excretion (UIE). However, during pregnancy the values of UIE may vary, this could be due to an increased glomerular filtration rate and possibly renal iodine clearance (Zimmerman M 2009). Hence, it becomes necessary to assess thyroid hormone profile as an additional indicator towards assessing circulatory iodine fluctuations in the system.

The urinary iodine levels of our study population revealed that, the population is falling into the above adequate levels (297.14µg/l) (According to WHO 2007 guidelines) **(Table 4.6)** of iodine excretion in urine. Respite these UIE levels study considered the need for assessing thyroid hormone profile during pregnancy, as an additional indicator. Serum samples were subjected to hormone assays. These metabolic hormones also contributes to the fetal development and thereby respective outcomes.

The serum levels of thyroid stimulating hormone (TSH) and free thyroxine (FT_4) are understood to change during the course of progressive stages of pregnancy. During a

normal pregnancy the changes in maternal thyroid function occurs as a balance between hormone requirements and the availability of iodine. The increase in demand may occur due to thyrotrophic action of hCG, which in turn tends to transiently elevate FT_4 level and decrease TSH at the initiation of first trimester and later TSH levels starts elevating to come to the normal levels and FT_4 begins to decrease (Glinoer, 1997).

The TSH levels decreases at the beginning of first trimester and later normalizes. (Berghout et al 1998 and Glinoer et at 1990). Similar pattern was observed in our study also, where TSH levels started elevating towards the end of 1st trimester and FT_4 levels started decreasing **(Table 4.6).**

Table 4.6: Thyroid hormone profile and iodine status of the subjects enrolled at different months of first trimester

Gestational Age	Subjects N (%)	Mean	SD	Median	95th Percentile	5th Percentile
Urinary Iodine Excretion (UI) (µg/l)						
1st Month	4 (1.56)	234.94	67.70	247.62	295.52	156.59
2nd Month	77(30.07)	303.03	187.45	296.64	485.13	98.48
3rd Month	175(68.35)	301.59	132.47	299.00	484.04	101.51
Total	256(100)	300.94	150.58	297.14	484.04	101.44
Thyroid Stimulating Hormone (TSH)					**(Normal Range : 0.00-2.49 µIU/ml)**	
1st Month	4 (1.56)	1.86	1.97	1.37	4.21	0.21
2nd Month	77(30.07)	1.93	1.42	1.69	3.90	0.18
3rd Month	175(68.35)	2.35	1.68	2.02	5.61	0.49
Total	256(100)	2.22	1.63	1.88	5.06	0.35
Free Thyroxine (FT_4)					**(Normal Range : 0.650-2.10 ng/dl)**	
1st Month	4 (1.56)	1.10	0.14	1.11	1.24	0.94
2nd Month	77(30.07)	0.87	0.19	0.87	1.18	0.58
3rd Month	175(68.35)	0.77	0.21	0.79	1.06	0.45
Total	256(100)	0.81	0.21	0.83	1.13	0.46
Total Thyroxine (TT_4) (µg/dl)						
1st Month	4 (1.56)	7.72	5.47	9.60	11.68	1.15
2nd Month	77(30.07)	10.62	2.68	10.23	15.62	6.06
3rd Month	175(68.35)	10.23	2.37	10.24	13.95	6.93
Total	256(100)	10.29	2.54	10.24	14.59	6.66

Values in parenthesis depicts percentage

FT_4 concentration during pregnancy is partly affected by inflow of iodine (cellular) and the duration of pregnancy (Springer et al 2009). In our study results, mean serum TSH of the population was observed to be 2.22 µIU/ml, FT4 was 0.81 ng/dl and TT_4 was 10.29 µg/dl. The range of ± 2SD of each thyroid analyte has been depicted as 95^{th} and 5^{th} percentile.

The TSH level rises further during second and third trimesters, moderately in iodine sufficient areas and more markedly in iodine deficient areas. Since median UI of the population reveals sufficient intake of iodine, hypothetically increase in TSH levels could be expected to be at moderate levels (within normal range) and hypothetically lower incidence of subclinical hypothyroidism (SCH) or overt hypothyroidism (OH) in the population could be expected.

During the first trimester median values for serum concentration of TSH, TT_4 and UI were observed to be 1.35µIU/ml, 10.45µg/dl and 175.6µg/l respectively in Nigeria (Ujowundu et al 2011). In our study thyroid hormones showed similar results to these values, but urinary iodine levels were observed to be higher in our population (297.13 µg/l) **(Table 4.6)** when compared to Nigerian population.

A Departmental study conducted (Nair S and Sarraju P 2008) in Vadodara city, revealed prevalence of urinary iodine insufficiency amongst pregnant women was 62.17%. This depicted a very high prevalence of iodine insufficiency after two years of USI reimplementation. However, study conducted (2011) in Vadodara city by Agarwal J and Nair S revealed prevalence of urinary iodine insufficiency to be <20%. This is suggestive of the success of our efforts towards improving salt iodization at production level and usage of iodized salt at consumer level amongst population in Vadodara city (Nair S and Joshi K 2007-2011). Considering sustained salt iodization program in the city, there were 16.79% **(Table 4.7)** subjects found on insufficient levels of urinary iodine, which is significantly lower compared to previous study (Year 2008).

Table 4.7: Classification of Iodine Deficiency (Based on UI) (µg/l)

Severity	Cutoffs	Subjects N (%)	95%CI
Insufficient	<150	43 (16.79)	12.13 - 21.45
Adequate	150 – 249	50 (19.53)	14.59 - 24.47
>than adequate	250 – 499	145 (56.64)	50.46 - 62.82
Excess	>500	6 (2.34)	0.46 – 4.22

Values in parenthesis depicts percentage

The access of iodized salt was assessed as part of the study. Based on urinary iodine levels, almost 83% population was observed consuming adequate levels of iodine from their daily diet; it might be the result of sustainable salt iodization program running successfully in the region. The availability of adequately iodized salt in the city is >71%.

4.1.4 INTERRELATION BETWEEN PARAMETERS

Baseline parameters were correlated with iodine and iron status of the subjects, **Table 4.8** reveals a significant correlation between weight with age of the pregnant women enrolled ($r=0.171$, $p<0.01$), height ($r=0.415$, $p<0.01$) and BMI ($r=0.883$,$p<0.01$).

Table 4.8: Correlation coefficient for baseline characteristics to iodine and iron status in the population

	Age	Gravida	Height	Weight	BMI	Education	Hb	UI
Age	1							
Gravida	0.523	1						
Height	0.085	0.099	1					
Weight	0.171**	0.090	0.415**	1				
BMI	0.152	0.052	-0.026	0.883**	1			
Education	-0.010	-0.167	0.048	-0.004	-0.034	1		
Hb	0.083	0.038	0.125*	0.223**	0.174**	0.139*	1	
UI	-0.063	0.049	-0.130*	-0.056	-0.005	0.007	0.075	1

*Correlation is Significant ** p<0.01 level, *p<0.05 level*

It was also observed that, hemoglobin levels showed significant positive correlation with education level of the subjects ($r=0.139$, $p<0.05$), weight ($r=0.223$, $p<0.01$), BMI ($r=0.174$, $p<0.01$) and height ($r=0.125$, $p<0.05$) of the subjects. Thus, it can be concluded that, there is a positive linear correlation between anthropometry and iron status of the subjects, which is a direct indication for need of improving nutritional status of the

subjects to achieve sufficient levels of iron (hemoglobin level) to meet iron requirements of both mothers and fetuses. A study conducted by (Skjelbkkan T 2006) also revealed a positive correlation between hemoglobin concentration and BMI of the subjects.

Table 4.9: Correlation coefficient for month of enrollment and thyroid analytes –UI

	Month of Enrollment	**TSH**	**FT_4**	**TT_4**	**Tg**	**UI**
Month of Enrollment	1					
TSH	0.131*	1				
FT_4	-0.248**	-0.178**	1			
TT_4	-0.027	-0.090	0.086	1		
Tg	-0.073	0.138	0.192*	0.047	1	
UI	0.025	-0.043	-0.010	-0.046	-0.142	1

*Correlation is Significant at the **p< 0.01 level and * p<0.05 level*

As pregnancy progresses there are circulatory level adjustments in thyroid hormones. Generally, TSH and FT_4 levels fluctuate very drastically during pregnancy, which was also observed at the time of enrollment. Subjects enrolled varied from 1^{st}, 2^{nd} or 3^{rd} month of their pregnancies. As per spearman's correlation coefficient, month of gestation in first trimester gave the significant negative correlation ($r= -0.248$, $p<0.01$) with FT_4 levels and positive correlation with ($r= 0.131$, $p<0.05$) TSH levels. This depicted normal physiological fluctions at the time of initial stage of gestation. This was also proved by TSH and FT_4 levels indicating reciprocal pattern of relation($r= - 0.178$, $p<0.01$) **(Table 4.9).**

Other than iron status, BMI also correlated with TSH. It was observed that TSH correlated negatively with BMI ($r= -.143$, $p<0.05$). On the contrary, studies conducted by (Figueroa et al 2008) and (Knudsen N et al 2005) revealed a significant ($p<0.001$) positive relationship between BMI and TSH levels amongst obese population. Our study findings could have been due to lower to normal ranges of BMI amongst the subjects and the pregnancy induced thyroidal hormones adjustments. Thus, there could be many factors affecting thyroidal economy and thus the inverse relation could have been amongst our study population.

4.1.5 SUBSAMPLING OF THE POPULATION

Out of total 256 subjects, the subjects who were to migrate to other places for delivery or different hospital at a longer distance from Vadodara city, were excluded to maintain consistency and reduce drop outs towards the end of the study. Owing to drop out rate due to other reasons like abortion, miscarriages, missing visits during pregnancy, unwillingness to participate and non-reachability. Thus, the number of the study subjects was one hundred and fifty (N=150).

4.1.5.1 General profile of the subjects (N=150)

Baseline characteristics of these subjects was also analyzed to assess the distribution pattern, as well as to maintain consistency while choosing a subsample subsistent population. Thus, ensuring comparable characters of the subsamples (N=150).

Majority of the subjects were residing in urban or periurban areas. Further analysis on type of family revealed that, 80.2% of the subjects were living in joint families and 19.8% belonged to nuclear families. Distribution of the subjects based on their regions, revealed that, 67.33% of the subjects were Hindus and 32.66% were Muslims. However, literacy level assessment revealed that, majority of the subjects had attended schools up to primary (41.33%) and secondary (38%), where as two extremes in the classification named illiteracy and higher secondary education shared 10% of the population each.

Majority of the subjects were homemakers (98.66%) and 1.33% were employed as laborers or servants. Analysis on their monthly income revealed that, 62.8% had monthly income < 5000/- and 37.2% had >5000/-. With respect to monthly income, 55.4% of the subjects were found belonging to LIG and 14% of the families had per capita income $\leq$ 750/-. With reference to all baseline characteristics and socio-economic aspects, sub sampled group is representative of the entire population enrolled. **(Table 4.10)**

Table 4.10 General Profile of the subjects

Details	Subjects (N=150)	95% CI
Area of Residence		
Urban	137(91.33)	86.73 - 95.93
Rural	13 (8.66)	4.06 - 13.26
Religion		
Hindu	101 (67.33)	59.67 - 74.99
Muslim	49 (32.66)	25.0 - 40.32
Education		
Illiterate	15 (10)	5.1 - 14.9
Primary	62 (41.33)	33.26 - 49.34
Secondary	57 (38)	30.08 - 45.92
Higher Secondary	15 (10)	5.1 - 14.9
Graduate	1 (0.66)	-0.66 - 1.98
Occupation		
Working	2 (1.33)	-0.53 - 3.19
Housewife	148 (98.66)	96.8 - 100.52
Type of Family		
Joint	97(80.2)	73.7 - 86.7
Nuclear	24(19.8)	13.3 - 26.3
No. of Family members		
2-5	87 (71.9)	64.56 - 79.24
>5-9	28 (23.1)	16.22 - 29.98
$\geq$10	6 (5.0)	1.44 - 8.56
Monthly income		
<5000	76 (62.8)	54.9 - 70.7
$\geq$5000	45 (37.2)	29.3 - 45.1
Income Group		
LIG	67 (55.4)	47.28 - 63.52
MIG	54 (44.6)	36.48 - 52.72
Per Capita Income		
$\leq$ 750	17 (14)	8.34 - 19.66
> 750	104 (86)	80.34 - 91.66

Values in parenthesis depicts percentage

4.1.5.2 NUTRITIONAL STATUS ASSESSMENT

Mean age, height and weight of the subjects were 23.35± 3.54 years, 150.63± 5.51 cm and 45.58± 8.91 kg respectively. Incidence of having history of abortion was observed to be 0.2. **(Table 4.11)**

Table 4.11 Baseline Characteristics of the subjects

Characteristics	Subjects (N=150)			95% CI
	Mean	SD	Median	
Age (Yrs.)	23.35	3.54	22.5	22.78 - 23.92
Height (Cms)	150.63	5.51	150.5	149.75 – 151.51
Weight (Kg)	45.58	8.91	45	44.15 – 47.01
Parity	0.8	0.8	1	0.67 – 0.93
Abortion	0.2	0.4	0	0.17 – 0.23

Percent distribution of BMI of the subsampled population is depicted in **Table 4.12.** It was observed that, the subjects enrolled during 2nd and 3rd month, had similar percentages (8%) in the overweight category, where as majority of the subjects were falling into normal category, in our total population (n=256). One third of the subjects were falling into undernourished category **(Table 4.12).**

Mean BMI was falling into the normal category of the classification **(WHO 2004)**, which ranged from 19.54 to 20.60 kg/m^2 **(Table 4.12).**

Table 4.12 Distribution of subjects with respect to BMI

Gestational Age	Subjects N (%)	Level of BMI (Kg/m^2)			Mean ± SD	95% CI
		<18.5 (UW)	18.5-25 (Nor)	>25 (OW)		
2nd Month	25(16.66)	8 (32)	15(60)	2(8)	20.41 ± 3.17	19.17 – 21.65
3rd month	125(83.33)	8 (32)	70(56)	10(8)	20.00 ± 3.37	19.41 – 20.59
Total	150(100)	53(35.33)	85(56.66)	12(8)	20.07 ± 3.33	19.54 – 20.60

Values in parenthesis depicts percentage

UW- Underweight, Nor- Normal weight, OW- overweight according to WHO 2004 classification.

4.1.5.3 MICRONUTRIENT STATUS ASSESSMENT

(A) Iron status assessment

Hemoglobin levels of the sub sampled group (n=150) depicted that, majority of the subjects were mildly iron deficiency anemic with mean hemoglobin level at 9.38 ± 1.04 g/dl, irrespective of the month of enrollment.**(Table 4.13).** 95^{th} percentile and 5^{th} percentile depicts the mean hemoglobin range for 2SD on both the ends of the distribution.

Table 4.13 Iron status of the subjects using hemoglobin as an indicator

Gestational Age	Subjects N (%)	Mean	SD	Median	95^{th} Percentile	5^{th} Percentile
2^{nd} Month	25 (16.66)	9.63	1.03	10	11	8.0
3^{rd} Month	125 (83.33)	9.34	1.05	9	11	7.6
Total	150 (100)	9.38	1.04	9.5	11	7.72

Values in parenthesis depicts percentage

Table 4.14: Classification of iron deficiency anemia among the subjects

Category	Standard range cutoffs	Subjects N (%) (N=150)
Normal	$\geq$ 11	16 (10.66)
Mild	10 – 10.99	41 (27.33)
Moderate	7 – 9.99	89 (59.3)
Severe	< 7	4 (2.7)

Values in parenthesis depicts percentage

Based on the classification (WHO 2001) for anemia, it was observed that majority (59.3%) **(Table 4.14)** of the subjects were moderately iron deficient. The prevalence is similar to that of enrolled population (n=256). Thus, justifying for the representative samples.

(B) Iodine status and thyroid hormone profile assessment

Observed median urinary iodine level was 344.43µg/l, which indicates sufficient iodine intake by the subjects. Median urinary iodine levels ranged between 108.15 – 491.39 µg/l **(Table 4.15).** A review study conducted by (Yadav K, Srivastav R., S Badhal et al 2011) depicted median urinary iodine levels of pregnant women in different states of India ranges from 95-178 µg/l, which is comparatively lower than our study results.

Table 4.15: Iodine status of the subjects using UIE as an indicator

Gestational Age	Subjects N (%)	Mean	SD	Median	95th Percentile	5th Percentile
2nd Month	25 (16.66)	383.12	272.02	351.51	488.86	113.41
3rd Month	125 (83.33)	320.82	135.00	342.61	491.39	107.84
Total	150 (100)	331.20	166.11	344.43	491.39	108.15

Based on WHO classification (2007) iodine deficiency revealed that, 14% **(Table 4.16)** of the subjects had insufficient levels of urinary iodine (<150 µg/l). However, majority of the subjects were on the sufficient levels of urinary iodine. A review study conducted by (Yadav K, Srivastav R., S Badhal et al 2011) revealed prevalence of iodine deficiency based on UI levels ranging from 44.4%-95%. However, our study results revealed comparatively lower prevalence. This could be due to successful operation of salt iodization program by 2007 (Nair S and Joshi K 2007- unpublished data) and availability of adequately iodized salt in the region indicating sustainability of program indicators in place (Joshi K and Nair S 2008-2011- unpublished data).

Table 4.16 Classification of iodine deficiency among pregnant women

Severity	Standard range cutoffs	Subjects N (%) (N=150)	95%CI
Insufficient	<150	21 (14)	8.34 – 19.66
Adequate	150 – 249	31 (20.66)	14.05 – 27.27
>than adequate	250 – 499	87 (58)	49.94 – 66.05
Excess	>500	11 (7.33)	3.09 – 11.57

Values in parenthesis depicts percentage

(Table 4.17) Thyroid hormone profile of the subsampled group (N=150) again revealed a similar reciprocal pattern for the levels of TSH and $FT_{4,}$ where TSH increased with the progression of gestation and FT_4 reduction was observed with the progression of gestation and then stabilization of the hormones during normal pregnancy.

One of the initial biochemical signs of poor iodine nutrition is less than normal serum TSH concentration. (Smith & Bold 2005). Based on TSH values at 1st trimester 81% had normal thyroid status indicating optimal iodine nutrition in pregnancy (Ujowundu et al 2011). In our study 62.77 % of the subjects had normal TSH values (01.-2.5 µU/ml) which revealed adequate iodine nutrition, required for optimal thyroid function of the subjects.

Table 4.17 Thyroid hormone profile of the subjects enrolled at different point in first trimester

Gestational Age	Subjects N (%)	Mean	SD	Median	95th Percentile	5th Percentile
Thyroid Stimulating Hormone (TSH)				**(Normal range : 0-2.49 µIU/ml)**		
2nd Month	25 (16.66)	1.73	1.85	1.22	3.63	0.11
3rd Month	125 (83.33)	2.27	1.47	1.99	5.21	0.47
Total	150 (100)	2.18	1.55	1.86	5.16	0.22
Free Thyroxine (FT_4)				**(Normal Range : 0.650-2.10 ng/dl)**		
2nd Month	25 (16.66)	0.88	0.23	0.86	1.21	0.50
3rd Month	125 (83.33)	0.76	0.22	0.80	1.06	0.44
Total	150 (100)	0.78	0.22	0.81	1.09	0.45
Total Thyroxine (TT_4) (µg/dl)						
2nd Month	25 (16.66)	10.35	2.75	9.94	15.77	6.09
3rd Month	125 (83.33)	9.93	2.29	10.18	13.07	6.88
Total	150 (100)	10.00	2.37	10.15	13.49	6.67

Values in parenthesis depicts percentage

The range of normal serum TT_4 is modified during pregnancy under the influence of a rapid increase in serum TBG levels. Hence, if TT_4 is used for estimation of thyroid

functions, it is therefore reasonable to adapt the non pregnant reference range (5-12 µg/dl) by multiplying this range with 1.5 (i.e. 7.5 – 18 µg/dl) during pregnancy. (Soldin et al 2004; Kahric-Janicic et al 2007; Demers LM and Spencers CA 2009). Thus, it can be reasoned as pointed by the above authors, during pregnancy TT_4 increases with the progression of gestational age and sometimes may increase 2-3 folds. Considering the justification these authors contributed to adaptation of specific ranges. These reference ranges were used as a standard approach to compare the total thyroxin levels. Based on these standards, our subjects had normal levels of TT_4.

Thus from the data, it is clearly evinced that homogeneity of the population was maintained barring all confounding factors which brought a unique cohesive pattern in the study. The results of the subsampled group (n=150) represented close similarities in range values to that of the population enrolled (n=256). Hence, the justification that, these subjects could be considered as representative samples of the population.

4.2 LONGITUDINAL PHASE WITH INTERVENTIONS AND MONITORING

4.2.1 DISTRIBUTION OF SUBJECTS INTO EXPERIMENTAL AND CONTROL GROUPS

The subgrouped population was randomly divided into experimental (n=75) and control group (n=75). Both the groups were matched for month of enrollment and level of hemoglobin.

4.2.2 INTERVENTIONAL STRATEGIES

(A) Double Fortified Salt Supplementation

During the study phase, to achieve the desired objective of assessing efficacy of DFS on improving iron and iodine status of the subjects, two interventional strategies were implemented to match the population except supplementation. Experimental group was supplemented with double fortified salt (NIN formula) till the time of delivery and control group was effectively communicated to consume iodized salt and the supply was ensured assessing household salt samples of the control group.

(B) Nutrition Health Education

The sessions for NHE and BCC were delivered based on the details of knowledge; attitude and practices (KAP) availed at the time of enrollment using semi structured and pretested questionnaires. Data on KAP revealed many of the aspects to be focused towards improving iodine and iron nutrition especially, including cooking practices, storage practices and dietary consumption- combination practices. Nutrition health education to both the groups was provided equally and effectively to improve on their dietary intake.

4.2.2.1 DOUBLE FORTIFIED SALT AS A SUPPLEMENT

NIN has developed a formula using SHMP as a stabilizer and Ferrous sulphate as a source of iron in the salt. The composition of premix for DFS is provided in **(Table 4.18)**

Table 4.18: Composition of Double Fortified salt

Ingredients	Amount
Common Salt	1000 gm
Ferrous Sulfate Heptahydrate	1000 ppm
Sodium Hexametaphosphate (SHMP)	10 g
Potassium iodate	40 ppm

Double fortified salt which was supplemented amongst experimental group was purchased from one of the salt production unit in Gujarat. It was produced based on the technical formula developed by NIN for double fortified salt into two batches.

At production base iodine and iron contents of DFS were estimated at the laboratory by titrimetric method provided by BIS (Bureau of Indian standards). The content has been mentioned in **Table 4.19**.

Table 4.19 Content estimation of Double fortified salt

Sr.No	Parameters	Unit	Standard	Batch 1	Batch 2
1	Iodine (I_2)	ppm	Min. 30	35	36
2	Iron (Fe)	ppm	Min. 850	1150	1050

Double fortified salt was supplemented amongst experimental group, from the time of enrollment till the end of gestation (7 months). Control group subjects were counseled for iodized salt consumption and nutrition health education was imparted towards the achievement of healthy dietary practices in a motivational way to improve on their nutritional aspects.

Table 4.20: Stability of iodine and iron in double fortified salt (In house quality check)

Contents	Levels
Initial	
Iodine	40 ppm
Iron	1050 ppm
After 1 year	
Iodine	37.5 ppm
Iron	979 ppm

At the time of purchase of salt, quality of the salt was also tested using BIS method and iron-iodine contents were tested for their stability at after one year also. **(Table 4.20)**

Since, DFS was to be supplemented for consumption throughout gestation as well as by entire family, it was essential to assess the acceptability of DFS by the subjects and their families. Initially subjects, who observed colour change in the food preparation due to interaction of iron, were provided complete information regarding the usage of DFS while cooking. They were advised to add salt at a later stage of the preparation than adapting general method to reduce cooking time for DFS, which would reduce the possibilities of colour change. On adoption of recommended changes while cooking, the acceptability of DFS increased significantly among subjects and their families.

Assessment of acceptability of DFS **(Table 4.21)** by the subjects and families at baseline revealed that, the acceptability was 95.5% and 82.1% respectively. However, 70.1% of the subjects observed colour change while cooking and majorly (43.3%), it was black. Further, counseling and BCC while on DFS usage practices while cooking was provided, which increased the acceptability of DFS significantly with decrease in the incidence of observed colour change with the progression of the study.

Table 4.21: Acceptability of Double Fortified Salt at baseline

Characteristics	Experimental Group
1. Any color change in cooked food	
• Yes	70.1
• No	29.9
2. Colour Change observed	
• Black	43.3
• Dark Green	10.4
• Brown	23.9
• Yellow	14.9
3. Acceptability by,	
• Subject	95.5
• Family	82.1

Of the one hundred fifty subjects (n=150), one hundred twenty one (n=121) could complete the study due to further drop outs owing to migration to maternal place for delivery, non reachability or false contact details; missing visits to the hospital; abortions or premature deliveries; family's influence on the withdrawal; deliveries in other hospitals, home deliveries, deliveries on odd timings or on the way to hospitals etc. Of these remaining 121 subjects, n=67 in experimental and n=54 in control group could complete the study successfully.

4.3 IMPACT ASSESSMENT OF INTERVENTIONS ON THE SUBJECTS WITH COMPLETE THREE TRIMESTER DATA (N=121)

4.3.1 IMPACT ASSESSMENT ON NUTRITIONAL STATUS OF THE SUBJECTS

The supplemented group of subjects showed mean age and height as 23.17± 3.4 years and 151.4 ± 5.12 cms. Mean weight observed was 45.34 ± 7.4 kg, which also depicted prevalence of under nutrition amongst 35.80% (BMI <18.5 kg/m^2) of the subjects at the time of enrollment. There were 58.20% of the subjects with normal BMI (18.5-24.99 kg/m^2) and 6% were overweight ($\geq$ 25 kg/m^2) in experimental group. Percent distribution in control group was almost similar to the experimental group **(Table 4.22)** depicting non significant difference between both the groups at all the three trimesters.

Table 4.22: Percent distribution of subjects according to BMI classification in all three trimesters

Categories	Percent subjects			Chi Square
	Experimental (n=67)	Control (n=54)	Total (N=121)	*Experimental v/s Control*
1st trimester				
<18.5	24 (35.8)	21 (38.9)	45 (37.2)	0.27^{NS}
18.5- 25	39 (58.2)	29 (53.7)	68 (56.2)	
>25	4 (6)	4 (7.4)	8 (6.6)	
2nd trimester				
<18.5	19 (28.4)	16 (29.6)	35 (28.9)	0.04^{NS}
18.5- 25	41 (61.2)	32 (59.3)	73 (60.3)	
>25	7 (10.4)	6 (11.1)	13 (10.7)	
3rd trimester				
<18.5	7 (10.4)	10 (18.5)	17 (14)	2.77^{NS}
18.5- 25	49 (73.1)	32 (59.3)	81 (66.9)	
>25	11 (16.4)	12 (22.2)	23 (19)	

Values in parenthesis depicts percentage

Table 4.23: Mean BMI of the subjects according to the classification

trimester	BMI [Weight(kg)/Height(meter^2)] (Mean ± SD)			
	Experimental (n=67)	Control (n=54)	Total (N=121)	't' value *Experimental v/s Control*
1st trimester	19.75 ± 2.95	20.08 ± 3.46	19.90 ± 3.18	0.583^{NS}
2nd trimester	20.68 ± 2.96	21.02 ± 3.45	20.83 ± 3.18	0.562^{NS}
3rd trimester	21.98 ± 3.00	22.31 ± 3.45	22.13 ± 3.20	0.566^{NS}

Mean BMI of the subjects in all three trimesters based on classification is depicted in **(Table 4.23).** There was no significant difference observed between both the groups for all three categories of BMI during all three trimesters, since nutrition health education was provided to all the subjects irrespective of their group.

It is a proven fact that, lower weight before pregnancy and inadequate weight gain during pregnancy are significantly correlated with low birth weight of the neonates (Leader et al, 1981 and Neggers et al, 2003). Lower pre pregnancy BMI and inadequate weight gain during pregnancy are risk factors for LBW, prematurity and small for gestational age.

In our study, more than one third of the subjects required to improve on their nutritional status during their gestational period to prevent compromised nutrition and damage to fetus. Bearing these consequences in mind, the experimental group was also considered for providing nutrition health education (NHE) and behavioral change communication (BCC) sessions along with the supplementation.

4.3.2 IMPACT ASSESSMENT ON MICRONUTRIENT STATUS

4.3.2.1 Impact assessment on Iron Status of the subjects

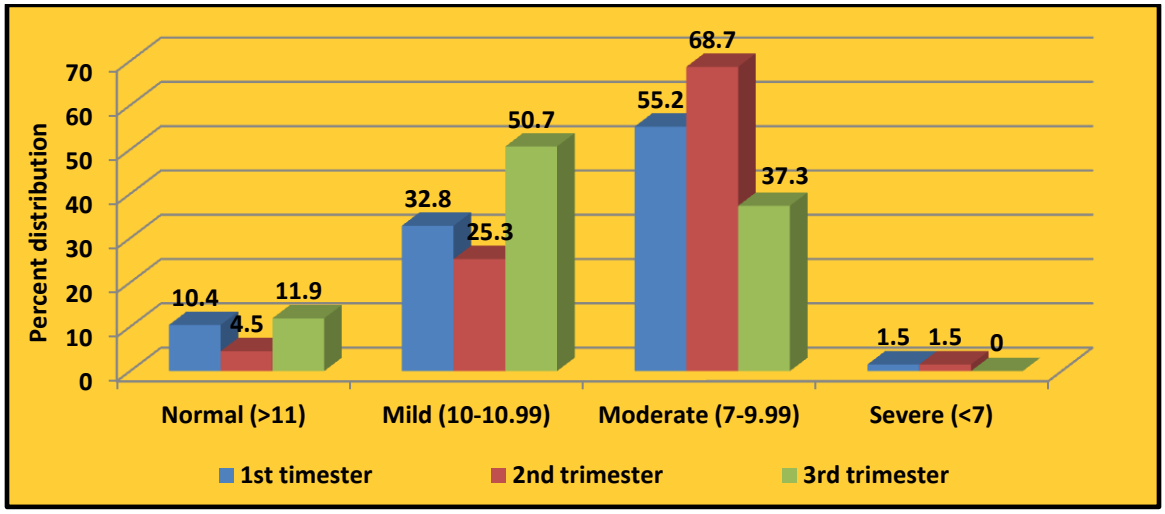
Majority of the subjects were anemic, distributed into categories for anemia from both the study groups. There were 89.6% anemic subjects in experimental and 85.2% amongst control group. Towards the end of the phase, the results were motivating amongst experimental group. There was a positive shift with 10% reduction from moderate levels to mild levels of anemia over the period of gestation (1^{st} trimester to 3^{rd} trimester) amongst DFS supplemented (experimental) group.

During second trimester, a general reduction in hemoglobin level occurs due to hemo dilution with the progression of gestational age. Hence, according to WHO classification 1998, standard cutoffs for 2^{nd} trimester has been set lower than the 1^{st} trimester and 3^{rd} trimester (Shobeiri F., Begum K., Nazari M 2006). However, there is no trimesterwise difference in cutoffs by WHO 2001. Following the WHO 2001 classification, 6% of normal subjects shifted to anemic category in second trimester. However, towards the end in the prevalence of anemia in each category of the classification decreased in

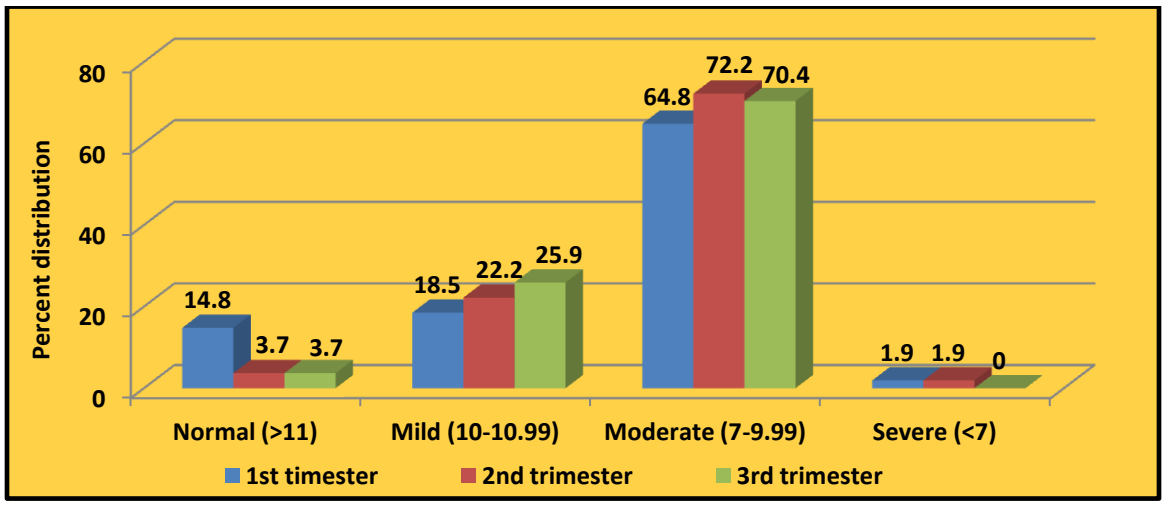
experimental group. Thus it can be stated that the situation reverted back with improvement in hemoglobin levels during third trimester.

Figure 4.1: Distribution of the subjects according to categories of anemia

(A) Experimental Group



(B) Control Group



It was very motivating to observe in experimental group that, the percentage of normal subjects improved from 10.4% at enrollment to 11.9% towards the end of gestation. There was a positive shift in the levels of anemia, 17.9% (55.2% to 37.3%) of moderately

anemic subjects shifted to mild or normal category in experimental group. Since the values shifted towards right, there was an improvement in the percentages of mildly deficient subjects from 32.8% to 50.7% **(Figure 4.1 A).**

In control group, with progression of gestation, 11.1% normal subjects shifted to anemic category till the end of gestation. Average >60% of the subjects remained moderately anemic throughout gestation and app. 20% remained moderately anemic **(Figure 4.1 B).**

Various studies have showed improvement in iron status using DFS, but impact assessment of DFS supplementation during pregnancy has not been reported, except one study by NIN 2001. Thus this study aimed in meaningful contribution on the usage of DFS during pregnancy. From our results there is a clear cut demarcation that, the experimental group improved in their iron status by 1.5% in the population. This is indicative that, there was a sufficient circulating pool of iron available for the mother and baby to combat with their daily physiological needs. This could be attributed due to sustained release of iron from DFS. Owing to the simultaneous reduction observed - 11.1% in the population of the control group, our results reflect the role and contribution of DFS during pregnancy.

This improved iron status in our study is suggestive of efficacy of DFS in improving iron status amongst the deficient subjects since there is a significant improvement in hemoglobin levels of the supplemented group compared to control group. It is important to note that, both the groups were on Iron-Folic acid (60 mg elemental iron) supplements under the government scheme, minimum for 120 days.

During first trimester there is only a small increase in iron requirement, which can be met by the cessation of menses (Gambling L et al 2011). However, when maternal blood volume expands and the fetus grows the need for iron increases. It was also concluded by (Bothwell et al 2000) that, iron balance in pregnancy can be maintained only when there are adequate stores (need to be ≈ 300 mg), if the mother continues to consume a diet with bioavailable iron. However, more iron stores will be required if the diet is suboptimal. In our study subjects, who belonged to lower strata of the community- irrespective of urban

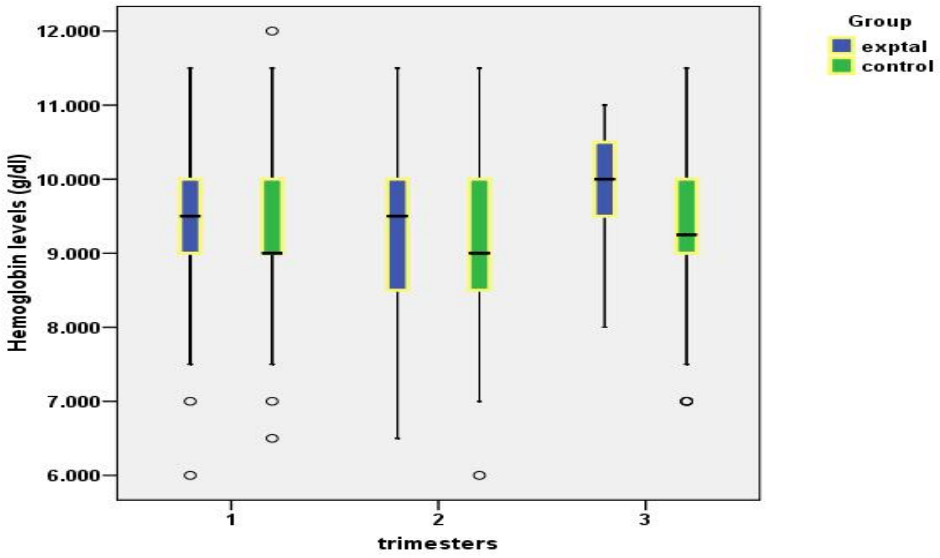
or rural localities, the dietary pattern lacks in meeting iron requirement during normal conditions and thus leading to obvious dietary deficit during pregnancy.

Table 4.24: Overview of iron deficiency anemia classification amongst subjects (N=121)

	Severe	Moderate	Mild	Normal
1st trimester	2 (1.7)	72 (59.5)	32 (26.4)	15 (12.4)
2nd trimester	2 (1.7)	85 (70.2)	29 (24.0)	5 (4.1)
3rd trimester	-	63 (52.1)	48(39.7)	10 (8.3)

Values in parenthesis depicts percentage

Figure 4.2: Comparison of haemoglobin levels between both groups in all three trimester



On the whole, the population reflected that, 75.2% of the subjects were mildly anemic towards the end of gestation **(Table 4.24)**. The effect was reflected on hemoglobin levels and nutritional status of the subjects from first trimester, indicating their pre pregnancy status. **Figure 4.2** reflects variations in mean hemoglobin levels of the subjects of both the groups along with the standard error bars to depict the lower and upper ranges. Observed mean hemoglobin level itself was falling into moderate category of anemia according to WHO (2001) classification. Hence, it is necessary to provide awareness regarding impact of maternal hemoglobin levels during gestation on fetal outcome. Dietary approaches along with IFA supplementation may prove to be an effective

strategy. Hence, a food item which is easily consumable, affordable and part of traditional food items with universal consumption, like salt- with iodine and iron fortification was used as an effective tool**.**

Table 4.25: Hemoglobin profile of the subjects for all three trimesters

Stage of Gestation	Group	Hemoglobin Levels (g/dl)		95% CI
		Mean ± SD	Median	
1st Trimester	**Experimental**	9.44 ± 1.07	**9.5**	9.18 – 9.7
2nd Trimester		9.18 ± 1.03	**9.5**	8.93 – 9.43
3rd Trimester		9.86 ± 0.76***†††	**10.0**	9.61 – 10.11
1st Trimester	**Control**	9.35 ±1.10	**9.0**	9.06 – 9.64
2nd Trimester		9.00 ± 1.06*	**9.0**	8.72 – 9.28
3rd Trimester		9.15 ± 0.95$^{£££}$	**9.25**	8.90 – 9.40
1st Trimester	**Total**	9.40± 1.08	**9.5**	9.21 – 9.59
2nd Trimester		9.10 ±1.05**	**9.0**	8.91 – 9.29
3rd Trimester		9.54 ±0.92†††	**9.5**	9.38 – 9.70

**p<0.05, ** p<0.01, *** p <0.001 v/s 1st trimester and † p <0.05, ††† p <0.002 v/s 2nd trimester, $^{£££}$p<0.001 v/s 3rd trimester conc. difference between experimental and control group.*

There was no significant difference observed in mean Hb between both the groups at the time of enrollment. Towards the end of gestation, the results were in favor of supplemented group. It was observed that there was a significant ($p<0.001$) improvement in hemoglobin conc. of DFS supplemented (experimental group) subjects. However, in control group, hemoglobin conc. decreased significantly ($p<0.05$) in second trimester compared to first trimester and did not improve significantly towards the end. **(Table 4.25)**

Comparison between first and second trimester mean hemoglobin level, revealed non significant reduction in experimental group ($p<0.072$), where as in control group the reduction was significant ($p<0.05$). These results indicated that, there might have been sustained release of iron in the gut of supplemented group after consumption of DFS on a regular dietary basis. Towards the end of gestation, improvement in mean hemoglobin levels of both the groups was observed compared to second trimester. This rise was significantly higher in experimental group when compared with first trimester ($p<0.001$) and second trimester ($p<0.001$). As per normal iron turn over during pregnancy, when first and second trimester hemoglobin levels were compared for total subjects (n=121),

significant reduction ($p<0.01$) was observed. On comparing mean hemoglobin level of third trimester with second trimester, higher level of significant improvement was observed. ($p< 0.001$) where as the rise was not significant compared to first trimester. **(Table 4.25)**

While comparing mean hemoglobin levels of both the groups (experimental and control) at each time point, there was no significant difference during first and second trimester hemoglobin conc. (Independent "t" test). However, third trimester results showed higher degree of significant difference between both the groups ($p<0.0001$)

The only data available on DFS supplementation amongst pregnant women in India by (Shivkumar B, Brahmam GNV, Nair M et al 2001) revealed no difference in hemoglobin conc. of the experimental and control group after 2 years of supplementation of DFS in tribal villages of Andhra Pradesh. This population was rice based diet. However, in our study a significant difference was observed between both the groups at the end. Our population was on wheat based diet. Another study conducted by NIN (Report of working group on fortification of salt with iron 1982) in 4 centers of India on effect of Iron fortified salt supplementation on the hemoglobin concentration included pregnant women as subjects but separate data of pregnant women has not been presented. Data of reproductive age group of females thus may bring an over or under estimated effect.

4.3.2.2 Impact assessment on Iodine status of the subjects

Urinary iodine excretion pattern of first trimester data revealed that, 86% of the subjects had sufficient levels of iodine nutrition in both the groups, where as 14% subjects were still to meet their daily iodine requirement for themselves and hence it is also expected that there might have been a compromise for the fetal iodine nutrition **(Table 4.26).**

Table 4.26: Overview of urinary iodine classification amongst subjects (N=121)

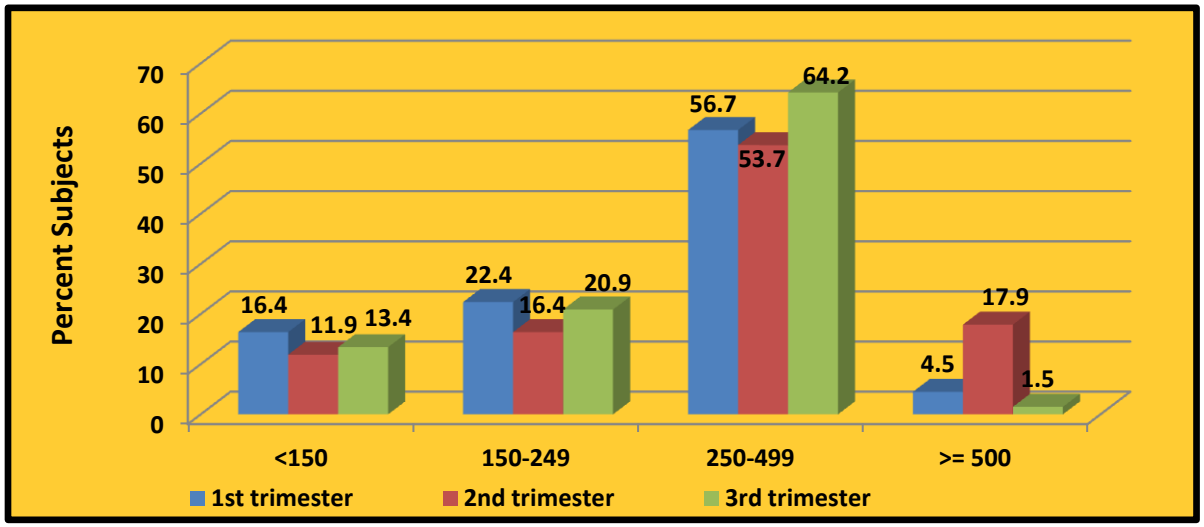
	<150 µg/l	**150-249 µg/l**	**250-499 µg/l**	**≥ 500 µg/l**
1st trimester	17 (14)	26 (21.5)	70 (57.9)	8 (6.6)
2nd trimester	13 (10.7)	23 (19)	71 (58.7)	14 (11.6)
3rd trimester	17 (14)	28 (23.1)	71 (58.7)	5 (4.1)

Values in the parenthesis depicts percentage

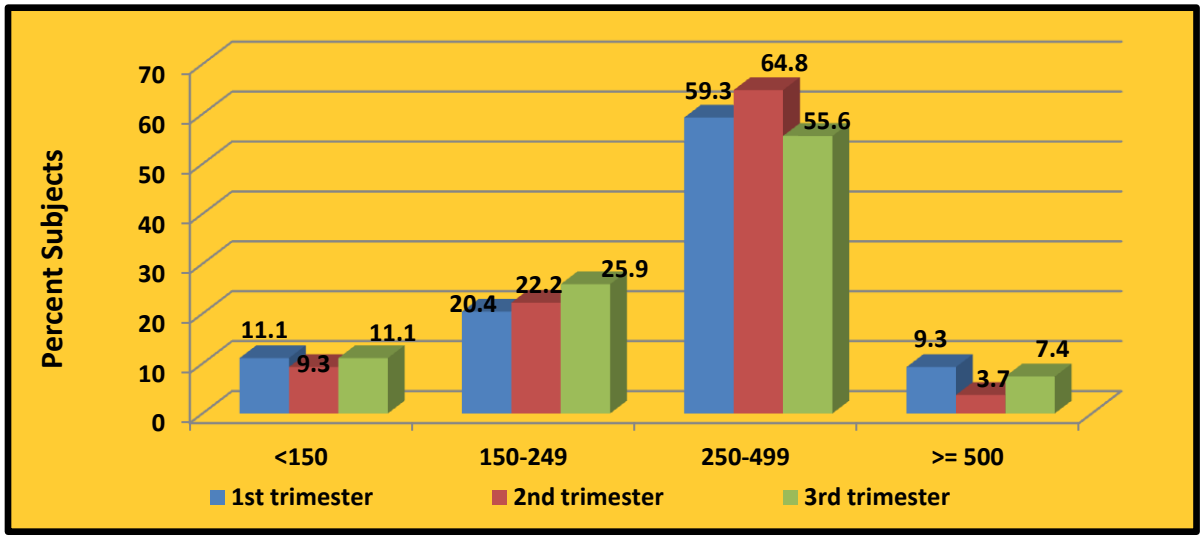
During second trimester the percent prevalence of overall iodine insufficiency reduced by 4.5% in experimental and 1.8% in control group. **(Figure 4.3-A & B)**. Since both groups were consuming sufficiently iodized salt, on comparing medians for urinary iodine values amongst both the groups, the difference was non significant for all three trimesters. Similar study reported by (Azizi F et al 2003) reported supportive evidence to our study results indicating no significance difference in UI between three trimesters of pregnancy in women residing in iodine sufficient region. This is reflective indication that, population is availing adequately iodized salt in the region.

Figure 4.3: Distribution of subjects based on urinary iodine excretion for all three trimesters

(A) Experimental Group



(B) Control Group



Urinary iodine excretion during the course of pregnancy has been presented in (**Table 4.27)**. Reports by (Ardawi, Nasrat and Mustafa 2002) revealed increased UI in pregnancy compared to non pregnant women but the level decreased during second and third trimester compared to first trimester. A supplementation study carried out by (Romano et al 1991) among the Italian pregnant women (median UI: 31-37 µg/l) with supplemented 120-180 µg iodine as iodized salt and reported threefold increase in UI.

Table 4.27: Urinary Iodine concentration of the subjects for all three trimester

Stage of Gestation	Group	UI (µg/l)		5th Percentile	95th Percentile
		Mean	Median		
1st trimester	**Experimental**	312.17	278.60	105.30	489.74
2nd trimester		437.48	347.60*	123.56	960.50
3rd trimester		314.89	299.01†	121.37	492.70
1st trimester	**Control**	404.15	376.59NS	131.95	918.01
2nd trimester		327.14	314.68 NS	116.94	492.19
3rd trimester		329.14	288.66* NS	106.61	783.00
1st trimester	**Total**	353.22	329.00	108.99	563.52
2nd trimester		388.35	333.20	122.61	805.00
3rd trimester		321.33	294.56†	116.00	499.06

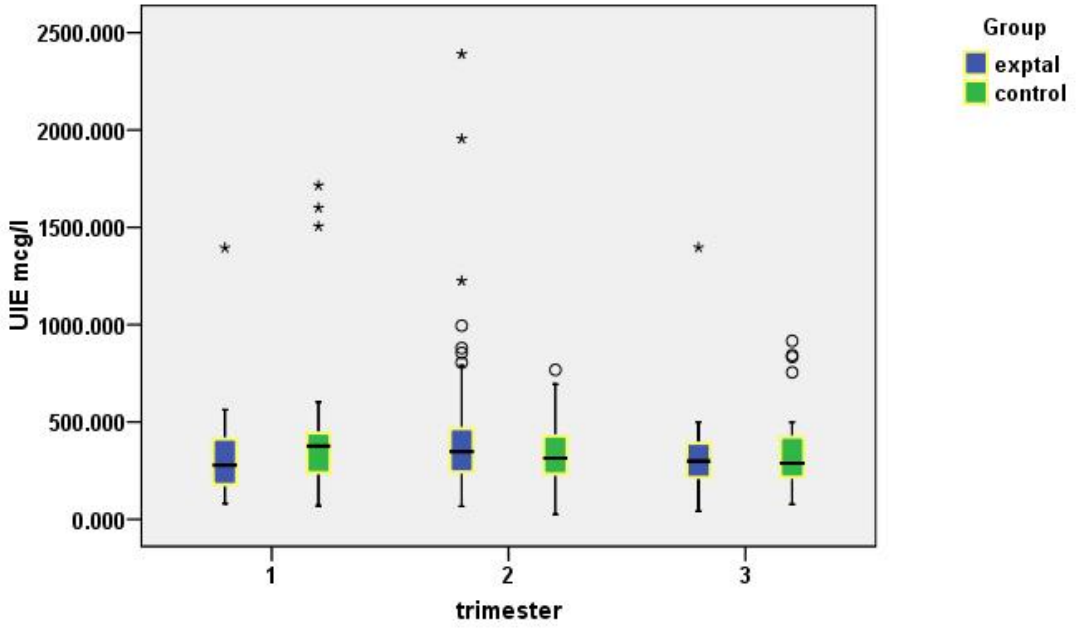
*$p<0.05$ *vs* 1st trimester and † $p<0.05$ *vs* 2nd trimester, NS$p>0.05$, no significant difference between experimental and control group at each trimester

Our study results **(Table 4.27)** based on median urinary iodine levels, indicated iodine sufficiency amongst majority of the subjects. Median levels revealed 278.60 µg/l, 376.59 µg/l and 329 µg/l respectively in experimental, control and overall subjects in first trimester. Non parametric "t" test between trimester wise UI of experimental group revealed significant ($p<0.05$) improvement during second trimester compared to first trimester. However, UI reduced non significantly during second trimester and significantly ($p<0.05$) during third trimester compared to first trimester in control group. Overall, significant reduction in UI was observed compared to second trimester ($p<0.05$).

Our results were supported by (Pedersen et al 1993, Liesenkotter et al 1996, Nohr et al 2000, Antonangeli et al 2002, Glinoer et al 1995), whose findings indicated higher levels of UI in supplemented group compared to control group. In our study groups also, experimental group (supplemented with DFS) indicated higher conc. of UI during

supplementation phase (2nd and 3rd trimester) compared to control group, although the difference was non significant between both the groups at each time point.

Figure 4.4: Comparison of urinary iodine excretion level between both groups in all three trimesters



This difference could have been due to differences in the strategy to emphasis on iodine consumption. Both the groups were imparted NHE for iodine nutrition but experimental group was supplemented with DFS which was optimally iodized salt where as control group was advocated to consume iodized salt, which might have been the reason for the difference in UI between both the groups, respite majority of the subjects (83%) being iodine sufficient.

Figure 4.4 depicts trimester wise comparison between both the groups, where outliers and extreme values were observed to be highest during second trimester in experimental group. However, during second and third trimester control group showed more variations.

4.3.2.3 Impact assessment on Thyroid analytes

Data on comparison of thyroid function tests between experimental and control group has been depicted in **Table 4.28.** When the supplemented group versus non supplemented

group were compared, no significant difference in values of mean, median levels of TSH, FT_4 and TT_4 were observed in our study.

Table 4.28: Thyroid hormone levels of the subjects throughout gestation

Parameters	Trimester	Mean ± SD	Median	5th Percentile	95th Percentile
Experimental group					
TSH	**1st Trimester**	2.07 ± 1.28	1.92	0.58	4.14
	2nd rimester	2.25 ± 1.05*	2.19	0.86	4.21
	3rd Trimester	2.39 ± 1.21**	2.07	1.01	4.75
FT_4	**1st Trimester**	0.81 ± 0.25	0.84	0.43	1.19
	2nd rimester	0.84 ± 0.18	0.85	0.60	1.04
	3rd Trimester	0.89 ± 1.14	0.80	0.51	1.00
TT_4	**1st Trimester**	10.14 ± 2.10	10.07	7.37	13.25
	2ndTrimester	10.73 ± 1.64*	10.70	8.32	14.48
	3rd Trimester	11.43 ± 1.85†††***	11.46	8.48	14.48
Tg	**1st Trimester**	4.22 ± 5.78	2.50	0.00	16.02
	2ndTrimester	14.97± 35.03**	3.96	0.00	71.26
	3rd Trimester	8.91 ± 46.70	0.26	0.00	27.28
Control Group					
TSH	**1st Trimester**	2.15 ± 1.44	2.04	0.18	4.45
	2ndTrimester	2.72 ± 2.45	2.30	0.98	5.19
	3rd Trimester	3.39 ± 5.06	2.46	0.94	7.21
FT_4	**1st Trimester**	0.76 ± 0.21	0.81	0.44	1.07
	2ndTrimester	0.87 ± 0.16*	0.86	0.65	1.17
	3rd Trimester	0.84 ± 0.21*	0.84	0.58	1.09
TT_4	**1st Trimester**	10.03 ± 2.63	10.56	5.70	13.25
	2ndTrimester	10.82 ± 2.21**	10.80	7.87	14.62
	3rd Trimester	11.85 ± 2.32†††***	11.86	8.26	15.57
Tg	**1st Trimester**	4.07 ± 4.95	2.75	0.00	16.97
	2ndTrimester	24.94 ± 49.51**	5.90	0.00	153.82
	3rd Trimester	6.39 ± 26.38†**	0.00	0.00	21.35

** p<0.05, **p<0.01 v/s 1st trimester, †p<0.05, ††p<0.01 v/s 2nd trimester*

Serum TSH : Our results were in agreement with a study conducted by Boas et al (2009), which reported non significant difference in serum TSH levels and thyroid hormones between women with and without iodine supplementation. Another study by Marwah et al (2008) reported no difference in the groups which is from a homogenous population and are not on hormone therapy.

In pregnant women from iodine sufficient areas, such as Switzerland (Stricker et al 2007) and Sweden (Elnagar et al 1998), TSH level was reported to rise from the first to the third trimester. In our study results, gradual increase in mean TSH levels was observed throughout gestation in experimental group and TSH values in third trimester were significantly higher in the experimental group compared to first trimester ($p<0.01$). However, in control group there was an increase in TSH level but the rise was non significant for all trimesters (2.04 µU/ml to 2.46, $p<0.07$) **(Table 4.28)**. This result was supported by the findings of (Marwah et al 2008) indicating a similar trend of rise in TSH level throughout gestation but that was non significant (2.1 µU/ml to 2.6 µU/ml, $p<0.11$).

Studies reported by (Romano et al 1991, Liesenkotter et al 1996, Nohr et al 2000 and Antonangeli et al 2002) reported no difference in maternal FT_4, TSH, Tg between supplemented and non supplemented groups. Similar studies by (Pedersen et al 1993 and Glinoer et al 1995), reported maternal Tg and TSH , Cord blood Tg to be significantly lower in supplemented group compared to control group. Our study results depicted a significant difference in TSH values during third trimester, when experimental and control group were compared ($p<0.05$). These results were further comparable to study results reported by (Pedersen, 1993 and Glinoer, 1995), which were found significant.

Serum FT_4: Median serum FT_4 value between groups (experimental and control) did not vary significantly. Fister et al (2011) reported changes in the levels of both free thyroid hormone during pregnancy and stated that, they are not in correlation with iodine intake but are due to physiological changes during pregnancy. Similarly Lauberg et al (2007) concluded that, a moderate fall in FT_4 during pregnancy is not a sign of iodine deficiency.

However, within group between all three trimesters, variation in mean serum FT_4 level was observed among control group throughout gestation. FT_4 level decreased significantly in second trimester compared to first trimester ($p<0.003$) and remained constant towards the end. Hence, there was also a significant difference observed between first and third trimester. ($p<0.04$) **(Table 4.28)**

Our study results followed similar lines, as reported by many authors indicating, a gradual decrease in FT_4 level during pregnancy, with its lowest level in the third trimester

has been reported in iodine-sufficient areas too (Marwah et al 2008; Stricker et al 2007 ; Kurioko et al 2005 and Elnagar et al 1998).

Berghout and Wiersinga (1998) revealed that, FT_4 significantly decreased by about 30 percent in lower range of normal values in the second and third trimester of pregnancy in both iodine-deplete and iodine- replete areas. A study conducted in (2002) where the changes in thyroid hormones were related to energy balance during pregnancy. It can be calculated that the extra energy needs of pregnancy can be 1020 KJ/day, which comprises the energy required for the synthesis of new tissues together with related increments in basal metabolism. However, in pregnancy, the increase in energy intake was found to be very small, approximately 80 KJ/day, giving rise to an estimated energy gap of 940 KJ/day. This is only partial fulfillment, 585 KJ/day has still to be met, and it is in this respect that down-regulation of thyroid hormone action as indicated by the decrease in the levels of FT_4 and FT_3 may contribute to the saving of energy. (Ardawi, Nasrat and Mustafa 2002).

***Serum TT_4*:** Majority of the researchers have considered range provided by commercially available kits and few have used laboratory based reference range. In our study also reference range was considered based on the laboratory range, 4.20 -13µg/dl as standards. Subjects with TT_4 values between 0.0 -4.19 µg/dl as below normal thyroid status. During first trimester 1.5% and 1.9% respectively in experimental and control group were observed to fall into the below normal thyroid status. However, during second and third trimester none of the subjects had below normal thyroid status. Our findings were supported by (Ujowundu et al 2011), who observed none of the subjects with <5 µg/dl in any of the trimester. During second trimester, significant improvement in TT_4 levels compared to first trimester was observed; level of significance was ($p<0.05$) in experimental and ($p<0.01$) in control group. During third trimester the improvement was highly significant compared to both the trimesters for both the groups ($p<0.001$) **(Table 4.28).**

Serum Tg: Median Tg concentration was substantially higher (19.42%) in subjects during 2^{nd} trimester compared to 1^{st} trimester in our study. Soldin (2004) reported median concentration to be approximately 25% higher in third trimester compared to first and

second trimester. Median Tg was 9.7 µg/l in first half of pregnancy (gestational age < 20 weeks) and it was 12 µg/l in the last half of pregnancy (gestational age > 20 weeks) (Boas et al 2009). In our study, mean Tg during second trimester was observed to be significantly higher in control group compared to experimental group ($p<0.05$).

It is known that UI doesn't always provide direct information about thyroid function (Hallowell 2007 and Soldin 2005). However, women whose UI within 100-149 µg/L during the first trimester had lower levels of TSH and higher levels of FT_4 than women with UI below 50 µg/L (Alvarez-Pedrerol 2009). Our study results also revealed lower levels of TSH and higher FT_4 compared to the subjects who were classified as iodine deficient during 1st trimester **(Table 4.29)**.

Table 4.29: Thyroid function tests of experimental group based on urinary iodine sufficiency

Parameters	Trimester	Iodine deficient (N=11)		Iodine sufficient (N=56)	
		Mean ± SD	Median	Mean ± SD	Median
TSH	1st Trimester	2.19 ± 1.18	2.09	2.06 ± 1.31	1.87
	2nd Trimester	2.04 ± 0.90	2.06	2.30 ± 1.09	2.25
	3rd Trimester	2.28 ± 1.55	1.96	2.42 ± 1.14	2.07
FT_4	1st Trimester	0.704 ± 0.179	0.751	0.839 ±0.260	0.864
	2nd Trimester	0.866±0.333*	0.772	0.841 ±0.145	0.868
	3rd Trimester	0.646 ±0.266*†	0.700	0.839± 0.269	0.864
TT_4	1st Trimester	10.70 ± 2.29	10.64	10.02 ± 2.06	10.06
	2nd Trimester	10.99 ± 1.76	10.90	10.68 ± 1.63	10.70
	3rd Trimester	11.81 ± 2.39	11.37	11.35 ± 1.74	11.56

**p<0.05 v/s 1st trimester, † p<0.05 v/s 2nd trimester*

This difference was observed throughout gestation. UI deficient subjects showed significant increase in FT_4 levels during second trimester ($p<0.05$) compared to first trimester and the levels decreased significantly during third trimester ($p<0.05$).

Table 4.30: Correlation coefficients for the thyroid analytes and UI by trimester in experimental group

	TSH	FT4	TT4	TG	UI
1st Trimester					
TSH	1				
FT4	-.295*	1			
TT4	-.017	-.109	1		
Tg	.076	.096	-.035	1	
UI	.067	.063	.004	-.007	1
2nd Trimester					
TSH	1				
FT4	.059	1			
TT4	-.251*	.114	1		
Tg	-.033	.010	-.048	1	
UI	.018	.126	.167	-.024	1
3rd Trimester					
TSH	1				
FT4	-.067	1			
TT4	-.113	.088	1		
Tg	-.115	-.149	.071	1	
UI	.164	.036	-.220	-.157	1

There was a no correlation between UI and thyroid hormone analytes in any of the trimesters **(Table 4.30) (Figure 4.6)**. Similar findings were also reported by Fister et al (2011) as no correlation between TSH and UI, indicating that higher TSH levels in the third trimester inversely mirros hCG levels in pregnancy, which is much lower in the third trimester than in the first trimester (Glioner et al 1990), since after conception hCG level rises in circulation and competes with TSH to get attached to TSH receptors due to similarity in the structure. Thus, initial stage of pregnancy reports slight suppression of TSH and increase in FT_4 level, which gradually returns to normal in the iodine sufficient population **(Figure 4.5)**.

On correlating thyroid analytes in control group, no correlation was observed between the analytes and UI at any point of time (trimesterwise).

Figure 4.5: Experimental group TSH v/s FT4 during 1st trimester

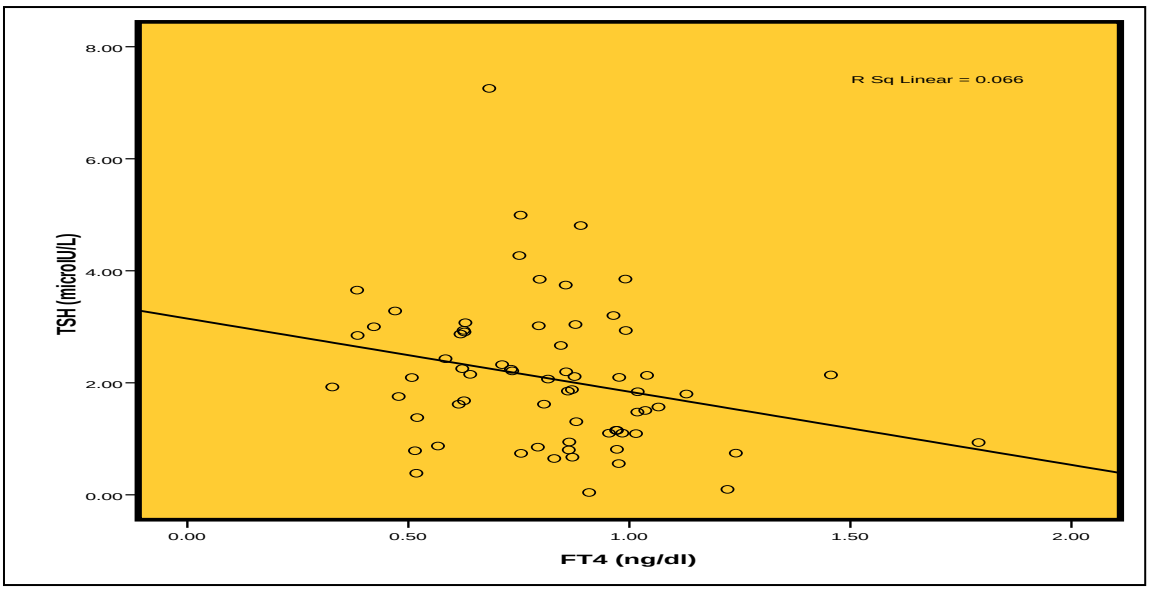


Figure 4.6: Experimental group TSH v/s UI during 1st trimester

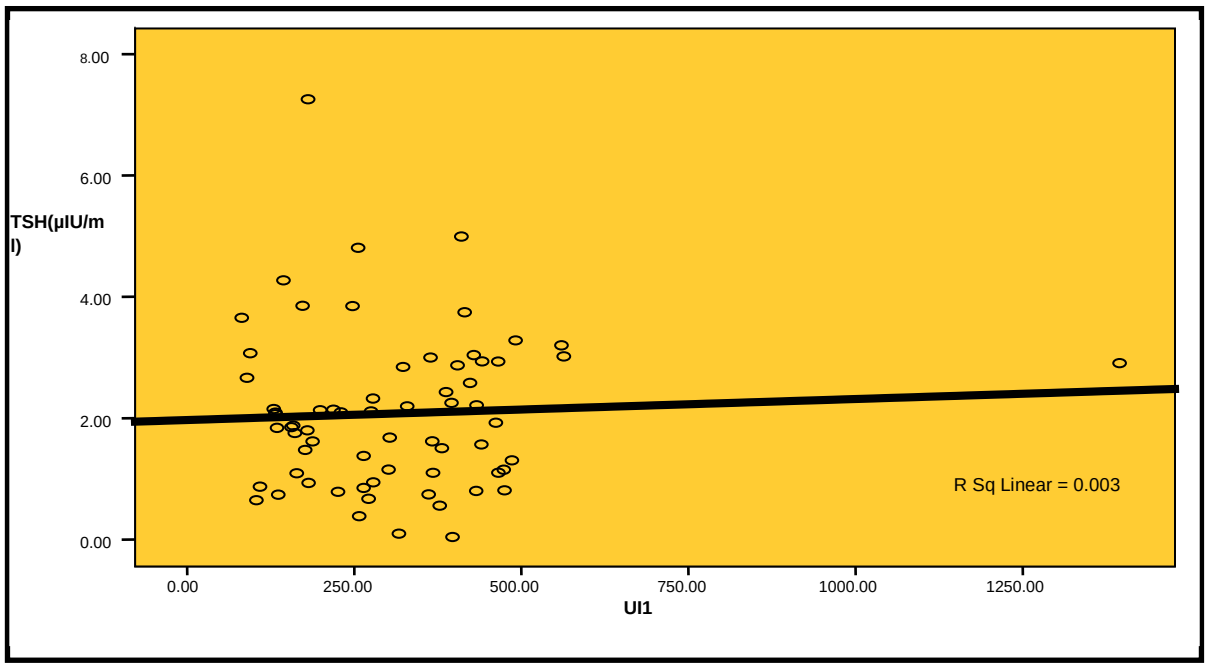


Table 4.31: Percent distribution of thyroid dysfunction in all three trimesters

Trimester	SCH	OH	Hypothyroxinemia	Euthyroidism
Experimental Group				
1st Trimester	12 (17.91)	8 (11.94)	20 (29.85)	27 (40.30)
2nd Trimester	12 (17.19)	1 (1.49)	7 (10.44)	47 (70.15)
3rd Trimester	10 (14.92)	4 (5.97)	17 (25.37)	36 (53.73)
Control Group				
1st Trimester	15 (27.77)	5 (9.26)	17 (31.48)	17 (31.48)
2nd Trimester	12 (22.22)	1 (1.85)	2 (3.70)	39 (72.22)
3rd Trimester	12 (22.22)	2 (3.70)	7 (12.96)	33 (61.11)
Total				
1st Trimester	27 (22.31)	13 (10.74)	37 (30.58)	44 (36.36)
2nd Trimester	24 (19.83)	2 (1.65)	9 (7.44)	86 (71.04)
3rd Trimester	22 (18.18)	6 (4.96)	24 (19.83)	69 (57.02)

Values in parenthesis depicts percentage

Table 4.31 is based on the clinical guidelines provided by "The Endocrine Society" (TES) in 2007 for management of thyroid function during pregnancy and postpartum, subjects were classified with subclinical hypothyroidism, overt hypothyroidism, hypothyroxinemia and euthyroidism.

Subclinical Hypothyroidism (SCH): As per the TES guidelines, subjects can be classified with SCH, when they have higher TSH and normal FT_4 levels. During first trimester the cutoff for TSH is >2.5 µIU/ml and for second-third trimester i.e. >3.0 µIU/ml- as per our reference standards. Normal range for FT_4 has not been mentioned and different researchers have used different cutoff values for FT_4. However, majority has followed reference range provided by commercially manufactured kit. For our analysis, the reference range for FT_4 has been considered as 0.650-2.10 ng/dl, which is provided by the kit. Hence, for screening of SCH, subjects with TSH- >2.5 µIU/ml or >3.0 µIU/ml based on their gestational stage and FT4- within reference range were considered.

SCH was observed in 17.91% subjects in experimental and 27.77% subjects in control group during first trimester **(Table 4.31)**. Percent prevalence remained same during second trimester in experimental group and 5% reduction was observed in control group.

Compared to second trimester, the status remained same for control group and 3% reduction was observed in experimental group during third trimester.

***Overt Hypothyroidism (OH)*:** Following TES guidelines, subjects with elevated TSH and lower FT_4 levels are considered as overt hypothyroidic. Based on the standard cutoffs 11.94% subjects in experimental group and 9.26% in control group were screened with OH during first trimester. During third trimester the percent prevalence reduced by 50% in both the groups.

***Hypothyroxinemia*:** It can be defined as lower FT_4 levels and normal TSH. It is the most critical condition compared to SCH and OH, for fetal growth, development and survival. Almost 30% of the subjects had hypothyroxinemia during first trimester, which reduced significantly in control group (app. 40% reduction) compared to experimental group with 15% reduction.

***Euthyroidism*:** There were almost 36.36% subjects inclusive of both the groups, with all hormone levels in normal range. Still the prevalence of thyroid dysfunction was very high. The difference compared to other researchers, would have been due to variability in reference ranges.

4.3.3 INTERRELATION BETWEEN PARAMETERS

(Table 4.32) On correlating maternal basic characteristics with anthropometry, it was observed that maternal age was positively correlated with gravida. It reveals that, with the progression in age genesis process increases. Our subjects are in their peak age range of reproduction and genesis is a general phenomenon of human life cycle. Hence, the correlation might have been significant ($r=0.492$, $p<0.01$).

Body weight during pregnancy- a different physiological condition- is expected due to maternal dietary intake, fetal growth and many other humoral factors. Hence, weight gain in such a state, vary individually. Correlation between all three trimester weights was significant ($p<0.01$).

Table 4.32: Correlation of baseline characteristics, anthropometric parameters with iodine and iron status (N=121)

	Age	Gravida	Education	Ht	Wt1	Wt2	Wt3	BMI1	BMI2	BMI3	Hb1	Hb2	Hb3	UIC 1	UIC2	UIC3
Age	1															
Gravida	.492**	1														
Education	-.067	-.229*	1													
Ht	.042	.093	.024	1												
Wt1	.126	.119	-.067	.384**	1											
Wt2	.100	.094	-.077	.381**	.945**	1										
Wt3	.097	.090	-.066	.379**	.933**	.983**	1									
BMI1	.140	.101	-.071	-.037	.890**	.835**	.822**	1								
BMI2	.113	.085	-.089	-.068	.826**	.877**	.862**	.942**	1							
BMI3	.098	.076	-.076	-.090	.800**	.851**	.869**	.920**	.978**	1						
Hb1	.077	-.040	.096	.144	.320**	.288**	.329**	.260**	.232**	.275**	1					
Hb2	.090	-.060	-.086	.076	.168	.192*	.227*	.153	.174	.211*	.368**	1				
Hb3	-.067	-.148	.096	.056	.113	.105	.120	.076	.060	.080	.281**	.560**	1			
UIC1	-.090	.031	.041	.080	.062	.041	.045	.087	.071	.083	.044	-.241**	-.095	1		
UIC2	.037	.071	.024	-.089	-.093	-.092	-.085	-.063	-.055	-.040	.049	-.057	.011	.182*	1	
UIC3	-.084	.021	.045	.034	.127	.103	.101	.139	.108	.105	.028	-.102	-.025	.263**	.302**	1

Correlation significance*P<0.05 and ** p<0.01

Maternal height was significantly correlated with weight throughout gestation ($p<0.01$) and thus with BMI.

4.3.3.1 Correlation between baseline characteristics and iodine- iron status of the subjects

On correlating maternal baseline hemoglobin with maternal weight and BMI, highly significant correlation was observed throughout gestation ($p<0.01$), which in turn indicates an impact of maternal dietary intake and change in requirement and physiology of iron during fetal growth phase **(Table 4.32)**.

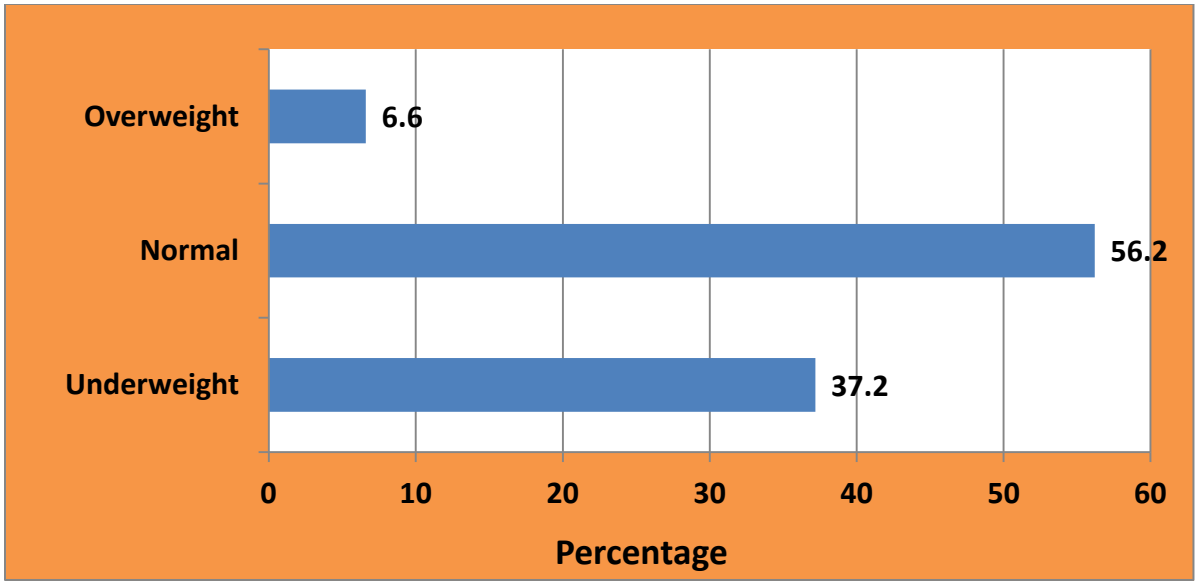
Maternal urinary iodine did not show any significant correlation with other parameters like basic characteristics, anthropometry and iron status. All three trimester urinary iodine showed significant dependence on each other ($p<0.05$) and third trimester values were correlated with first and second trimester UI values with high significance ($p<0.01$).

4.3.3.2 BMI and thyroid hormone levels

The subjects were subdivided as underweight, normal weight and overweight according to BMI at the time of registration and data is presented in **(Figure 4.7)**. Higher risk or thyroid dysfunction during pregnancy is generally expected amongst overweight or obese women during pregnancy. Hence, they need to be considered as at risk pregnancy for adverse outcome. Overweight and obesity have been studied as independently and have shown effect as intervening factors for adverse outcome due to thyroid dysfunctions (Raatikainen, Heiskanen and Heinonen 2006). Results of our data also supported the statement. Since, first indication towards thyroid insufficiency is fluctuations in TSH levels.

It was observed that, serum TSH increased with BMI and FT_4 decreased significantly with BMI reported by Ashoor et al (2010) and Mannisto et al (2011). In our study results, serum TSH showed a similar pattern of increased TSH, but the difference was non significant, where as FT_4 decreased with improvement in BMI ($p<0.02$). TSH was at its highest level in the overweight group, but when compared to other groups; the difference was not achieving levels of significance ($p=0.548$).

Figure 4.7: Distribution of subjects into BMI categories at baseline



Serum FT_4 showed gradual decrease in percentage when compared with BMI categories, a posthoc analysis revealed a significance at ($p<0.05$, H test) during first trimester only. Later stages of gestation did not show the similar pattern at significant level. Comparison of FT_4 concentration in different BMI categories revealed that FT_4 levels were significantly different in underweight and normal group (U test, $p <0.005$). No significant difference was observed when underweight and normal BMI categories were compared with overweight category (data not presented).

Table 4.33: Average weight gain in subjects by baseline BMI classification

BMI at the time of registration	Weight gain (kg) Mean ± SD	% gain compared to recommendation	Recommended weight gain (kg)
Experimental Group			
Low (<18.5)	5.37 ± 1.64	42.96	12.5 – 18
Normal (18.5-25)	4.84 ± 1.78	42.08	11.5-16
High (>25)	5.75 ± 1.26	82.14	7-11.5
Control Group			
Low (<18.5)	5.42 ± 2.03	43.36	12.5 – 18
Normal (18.5-25)	4.86 ± 1.84	42.26	11.5-16
High (>25)	4.25 ± 2.50	60.71	7-11.5
Total			
Low (<18.5)	5.40 ± 1.81	43.2	12.5 – 18
Normal (18.5-25)	4.85 ± 1.79	42.17	11.5-16
High (>25)	5.00 ± 2.00	71.4	7-11.5

(Table 4.33) reveals mean weight gain during pregnancy amongst both the groups. According to WHO Health Statistics 2010, there are 35.6% pregnant women in India with lower BMI and they tend to gain lower weight during pregnancy.

There was no significant difference observed between both the groups in any of the categories of BMI, which revealed equal impact of nutrition health education on the nutritional status of the subjects, irrespective of the groups. However, in both the groups, subjects were highly deficit in weight gain compared to standard weight gain recommended by IOM, 2009. Among experimental group, the subjects who were below normal BMI were 57.04%, normal BMI subjects were 57.92% and above normal were 17.86% deficit compared to lower limit of standard range for weight gain. These percentages were 56.64, 57.74 and 39.29 respectively for subjects belonged to control group. These percent deficits can be attributed due to negligence, limited purchase power and food insecurity for women in the families.

4.3.4 IMPACT ASSESSMENT ON PREGNANCY OUTCOME OF THE SUBJECTS

As a result of pregnancy outcome, data from eighty three neonates of the subjects belonged to both the groups could be collected on baseline characteristics and anthropometry. This data represented neonatal data on fifty from experimental group and thirty three from control group subjects **(Table 4.34)**. All details of sixty four subjects could be collected, including cord blood sample, since some of the subjects had neonatal death, delivered at other hospitals or home deliveries.

The data on the gender distribution of neonates revealed that, 45.78 % neonates were males and 54.21% were females. Majority of the deliveries were normal and 20.48% deliveries were cesarean. There were 13% deliveries before 37 weeks of gestation i.e. preterm deliveries and the percentages were higher (18.36%) in experimental group, almost double than control group (9.37%) **(Table 4.34)**.The difference might have been due to unequal samples in both the groups and many a times the mothers have delivered at home or on the way to hospitals. So the babies whose cord blood was not collected have been omitted. Hence, the difference does not reveal the actual percentage and any correlation with supplemented or non supplemented groups.

One baby had cleft palate and lip. Four babies (4.81%) reported death due to cyanosis/epilepsy or congenital heart disease. One still birth was reported.

Table 4.34: Characteristics of neonates of the subjects

Characteristics	Frequency (%)		
	Experimental (n=50)	Control (n=33)	Total (N=83)
Sex of the Baby			
Male	23 (46.00)	15 (46.87)	38 (45.78)
Female	27 (54.0)	18 (56.25)	45 (54.21)
Type of delivery			
Normal	36 (72.00)	29 (87.88)	65 (78.31)
Cesarean	13 (26.00)	4 (12.12)	17 (20.48)
Others	1 (2.00)	0 (0)	1 (1.20)
Preterm deliveries			
<34 weeks	1 (2.04)	0 (0)	1 (1.20)
<37 weeks	9 (18.36)	3 (9.37)	11 (13.25)
LBW			
<2.5 kg	10 (20)	7 (21.21)	17 (20.48)
2.5 kg	10 (20)	10 (30.3)	20 (24.09)

*Values in parenthesis depicts percentag*e

Based on anthropometric data available for n=83 neonates revealed 20.48% prevalence of low birth weight. Neonates with 2.5 kg weight were 30.3% in control group, which was 50% higher than the experimental group.

Table 4.35: Mean parameters of experimental and control group

Parameters	Mean± SD (Range)	
	Experimental Group	Control Group
Gestational Age at delivery (Weeks)	38.43 ± 2.12	38.68 ± 1.85
	(32.2 – 44)	(34.6 – 44.90)
Birth weight (kg)	2.74 ± 0.48	2.67 ± 0.39
	(1.5 – 3.7)	(2.0 – 3.75)
Head circumference (cm)	32.73 ± 1.95	33.17 ± 1.17
	(25 – 37)	(30 – 35)
Mid upper arm circumference (cm)	8.94 ± 1.11	9.28 ± 0.59
	(5 – 12)	(8 – 11)
Birth length (cm)	45.70 ± 3.30	45.89 ± 2.41
	(30 – 51)	(42 – 52)

On comparing neonatal parameters in experimental and control group, no significant difference was observed **(Table 4.35)**.

4.3.4.1 Relationship between Maternal and Neonatal parameters

There was no general trend observed among both the groups. In experimental group, it was observed that, maternal FT_4 of third trimester correlated significantly with neonatal FT_4 ($r=0.427$, $p<0.01$) and maternal TT_4 for second and third trimester negatively correlated with neonatal TT_4 at ($r= -.329$, $p<0.05$) and ($r= -.373$, $p<0.05$) respectively. Maternal TSH did not correlate with cord blood TSH, whereas first trimester TSH correlated with neonatal weight ($r = 0.327$, $p<0.05$).Third trimester Tg inversely correlated with neonatal height ($r = -0.371$, $p<0.05$), neonatal weight ($r = -0.304$, $p<0.05$) and neonatal head circumference ($r= -0.348$, $p<0.05$).

There was an interesting finding observed indicating highly significant correlation between maternal hemoglobin in third trimester of experimental group with neonatal height ($r = 0.468$, $p<0.01$), neonatal weight ($r= 0.322$, $p<0.01$) and neonatal head circumference ($r = 0.402$, $p<0.01$).

In control group, there was a correlation observed between maternal TSH level of second trimester and neonatal weight ($r= 0.389$, $p<0.05$) and neonatal MUAC ($r=0.378$, $p<0.05$). Neonatal TT_4 and maternal TT_4 correlation for first trimester ($r=0.433$, $p<0.05$) and second trimester ($r=0.431$, $p<0.05$) was also observed.

A study conducted by (Shobeiri F, Begum K and Nazari M 2006) reported similar findings. Birth weight of neonates correlated significantly with difference in hemoglobin concentration during third trimester. It was observed that, mean birth weight varied significantly (F-6.09, $p<0.01$) in relation with hemoglobin concentration in the three trimesters.

In our study results, multivariate regression analysis revealed that, the neonatal birth weight was significantly associated with hemoglobin concentration at third trimester ($p<0.05$). A study conducted by Laflamme E. (2010) also revealed a statistically significant association between hemoglobin concentration and gestational age ($p<0.02$),

parity ($p<0.001$). However, correlation between hemoglobin concentration at third trimester and birth weight was not found. In our study results, correlation between hemoglobin concentration and gestational age, parity was not observed.

Table 4.36: Correlation between neonatal anthropometry and cord blood analytes (experimental group)

	Ht	**Wt**	**HC**	**MUAC**	**G.age (Wks)**	**CB TSH**	**CB FT_4**	**CB TT_4**
Ht	1							
Wt	0.639***	1						
HC	0.688**	0.626**	1					
MUAC	0.398*	0.645**	0.524**	1				
G.age (Wks)	0.140	0.285*	0.169	0.176	1			
CB TSH	-0.387*	-0.569*	-0.456*	-0.273	-0.300	1		
CB FT_4	0.074	-0.168	-0.53	0.033	-0.252	0.311	1	
CB TT_4	0.258	0.183	0.212	0.286	0.205	0.135	0.454**	1

*Correlation significance *p<0.05, **p<0.01 and ***p<0.001*

(Table 4.36) There was a significant correlation between neonatal weight with gestational age (weeks) in experimental group ($r=0.285$, $p<0.05$). This indicates appropriate growth and development of the neonates during fetal development of the embryo. Cord blood TSH showed negative correlation with neonatal anthropometric parameters like height, weight and head circumference ($p<0.05$) in experimental group. Cord blood FT_4 was significantly correlated with cord blood TT_4 ($r= 0.454$, $p<0.01$). All neonatal anthropometric parameters were significantly correlated with each other ($p<0.01$).

There was no significant correlation observed between cord blood analytes with neonatal anthropometric parameters or amongst the analytes **(Table 4.37)**. Neonatal anthropometric parameters were significantly correlated with each other ($p<0.01$)

Table 4.37: Correlation between neonatal anthropometry and cord blood analytes (control group)

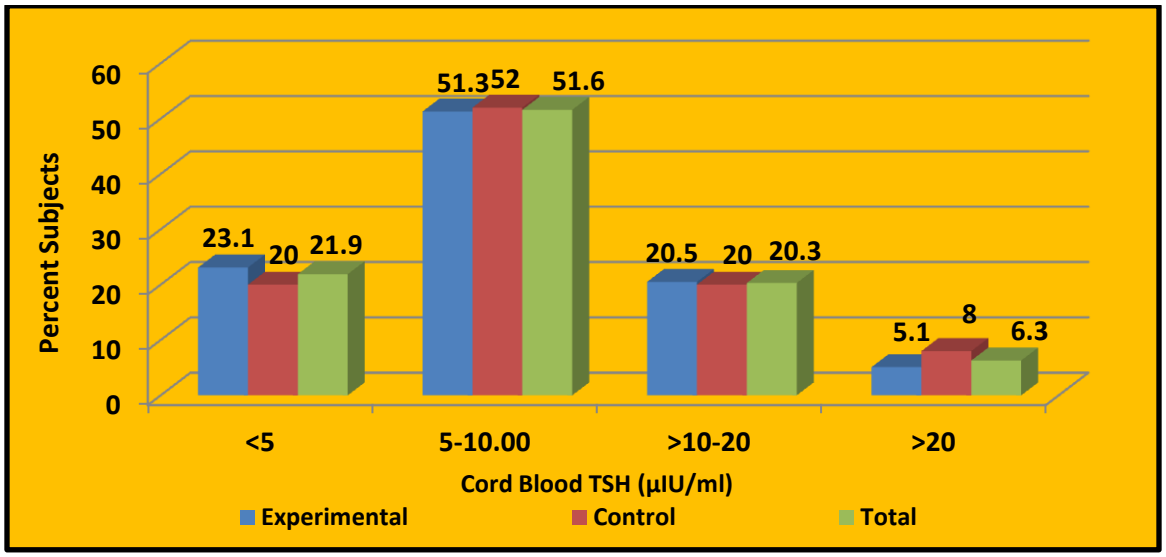
	Ht	Wt	HC	MUAC	G.age (Wks)	CB TSH	CB FT4	CB TT4
Ht	1							
Wt	0.722**	1						
HC	0.476**	0.495**	1					
MUAC	0.645*	0.730**	0.556**	1				
G.age (Wks)	0.212	0.100	0.079	-0.017	1			
CB TSH	0.187	0.108	0.011	0.092	0.215	1		
CB FT4	-0.034	0.019	-0.120	0.036	0.197	-0.123	1	
CB TT4	-0.071	-0.086	-0.124	-0.224	0.115	-0.214	0.214	1

*Correlation significance*p<0.05, **p<0.01 and ***p<0.001*

4.3.4.2 Thyroid function parameters of the Neonates

Cord blood TSH levels showed a wide scatter. Cord blood TSH levels were asymmetrical and skewed towards right side (positively skewed) which mean their trends shifted towards higher values. A study by Abbas et al (2003) also reported the similar trend.

Figure 4.8: Percent distribution of the neonates in all groups based on TSH levels



Elevated serum TSH for a longer period (after 72 hrs) in the neonates indicates insufficient supply of thyroid hormones to the developing brain, which is suggestive of thyroid dysfunction in the neonates. WHO/UNICEF/ICCIDD has included neonatal TSH

as one of the indicators of assessing IDD and their control in a population (Delange 1998). In the absence of iodine deficiency, the frequency of neonatal serum TSH above 10 µIU/ml is less than 3%. A frequency of 3%-19.9% indicates mild, 20%-39.9% indicates moderate and above 40% indicates severe IDD in the region.

In our study results, 26.6 % of the neonates were observed having TSH >10 µIU/ml which indicates moderate iodine deficiency **(Figure 4.8)** It means substantial numbers of fetuses were iodine deficient and thus might have been subjected to in utero hypothyroxinemia.

Table 4.38: Comparison between neonatal TSH and incidence of LBW

	Cord blood TSH		**Chi Square**
	> 10 µIU/ml	**<10 µIU/ml**	
Low Birth Weight			
≤2.5	11	16	4.81
>2.5	6	31	(p<0.02)

(Table 4.38) Neonatal TSH showed a significant correlation between incidences of LBW. On calculating odd's ratio and 95% CI limit, the results were (3.55; 0.97, 13.44). Relative risk of having LBW was higher in neonates with TSH < 10 µIU/ml indicating insufficient functioning of neonatal thyroid gland (RR, 95% CI: 2.51; 1.06 - 5.95). Cross tabulation of neonatal TSH levels also revealed that, birth weight varied significantly with variations in cord blood TSH (p<0.02). Neonatal birth weight was observed to be higher (>2.5 kg) amongst neonates with TSH <10 µIU/ml.

In control group, mean neonatal TSH was on the verge of reaching 10 µIU/ml, there was a non significant difference in all thyroid hormone parameters for both the groups. **(Table 4.39).**

Table 4.39: Neonatal parameters of cord blood analysis

Parameter	Mean ± SD (Median) (N=64)	
	Experimental Group	Control Group
Cord blood TSH (mIU/L)	9.20 ± 7.96	9.76 ± 7.07
	(6.90)	(7.40)
Cord blood FT4 (ng/dl)	1.20 ± 0.18	1.22 ± 0.16
	(1.17)	(1.21)
Cord blood TT4 (ng/dl)	7.96 ± 2.11	8.05 ± 2.45
	(8.13)	(8.20)

Neonatal nutritional status was assessed based on WHO child growth standards, using (WHO anthroplus for personal computers, version 3.1.2010). Nutritional status was classified for undernutrition based on three anthropometric indices: height for age (stunting), weight for height (wasting) and weight for age (underweight). They were categorized/grouped using sex of the child as criteria and then the nutritional status was assessed. **(Table 4.40).**

Table 4.40: Nutritional status of the neonates based on Z score (n=83)

Sex n (%)	WAZ			HAZ			WHZ		
	-2 to ±2SD	≤2 to -3SD	≤3SD	-2 to ±2SD	≤2 to -3SD	≤3SD	-2 to ±2SD	≤2 to -3SD	≤3SD
Female 45(52.21)	36 (80)	5 (11.1)	4 (8.89)	25 (55.55)	11 (24.44)	9 (20)	37 (82.22)	7 (15.55)	1 (2.22)
Male 38(45.78)	32 (84.21)	4 (10.52)	2 (5.26)	19 (50)	12 (31.58)	7 (18.42)	35 (92.10)	2 (5.26)	1 (2.63)
Total 83 (100)	68 (81.93)	11 (10.84)	6 (7.22)	44 (53.01)	23 (27.71)	16 (19.28)	72 (86.75)	9 (10.84)	2 (2.41)

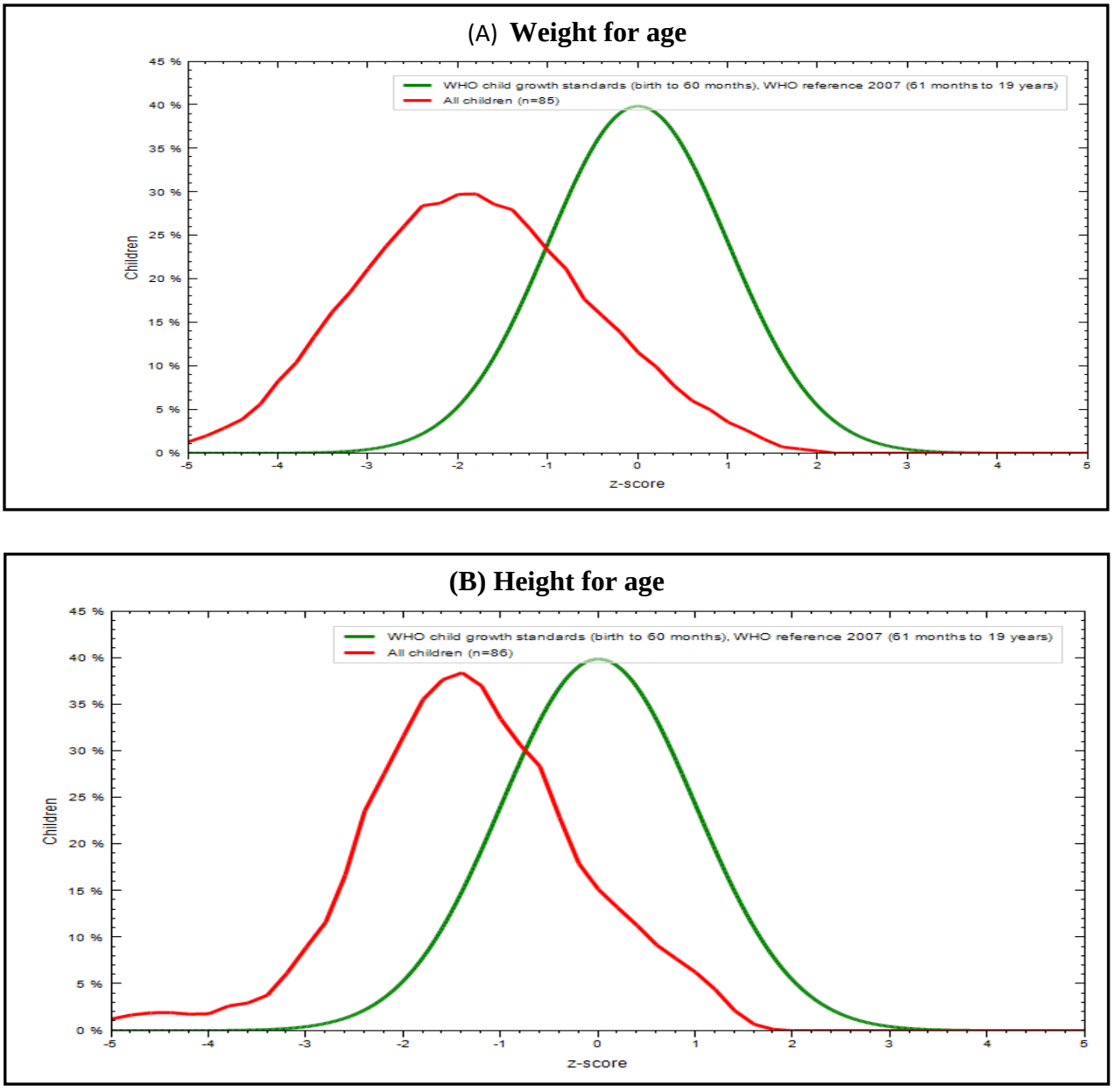
Values in parenthesis depicts percentage

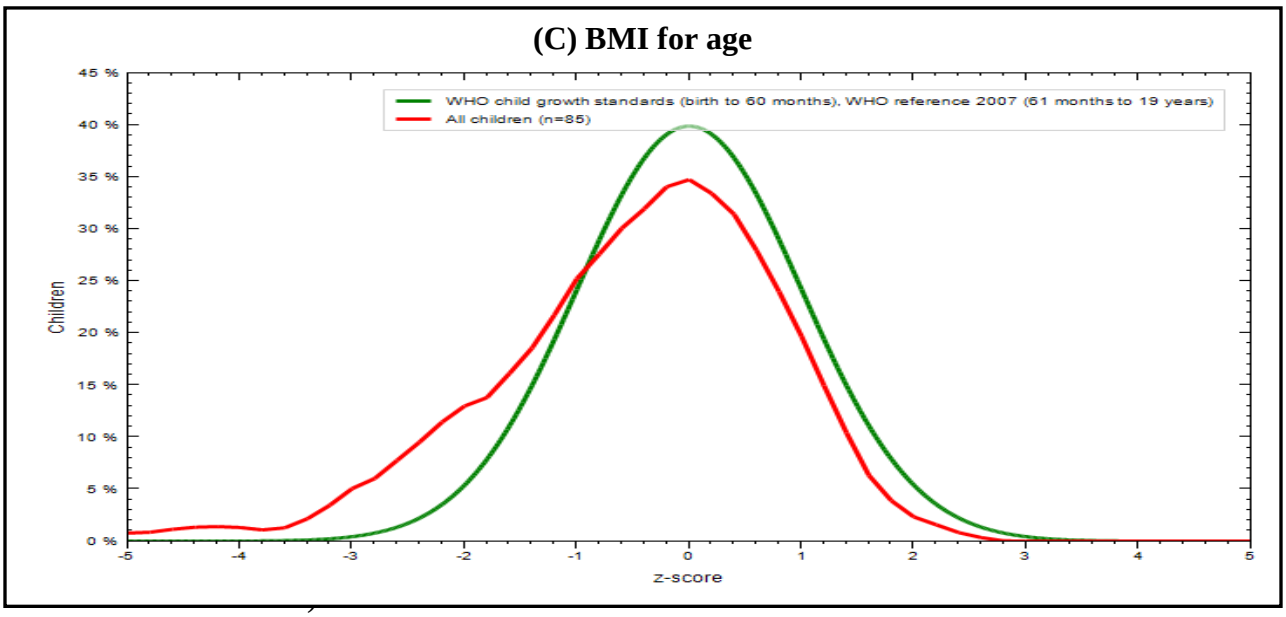
On comparing data on nutritional outcome of the neonates, it was observed that 52.21% of the subjects were females and 45.78% of them were males. Of the total subjects, 53.01% subjects were normal or mild stunted, 27.71% had moderate stunting and almost one fifth percent of the neonates were severely stunted.

On comparing subjects for wasting, 10.84% were observed to be moderately wasted and 2.41% were severely wasted. However, 81% of the neonates had normal or mildly lower Z scores for wasting.

Below are the graphs indicating skewness in the nutritional status of the neonates in our study compared to WHO child growth standards. All the graphs indicated by red line are on the left side of the standard curve which means, they are negatively skewed **(Figure 4.9).**

Figure 4.9: Comparison of neonatal nutritional parameters with WHO growth standards





4.3.5.1 Dietary Recall- 24 hours

The dietary intake of various nutrients amongst the subjects with RDA was compared before and after intervention to assess the impact on dietary intake in both the groups **(Table 4.41 and 4.42)**. In experimental group, subjects were observed to be 49.42%, 62.31%, 10.45 % and 84.42% deficit in energy, protein, carbohydrate and iron intake respectively compared to RDA **(Table 4.41)**. In control group, subjects were observed to be 52%, 64.34%, 14% and 85.97% deficit in energy, protein, carbohydrate and iron intake respectively compared to RDA **(Table 4.42)**. This deficit could be reduced upto certain levels by giving nutrition health education sessions on regular intervals.

On comparing dietary intake of macronutrients with initial deficits with RDA, it was observed that, mean dietary intake of calories could be improved by 10-11% ($p<0.001$). Mean protein could also be improved by 10-12% ($p<0.001$) towards end of the study. Carbohydrate intake was improved by 20% ($p<0.001$), where a shift from deficit levels to surplus intake was achieved. **(Table 4.41)**

Success in improving micronutrient intake - iron (p<0.001) through DFS was attained significantly in experimental group **(Table 4.41)**, ***since experimental group was***

provided additional 10 mg iron through diet, from the DFS supplemented to the group. This improvement was highly significant compared to control group also (p<0.001).

Table 4.41: Dietary intake of subjects before and after supplementation and NHE (Experimental group)

Nutrients	RDA	Initial (Mean ±SD)	Difference from RDA	Final (Mean±SD)	Difference from RDA	Difference (Initial-final)	't' value
Energy (K.cal)	2525	1277 ± 370.92	- 49.42 %	1536 ±399.4	-39.16 %	259***	5.837
Protein (gm)	65	24.5 ±10.63	- 62.31%	32.82±11.97	- 49.5%	8.326***	5.801
Fat (gm)	30	38.51 ±11.76	±22.10%	41.24±14.87	± 27.55%	2.726 (NS)	1.403
CHO (gm)	175	156.72 ± 51	- 10.45%	194.67±59.07	± 10.10%	37.95***	5.902
Iron (mg)	38	5.92 ± 5.12	-84.42 %	7.54 ±5.97	- 80.16 %	1.62**	2.675
Diet ± DFS				17.55 ± 13.25	-53.84%	11.62***	6.976

Mean dietary intake improvement was significant amongst control group also. Mean calorie intake improved by 10% (p<0.001) and 12% (p<0.001) for protein. Improvement in carbohydrate and iron intake was also significant (p<0.01) in control group **(Table 4.42).**

Fat intake improved non significantly in experimental and significantly (p<0.01) in control group with an improvement in dietary intake up to one to two meals per day.

Table 4.42: Dietary intake of the subjects before and after NHE (Control group)

Nutrients	RDA	Initial (Mean ± SD)	Diff with RDA	Final (Mean ± SD)	Diff with RDA	Difference	't' value
Energy (K.cal)	2525	1212.3± 325.72	- 52 %	1537.87± 430.63	- 39.30%	325.48***	5.785
Protein (gm)	65	23.18 ± 8.10	- 64.34%	30.61 ± 11.41	- 52.90%	7.425***	4.972
Fat (gm)	30	34.22± 12.41	± 12.33%	41.77 ± 14.61	± 28.17%	7.546**	3.215
CHO (gm)	175	150.37± 39.64	- 14.07%	187.22 ± 57	± 6.53%	36.85**	2.801
Iron (mg)	38	5.33 ± 3.00	- 85.97%	7.64 ±5.6	- 79.89%	2.31**	5.332

4.3.5.2 Food Frequency

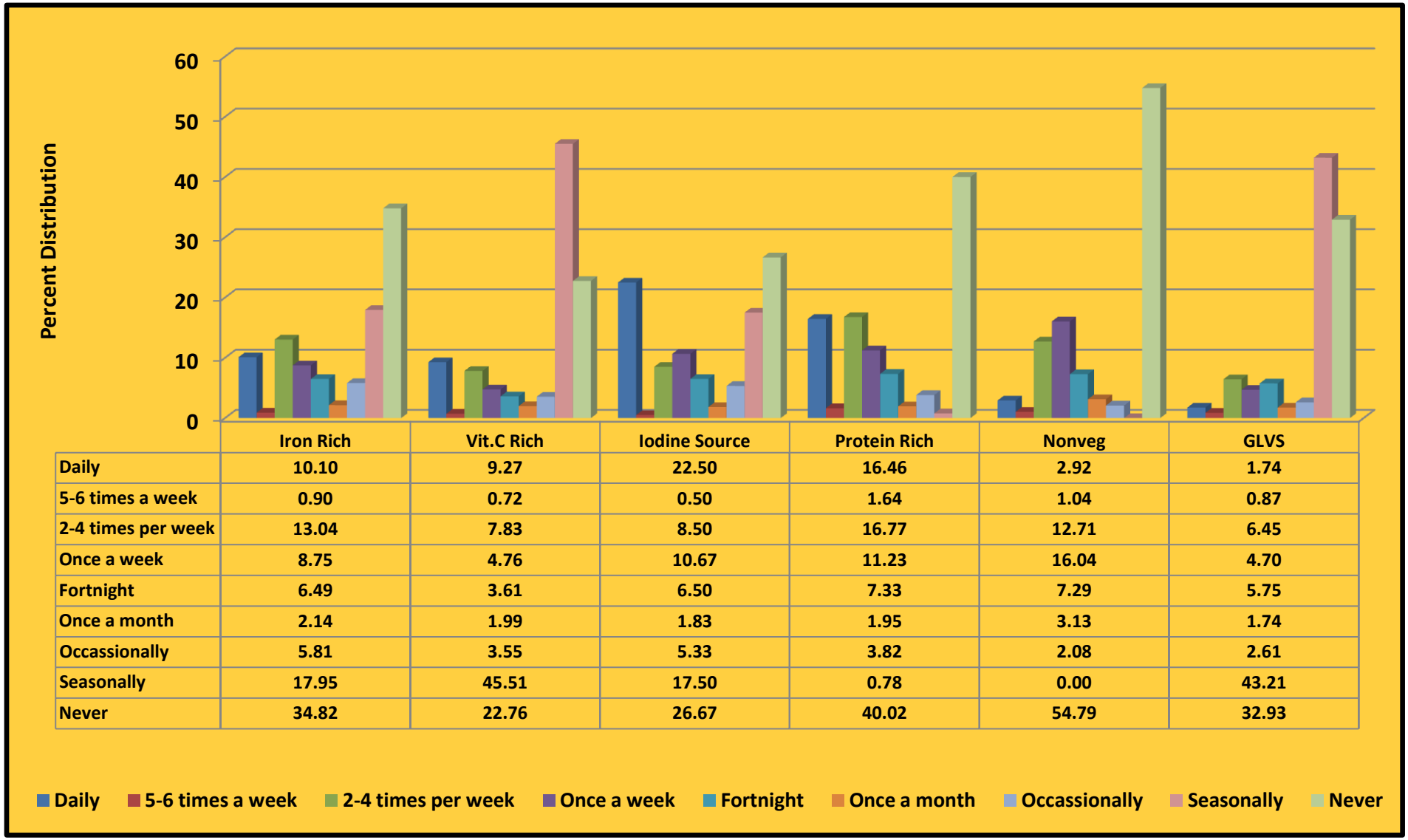
Food frequency pattern of the subjects revealed no significant difference in the nutritional intake and modifications, dietary habits or frequencies considering pregnancy as a special physiological condition. **(Figure 4.10)** revealed an overall scenario of the consumption of various food groups based on their major nutrient content.

Iron rich foods were consumed by 10.10% of the subjects on daily basis, 13.04% were consuming them 2-4 times a week and 18% subjects were consuming them seasonally. However, 34.82% subjects never consumed them. This group included Wheat, Bajra, Rice flakes-puff, Semolina, Lentil, Bengal gram (roasted), Jaggery, dates and GLVS etc.

Wheat, Bajra, Jowar and Rice flakes-puff were consumed on daily basis by 95.87%, 4.96%, 6.61% and 23.14% respectively. However, consumption of GLVS was notably lower amongst the subjects due to seasonal availability, cost and universal acceptability as a single vegetable by entire family.

Vitamin C rich foods were consumed by 9.27% subjects on daily basis, half (45.51%) of the subjects were consuming them seasonally and one fifth (22.76%) never consumed these foods. This group included Cabbage, Amla, Capsicum, Radish leaves, Citrus fruits, Lemon, Tomato, Onion stalks, Cauliflower, Guava etc. Tomato and Lemon are most frequently consumed by 81.82% and 38.84% of the subjects respectively on daily basis.

Figure 4.10: Overview of Food Frequency pattern of the subjects



	Iron Rich	Vit.C Rich	Iodine Source	Protein Rich	Nonveg	GLVS
Daily	10.10	9.27	22.50	16.46	2.92	1.74
5-6 times a week	0.90	0.72	0.50	1.64	1.04	0.87
2-4 times per week	13.04	7.83	8.50	16.77	12.71	6.45
Once a week	8.75	4.76	10.67	11.23	16.04	4.70
Fortnight	6.49	3.61	6.50	7.33	7.29	5.75
Once a month	2.14	1.99	1.83	1.95	3.13	1.74
Occassionally	5.81	3.55	5.33	3.82	2.08	2.61
Seasonally	17.95	45.51	17.50	0.78	0.00	43.21
Never	34.82	22.76	26.67	40.02	54.79	32.93

Based on their affordable economy, cabbage was most frequently consumed food item of lower income group, with a pattern of 40% consuming- 2-4 times a week and 25%-once a week consumption. Cauliflower was also consumed by 21.49% of the subjects 2-4 times a week. However, 10% were consuming capsicum once a week. Rest of the items were consumed seasonally or based on the availability at an affordable cost.

Iodine rich foods were consumed by 22.50% on daily basis, 10.67% consumed them once weekly, 17.50% subjects consumed these foods seasonally and one fourth of the subjects (26.67%) never consumed these foods. Other than iodized salt, foods like Milk, Milk products, Egg, Fish and Carrot were included in this group. Milk was consumed by 95.87% of the subjects on daily basis, it was generally consumed as tea. However, most frequently consumed milk products were curd and buttermilk. These products were consumed by 9.92% of the subjects on daily basis. However, 60% of the subjects consumed them frequently within week. Egg was consumed by 5.79% of the subjects on daily basis, Fish was consumed by 24% of the subjects at different frequencies and carrot is generally consumed based on the availability.

Protein rich foods were consumed daily and fortnightly by a similar percent (16%) of subjects. However, majority of the subjects never consumed these foods. More than half (54.79%) of the subjects were vegetarian, since non-vegetarian foods are not being consumed by the subjects. This group included Milk, Milk products, Pulses and legumes. Most frequently consumed pulse was redgram dal, which was consumed by half (52.89%) of the subjects on daily basis. However, 55.37% and 43.80% of the subjects respectively consumed Bengal gram dal and green gram dal 2-4 times a week.

Non vegetarian foods were considered as a different group, since half of the subjects were vegetarians. This group included Egg, Chicken, Mutton and Fish. Of the total non-vegetarian subjects, the highest frequency (20%) was observed to be 2-4 times per week and once a week by the subjects.

Green leafy vegetables (GLVS) were considered as iron rich and vitamin C rich foods. It was observed that, 43.21% of the subjects were consuming them based on the seasonal availability of GLVS. However, 32.93% of the subjects never consumed GLVS.

Based on the available information on food frequency, some of the food items were selected considering their cost, availability throughout the year and acceptability by the subjects. These items included Orange, GLVS, Lentil, Bengal gram (sprouted/roasted), Rice flakes-puff, Jaggery, Dates were recommended to improve upon their iron status. Post interventional impact was assessed with these foods only, since majority of the food frequencies were not expected to change within a span of six-seven months. Data on pre and post intervention consumption frequencies of the above mentioned food items are depicted in **Table 4.43.**

Table 4.43: Consumption of recommended foods

(A) Before Intervention							
	Jaggery	**Dates**	**Rice Flakes-puff**	**Bengal gram**	**Lentil**	**GLVS**	**Orange**
Daily	0	0	2.52	0.84	5.04	1.04	0.84
5-6 times	0	0	1.68	0	0	0.63	0.84
2-4 times	4.2	0	26.89	9.24	11.76	6.66	0
Once a week	1.68	0	20.17	11.76	6.72	5.83	2.52
Fortnight	0.84	0	13.44	6.72	10.92	5.41	1.68
Once a month	0	0	1.68	4.2	0.84	3.33	4.2
Occassionally	15.97	6.72	16.81	5.88	0.84	2.29	3.36
Seasonally	1.68	18.48	0	0	42.02	46.46	28.33
Never	75.63	74.78	16.8	62.18	70.58	28.33	39.5
(B) After Intervention							
	Jaggery	**Dates**	**Rice Flakes-puff**	**Bengal gram**	**Lentil**	**GLVS**	**Orange**
Daily	7.56	3.36	6.72	8.4	6.72	0	4.2
5-6 times	0	0	8.4	0	0	6.72	0
2-4 times	8.4	5.04	24.37	12.6	16.8	12.61	6.72
Once a week	5.88	3.36	8.4	16.8	10.08	28.57	8.4
Fortnight	2.52	0.84	10.92	8.4	10.08	16.8	8.4
Once a month	0	0	5.04	23.52	3.36	6.72	5.88
Occassionally	5.88	6.72	26.89	5.88	1.68	5.04	16.8
Seasonally	8.4	4.2	0	0	0	23.52	24.36
Never	61.44	76.37	9.24	24.36	51.26	0	25.21

It was observed that, more than two third (75%) of the subjects never consumed or stopped consuming Jaggery during pregnancy, which was improved by 14%. It was encouraging to note that, 7.54% of the subjects started consuming Jaggery on daily

basis in different food preparations- recommended while diet counseling, like, Raab, Golchana, Sheera etc. Consumption of rice flakes-puff also could be improved. Since 19% of the subjects never consumed rice flakes, it was of interest to observe that, 7% started to consume rice flakes-puff. There was a remarkable improvement in consumption of GLVS observed in the population. Initially 28.33% of the subjects never consumed GLVS, however, all the subjects started consuming GLVS at different frequencies indicating a success of nutrition health education although daily consumption reduced. Further, frequency for never consumed also reduced amongst rest of the foods from the recommended foods list.

4.3.6 IMPACT ASSESSMENT ON KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF THE SUBJECTS

Data on knowledge, attitude and practices regarding consumption and awareness regarding iodized salt, impact of iodine deficiencies and usage and storage of iodized salt was found helpful in developing education material to inculcate appropriate practices and achieve optimum iodine nutrition. Hence, a positive shift in behavior and practices could be achieved in both the groups towards end of the phase. **(Table 4.44).**

Initially there were average 60% of the subjects in both the groups, who were aware of iodine, iodized salt and sources of iodine, which has improved to almost 100% amongst both the groups. When information on knowledge regarding individual sources of iodine was availed, there has been a remarkable improvement amongst both the groups has been observed ($p<0.01$).

The subjects belonged to both the groups have been acquainted with the recognition of iodized salt by smiling logo and label compared to baseline ($p<0.001$); after enduring that, these were originally maintained in the standard levels for iodine. There has also been improvement in storage practices of the salt to prevent the loss of the fortified contents ($p<0.01$). However, the effective cooking practices to prevent the loss of iodine from the food being practiced by majority of the subjects after NHE was imparted regularly. The pregnant women also became aware of the consequences of chronic iodine deficiency on their fetuses and in general after provided intervention.

Table 4.44: Comparison of knowledge, attitude and practices regarding iodine nutrition between groups before and after imparting Nutrition Health Education

	Experimental		Control	
	Pre	**Post**	**Pre**	**Post**
1. Heard of Iodine				
• Yes	64.2	100	61.1	98.1
• No	35.8	-	38.9	1.9
2. Source of Iodine				
• Yes	64.2	100	59.3	98.1
• No	35.8	-	40.7	1.9
3. Source items				
• Iodized salt	65.7	98.5	57.4	96.3
• Milk and Products	3.0	37.3	-	38.9
• GLVS	-	29.9	-	44.4
• Carrot	-	10.4	-	13.0
• Egg	-	22.4	-	20.4
• Seafood	-	23.9	-	20.4
4. Source of Information				
• Newspaper	1.5	1.5	1.9	1.9
• Media	41.8	20.9	37	18.5
• AWW	20.9	1.5	20.4	1.9
• Researcher	-	97	-	96.3
5. Current Salt				
• Refined	56.7	82.1	59.3	74.1
• Crushed	43.3	17.9	40.7	24.1
• Loose	-	-	-	1.9
6. Cost of Salt				
• 1 – 3 Rs	34.3	7.5	37	7.4
• >3 – 6 Rs	14.9	13.4	9.3	16.7
• >6 Rs	50.7	79.1	53.7	75.9
7. Awareness regarding Iodized Salt				
• Yes	70.1	100	59.3	96.3
• No	29.9	-	40.7	3.7
8. Recognize Salt				
• Labels	7.5	68.7	3.7	72.2
• Sunlogo	-	31.3	-	27.8
• Don’t Know	92.5	-	96.3	-
9. Salt consumption per month				
• 1 kg	65.7		81.5	
• 2 kg	32.8		18.5	
• > 2 kg	1.5		-	
10. Point of addition of salt in preparation				
• Beginning	70.1	9.1	72.2	16.7
• Mid Way	28.4	24.2	27.8	27.8

• Towards end	1.5	66.7	-	55.6
11. Storage				
• Open Jar	1.5	-	1.9	-
• Closed Jar	97	98.5	98.1	94.4
• Packet	1.5	1.5	-	5.6
12. Storage place of Salt				
• Near cooking range	32.8	4.5	46.3	1.9
• Away from cooking range	67.2	95.5	53.7	98.1
13. Consequences of IDD				
• Still birth	-	3.0	-	3.7
• Abortions	4.5	10.4	-	13
• Mental retardation	14.9	79.1	13	77.8
• Deafmutism	-	10.4	-	3.7
• Cretins	-	3	-	5.6
• Goitre	9	22.4	11.1	9.3
• Physical defect	10.4	59.7	7.4	51.9
• Squint	-	-	-	2.4

Impact on KAP regarding iron nutrition was evaluated by questionnaire and the results were motivating in both the groups **(Table 4.45).** Baseline knowledge on iron and it's sources of information were limited, which has improved significantly towards the end. However, knowledge on individual sources of iron was also limited, which has increased upto significant levels. The study subjects who were aware of iron rich sources at baseline, the distribution on the pronounced names involved Spinach, beet and Chana depicting 41.8%, 20.5% and 31.3% respectively.

Table 4.45: Comparison of Knowledge, attitude and practices regarding iron nutrition between groups before and after Nutrition Health Education

	Experimental		Control	
	Pre	**Post**	**Pre**	**Post**
1. Heard of Iron				
• Yes	70.1	100	61.1	98.1
• No	29.9	-	38.9	1.9
2. Source of Information				
• AWW	16.4	3	18.5	1.9
• Doctor	37.3	13.4	29.6	5.6
• Media	4.5	-	1.9	-
• Researcher	-	97	-	96.3
• Others	20.9	3	13	3

3. Sources of Iron				
• Yes	68.7	98.5	57.4	96.3
• No	31.3	1.5	42.6	3.7
4. Source Items				
• GLVS	41.8	85.1	38.9	81.5
• Jaggery	1.5	28.4	1.9	22.2
• Chana	31.3	68.7	22.2	61.1
• NonVeg.	4.5	17.9	-	25.9
• Beet	20.5	55.2	17.8	31.5
• Poha	-	8.9	-	11.3
• IFA	-	6.7	-	10.4
• Dates	-	6.7	-	2.4
5. Consequences of Iron deficiency anemia				
• Anemic Child	-	68.7	-	61.1
• Low Birth Weight	-	31.3	-	13
• Weak Child	9	71.6	7.4	79.6
• Lower Immunity	-	9	-	3.7
• Poor Cognition	-	13.4	-	13

Compliance to iron folic acid supplementation is generally very poor in the mothers due to various reasons and negligence. Hence, NHE was also provided including IFA as a major aspect to generate sufficient iron stores in the mother's to provide adequate iron for their developing fetuses. Significant improvement was observed in both the groups **(Table 4.46).** It was also motivating to gather that, the pregnant women and their respective family members have become aware of individual consequences of maternal iron deficiency and survival of their fetuses. Their cognitive development and mental development also became an issue of concern amongst the would be mothers of the newborns.

Table 4.46: Impact assessment on consumption and compliance to IFA supplements

	Experimental		Control	
	Pre	**Post**	**Pre**	**Post**
1 IFA consumption regularity				
• Yes	74.6	79.1	57.4	83.3
• No	25.4	20.9	42.6	16.7
2 Frequency				
• Daily	71.6	77.6	51.9	79.6
• 3 – 4 times a week	14.9	19.4	27.8	18.5
• Irregularly	13.4	3.0	20.4	1.9

3	**Side Effects**			
• Yes	23.9	7.5	31.5	7.4
• No	76.1	92.5	68.5	92.6
4	**If Yes, What**			
• Nausea	6.0	4.5	9.3	1.9
• Vomiting	10.4	3	9.3	5.6
• Uneasiness	7.5	-	13.0	-

During pregnancy hyperemesis or gravidarum (vomiting and uneasiness) are very common physiological responses due to metabolic changes and increased level of hCG in the circulatory system during first trimester. Gradually it decreases with the progression of pregnancy, but till then the pregnant women ends up into restricting certain food items, which are actually beneficial for their nutritional status Eg. Non vegetarians stop eating non-veg. Hence, nutrition education on consuming adequate diet to meet dual requirements of nutrition- maternal and fetal- was very strongly provided and the results were highly motivating with reference to dietary intake **(Table 4.47)**, which was also reflected in the 24 hr dietary recall after intervention **(Table 4.41 & 42)**

Table 4.47: Changes in the diet quality and quantity of the subjects

	Experimental		**Control**	
	Pre	**Post**	**Pre**	**Post**
1 Change in Dietary intake				
• Yes	77.6	76.1	81.5	83.3
• No	22.4	23.9	18.5	16.7
2 Change in quantity				
• Increased	59.7	68.7	42.6	77.8
• Decreased	19.4	9	38.9	5.6
3 **Diet Pattern**				
• Vegetarian	25.4	16.4	31.5	25.9
• Non Vegetarian	74.6	83.6	68.5	74.1

Irrespective of socio economic status, majority of the families in India may have one or the other blind beliefs regarding food restriction during pregnancy. Sometimes there are strong arguments between old and new generations and that leads to sacrificing nutrition during pregnancy.

Table 4.48: Dietary restrictions and beliefs

	Experimental		Control	
	Pre	**Post**	**Pre**	**Post**
1 Following food taboos				
• Yes	73.1	29.9	85.2	35.2
• No	26.9	70.1	14.8	64.8
2 Foods restricted				
• Jaggery	52.2		57.4	
• Papaya / Banana	65.7		64.8	
• Brinjal / Ladies finger	28.4		25.9	
• Fats	6.0		11.1	
• Non – Veg	20.9		35.2	
• Curd	10.4		7.4	
3 **Foods Added**				
• Nuts	4.5		-	
• Milk Products	3.0		1.9	
• GLVS	7.5		7.4	
• Fruits	7.5		16.7	

Moreover, a pregnant woman is willing to consume but families are resistant enough on consumption of certain food items. Hence, baseline data on such food items was collected and nutrition health education was provided. This was a special session evolved for subjects who faced such problems in the family and the concerned family members were educated and counseled along with the subject. It was surprising to gather that majority of the subjects were willingly or forcefully subjected to such food taboos or blind beliefs restricting beneficial food items throughout gestation. However, towards the end of the gestation, it was encouraging to know that, the percentages could be reduced towards high significant level **(Table 4.48).**

Hence, the results on KAP and behavior towards micronutrient nutrition have improved significantly and in the positive way towards contribution of health development of the future of our Nation.

DISCUSSION

Our study results were obtained from N=256 pregnant women of lower strata of the community in a hospital based setting of Vadodara city. Longitudinal studies measuring thyroid status along with iodine and iron status estimations using population based survey indicators and from areas with sufficient iodine intake are not

frequent. Thus, the study may have been the first database in Gujarat and one of the few in India.

Micronutrient malnutrition along with the undernourished status during pregnancy is of great concern among the lower strata of the community in urban settings, since the people have got an exposure and little awareness towards their nutritional needs compared to their counterparts in rural settings. However, due to limited purchasing power, they may compromise upon their dietary part to meet their basic necessities. Hence, the prevalence of micronutrient malnutrition is unexpectedly high amongst lower section of the Indian economy.

To discuss our results of the phase in brief and at different levels, the discussion has been divided into 3 parts:

4.1 BASELINE DATA

It was observed that, majority of the subjects were residing in urban setup of the city. Hence, the limited availability of food items was not expected to be a confounding factor in meeting their dietary requirements. But majority of the subjects were uneducated, housewives and they belonged to LIG or lower MIG. Hence, their socio-economic status could have been a major confounding factor towards their sacrificed nutritional needs and lower nutritional status depicted by more than 1/3 (35%) of the population below the normal range of BMI- based on their height and weight data. This was reflected by mean weight with 45.58 kg was below the requirement for a woman to be called as healthy. Similar observation was also discussed by (Laraia B, Bodnar L & Siega- Riz A 2007), which mentioned that, a woman's nutritional status prior to and during pregnancy affects fetal growth and development and course of pregnancy, as well as her long term health status. Pre pregnancy weight has been proven to be a primary indicator for reproductive health.

Our study results on micronutrient status revealed that 14% of the population has iodine deficiency based on UIE levels and approximately 90% with iron deficiency anemia as a reflection of pre pregnancy status. If a woman with such a deficient status becomes pregnant, then there are possible chances of the mental and physical growth of the fetus may get hampered.

Further, to confirm the prevalence of iodine deficiency and thyroid insufficiency due to inadequate iodine pool and its amount in circulation, thyroid hormone profile was assessed, since UIE may not be an accurate indicator during pregnancy reported by (Zimmerman 2009)

As recommended by "The Endocrine Society" in 2007, the TSH value >2.5 µIU/ml to be considered as a standard cutoff for detecting thyroid fluctuations. Considering the cutoff, 31.1% of the population was observed to have SCH. On comparing with established reference ranges (0.6-5 µIU/ml: 1st trimester) for Indian pregnant women (Marwah 2008), 5% of the population had TSH value >5 µIU/ml. The results are comparable with the study carried out in other region of the country (Sahu M, Das V, Mittal S et al 2010). Hence, there was a need to focus on meeting iodine requirements of the population to provide sufficient iodine to their developing fetuses.

While assessing the data on iron nutrition, it is very clearly evident that, there is a need of iron supplementation from the time of enrollment, since 90% of the population were deficient and were below cutoffs recommended for classification of anemia by (WHO 2001). Majority of the population was mildly deficient but it is expected that, the situation may worsen with the progression of gestation due to hemodilution during first to second trimester and as a result the hemoglobin levels will naturally go down. According to NFHS III survey (2005-2006), 60.8% pregnant women in Gujarat are anemic compared to 47.4% in NFHS II survey reflecting worsened scenario of the iron status after 5 years also. This calls for an immediate action to initiate community based approaches to create awareness on the need and importance of micronutrient nutrition and providing a simple and cost effective fortified ingredient to the population. Therefore a single tool like ***Double fortified salt*** brought a strategic achievement in sustaining the iron levels throughout gestation.

The sub grouped (N=150) population represented the population (N=256) enrolled with respect to all biochemical parameters, nutritional status and socio-economic aspects with additional data on monthly income, type of family, number of family members and per capita income. Owing to the additional information, an estimate on expected per head expense could be drawn i.e. Rs. 750/- as a lower cutoff. This depicted that, 14% of the subjects were below the cutoff, which added another reason to the lacunae in meeting dietary requirements.

4.2 INTERVENTIONAL STRATEGIES

Based on the results availed at baseline, interventional strategies were planned to achieve optimum nutrition, in an affordable cost and acceptable manner to the community. Mainly two strategies: (1) Double fortified salt supplementation (2) Nutrition health education, were taken as steps to reach the population, who are pregnant women and their unborn infants.

Studies initiated by Narsingarao (1974) on iron fortification of common salt were a first step towards efficacy trials on fortified salt supplementation. Impact of DFS supplementation amongst adult population has been studied by many researchers and groups (Working group on fortification of salt with iron 1982; Sarma KV et al 1992; Sattarzahed M and Zlotkin S 1998; Rajgopalan S and Vinodkumar M 2000; Sivakumar B, Brahmam GNV, Nair M 2000; Diosady L et al 2006; Asibey-Berko et al 2007) from last four decades in different parts of the world including India. Respite this data on effect on iodine and iron status in pregnant women, who were supplemented with DFS is not available; except one study by NIN 2001. Hence, our study may prove to be a unique and useful data to the community to initiate consumption of DFS in regular diet and further researchers could initiate some more studies in these lines.

DFS content estimation

Content estimation for iodine and iron and stability of iodine and iron in DFS used for supplementation revealed stability of the formula with mean 40 ppm iodine and 1050 ppm iron from production to 37.5 ppm iodine and 979 ppm iron at the end (1 year).

Our results are supported by the study conducted by NIN in 2006 revealed iodine content of DFS was 40.3 ± 3.8 ppm at the time of production and 40.2 ± 2.1 ppm with high level of stability. However, another study by NIN in reported a marginal decrease in iodine content after 6 months from 30 ppm to >25ppm-<30 ppm range, while the iron content remained unchanged at 1000 ppm (Narasinga Rao 1994), after 6 months.

Acceptability

Our study results reported higher level of acceptability of DFS by the subjects (95.5%) and their family members (82.1%). A similar study reported by (Shashi V and Puri S 2008) also demonstrated the consumer acceptability criteria for DFS in two rural villages of Haryana. There was no change observed in colour and taste of the salt on storage, only there was some change observed in the texture during rainy season. This change in texture was also observed in our study results. This was observed due to humidity in the atmosphere during monsoon.

Haryana study revealed overall rating on appearance, colour, taste and texture as satisfactory, satisfactory, very good and unsatisfactory respectively. Majority of the respondents stated that, DFS is used in lesser quantity (77%) where as 19% opined for its equal consumption. In our study, the percentage of subjects using DFS in an equal quantity (70%), more than the iodized salt (5%) and lesser quantity was (25%).The appearance, texture, taste and colour were not found different than the refined salt by the subjects. However, longer cooking time or after cooking storage for more time, slight colour change was observed by 70.1% of the subjects. Based on this observation, counseling on the usage of DFS while cooking was provided and the problem of colour change could be reduced marginally. There is no data available for review on while or after cooking colour change in the preparations using NIN-DFS as per our knowledge.

4.3 IMPACT ASSESSMENT ON DIFFERENT PARAMETERS

4.3.1 Impact on nutritional status of the subjects

During pregnancy there is an expected weight gain as a result of fetal growth and development; and also as an indication of sufficient nutrition to satisfy the macro and micronutrient requirements of both.

On comparing experimental and control group for BMI in each category, there was non significant difference observed indicating similar impact of NHE in both the groups. Moreover, non significant gradual increase in mean BMI in each category was observed **(Table 4.23).** This could have been due to weight gain during pregnancy, which is a natural process. Our results on weight gained during pregnancy were

compared with the recommended weight gain by **IOM 2009.** Overall 56.7% deficit was observed among the subjects falling into underweight and normal categories of BMI during first trimester compared to lower limit of the range **(Table 4.33).** Subjects belonged to overweight category were also observed to be in 30% deficit in meeting the lower limit of the recommended range.

A similar study carried out by (Rao et al 2000) in lower strata of the city and rural outskirts of Pune city, reported a mean weight gain as 5.5 ± 2.9 kg at 28^{th} gestational week. This is a closer value to our study results.

4.3.2 Impact on micronutrient status : Iron and Iodine

4.3.2.1 Impact of iron status

There is a physiological reduction in hemoglobin concentration during the course of gestation due to increased requirement of iron caused by fetal transfer and storage, fetal growth and development. Hence, WHO recommends $\leq$ 60 mg iron/day for 6 months in pregnancy (Stoltzfus R and Dreyfuss M 1998), which was followed in our study also. As per the government program policy on controlling anemia, the pregnant women are provided iron-folic acid supplements from their first anetenatal visit till 3 months of postpartum. However, teratogenic effect of elemental iron has been demonstrated in pregnant women in early period. Hence, promoting iron rich foods from the beginning of pregnancy could be a worthwhile attempt (Shobeiri 2006).Considering this fact, half of the subjects were supplemented with DFS and NHE to all the subjects was provided to assess the expected impact using multiple approaches.

A hospital based cross sectional study in Tibet (Xing Y et al 2009) reported a significant reduction in hemoglobin concentration with increasing gestational age. However, another longitudinal study based on effect of IFA supplementation and food based approaches in Mysore city (Shobeiri 2006) demonstrated a significant increase in anemia during second trimester (49.4%) compared to first (45.6%) and significant decrease during third trimester (16.2%) compared to both the trimesters. This in turn suggests the beneficial effect of multiple interventional strategies compared to supplementing IFA only.

On comparing these reviewed literatures, we could come to a consensus in our study results on iron status of the subjects. This was reflected in hemoglobin concentrations of our both study groups, where improvement in hemoglobin concentration in third trimester compared to first and second trimester could be achieved. However, the improvement was significant in DFS supplemented (experimental group). **(Table 4.25).** There was also a shift observed in moderate and normal category of the anemia classification, towards mild category as a net result of the change in hemoglobin levels of both the groups **(Table 4.24).**

4.3.2.2 Impact on iodine status

The iodine requirement during pregnancy increases since there is an increment in thyroid hormone synthesis to provide supply for the needs of the fetus and there is an increased renal clearance during pregnancy. Moreover, it is important to ensure that whether an increase in iodine intake is required among pregnant women from iodine sufficient areas, since physiological adaptations in iodine metabolism take place among women and adequate iodine intake before pregnancy (Glinoer 2007).

Results on iodine nutrition based on urinary iodine excretion and thyroid analytes were assessed to ensure iodine and thyroid status amongst study subjects. Our results on urinary iodine excretion revealed optimum iodine nutrition with majority of the subjects having sufficient levels with median urinary iodine concentration as 329 µg/l, 333.2 µg/l and 294.56 µg/l for 1^{st} trimester, 2^{nd} trimester and 3^{rd} trimester respectively. Similar longitudinal study carried out in Bangladeshi pregnant women by (Mehdi et al 2009) revealed mean UI levels as 143.02, 132.65 and 120.48 µg/l respectively for 1^{st}, 2^{nd} and 3^{rd} trimester, which is comparatively lower than our study results though the salt iodization programme has improved in the country. Another study conducted by (Ujowundu 2010) in Nigerian pregnant women demonstrated 81% subjects with adequate or more than adequate values of UI which is similar to 86% sufficiency in our study results.

Our study subjects were observed to have adequate iodine nutrition. Hence, to maintain the daily intake of sufficient iodine level, DFS was used as a tool towards attainment of micronutrient sufficiency in experimental group; and control group was recommended to consume optimally iodized salt and iodine rich foods. Close monitoring for consumption of adequately iodized salt was carried out by estimating

iodine content from the household samples of the subjects from control group at varied point of time. It was observed that, 92% of the subjects were consuming iodized salt with $\geq$ 15 ppm iodine content.

4.3.2.3 Impact on thyroid analytes

Further, detailed assessment on iodine status was carried out by analysis results on thyroid analytes. It showed a variation in TSH and FT_4 levels when classified based on the urinary iodine sufficiency levels **(Table 4.29).** A similar study carried out by (Alvarez-Pedrerol M et al 2001) also demonstrated lower TSH and higher FT_4 levels in women with urinary iodine insufficiency.

Overall it can be stated that DFS supplementation did not bring significant changes in urinary iodine levels of the pregnant women compared to control group, since majority of the subjects were consuming adequately iodized salt.

4.3.4 IMPACT ON PREGNANCY OUTCOME

Maternal undernutrition, low pregnancy weight gain, anemia in pregnancy, and obstetric problems such as pregnancy-induced hypertension are major factors associated with low birth weight in all countries. Strenuous physical work and stress—physical and psychological - are emerging as important factors in pregnancy outcome and birth weight. Supportive supervision and counseling may play an important role in alleviation of the stress. Some of the available data from research studies suggest that the nutrition during adolescence may have a role as determinant of maternal nutrition and birth weight of the offspring (WHO statistics 2010). However, our study findings revealed higher prevalence of anemia and undernutrition, thus low pregnancy weight gain respite integrated counseling on maternal nutrition and dietary intake. Thus, it suggests a need for counseling at an early stage (adolescence and preconception stage).

On comparing pregnancy outcome as type of delivery, gestational age, incidence of LBW, neonatal anthropology **(Table 4.34)**, no significant difference was observed between both the groups. Thus, DFS is not expected to contribute upto that extent within a short span of supplementation, since its biomechanism involves slow/sustained release of iron to maintain iron status at the same time not causing iron toxicity. None of the maternal parameters/analytes correlated with neonatal

parameters except maternal hemoglobin at 3rd trimester with neonatal anthropometry. It was found significantly correlated with neonatal birth weight. One of the research on similar lines in Indian pregnant women reported by (Shobeiri 2006) also reported a higher incidence of LBW amongst anemic subjects. The percentages of LBW decreased in group where hemoglobin was in the reference range during first half of the pregnancy. In our study such discriminations were not feasible to derive since majority of the subjects were anemic from the beginning. Hence, there was a slight increase in birth weight observed with increase in hemoglobin concentration.

4.3.5 IMPACT ON DIETARY INTAKE

Research studies by (Rao et al 2000 and Shobeiri 2006) revealed inadequate dietary intake in pregnant women of lower strata compared to their counterparts from the other sections. Our study also reported a similar trend with more than 50% deficit in all macro and micronutrients assessed, except fat intake. In view of the low habitual dietary intake, lack of increase in dietary iron during pregnancy and poor bioavailability of iron from cereal based diet, iron supplementation along with appropriate health education is must. Hence, as a second line of approach to improve on maternal nutrition NHE to all the subjects and respective family members was provided, whenever required.

There was a significant improvement in mean dietary intake of each macro and micronutrient, which could be achieved by end of the study period **(Table 4.41 and 42)**. Moreover, the intake of dietary iron in 3rd trimester was observed significantly higher ($p < 0.001$) compared to 1st trimester, which is a net result of consumption of DFS by the experimental group.

Subjects were on IFA supplementation from 1st trimester till the end of gestation. Hence, diet plus an additional supplemental iron intake reached from 65 mg/day to 67 mg/day in control group and 77 mg/day in experimental group, which was again observed to be significantly higher than the control group ($p<0.001$). Food frequency analysis did not revealed a significant improvement in both the groups, except the recommended food items inclusive of iron, iodine and Vitamin C rich food items. Thus, analysis on the post interventional effect showed a significant improvement in the consumption frequency of these foods.

4.3.6 IMPACT ON KNOWLEDGE ATTITUDE AND PRACTICES OF THE SUBJECTS

Data on KAP revealed that, there was high significant improvement in KAP of the subjects with regards to all the aspects covered under the sessions of health education.

The subjects and their concerned family members are now well acquainted with the knowledge of iodine and iron sources, the storage and cooking practices for retaining maximum iodine content, the consequences of these micronutrient deficiencies on their fetuses and a few of them have started applying the knowledge into practice. A cautious desire towards improving nutritional quality of the diet has improved significantly amongst our study subjects. They are also aware of the recognition of the iodized salt from the packet.

Regularity in consuming IFA tablets and quantity and quality of dietary intake has improved markedly. There has also been motivating finding indicating a remarkable reduction in the blind beliefs and food taboos after creating awareness on the dietary facts. Thus, there has been a withdrawal of restricted food items during pregnancy with reversible pattern. Thus, ensuring better consumption of various foods.

This is an achievement in itself, since marginal improvement of micro and macronutrient status of our study subjects with beneficial effects on the health of their neonates and commendable improvement in nutrition awareness and motivation towards healthy eating habits could be handed over to them.

PHASE II

IMPACT ASSESSMENT OF DFS SUPPLEMENTATION AMONGST SCHOOL CHILDREN

In this phase children aged between 5- 15 years were included from rural villages of Waghodia Block, Vadodara district. This was further divided into 4 demographic regions based on the population and number of schools available. Four schools on a same belt were selected, out of total 172 government schools. Written consent at three levels was availed, which involved government education officials, school principals and from the parents of the students. Students were also explained the purpose and then enrolled for the study. Initially, 1184 subjects were enrolled for the study; however, 947 subjects could complete the study. Exclusion criteria included the children who were not been available in 3 consecutive visits. There was almost 30% absenteeism in rural schools. The higher rate of absenteeism is due to parental negligence towards their child's attendance or education and migration for livelihood. Few children, who were coming from the far off villages, were out of reach most of the times leading to increased absenteeism.

This phase is divided into 3 major sections:

4.4 Baseline survey
4.5 Interventional strategies
4.6 Impact assessment

4.4 BASELINE SURVEY

A baseline survey was carried out before initiating the interventions to assess the present nutritional status and to measure the IQ and cognitive function of the school children. Thus, helped to have a picture of the current status of the village children compared to the previous years. This section thus helped to derive hypothesis on the level of impact by giving interventions.

At baseline, subjects from all four schools were enrolled standard wise along with their roll numbers for the study. These subjects were distributed according to their gender and age categories before proceeding for further analysis.

Table 4.49: Percent distribution of subjects into sex and age categories

Age (yrs.) Categories	Group*	Boys (n=617)	Girls (n=567)	Total (N=1184)
<5	**1**	13 (2.11)	14 (2.47)	27 (2.28)
5 – 5.11	**2**	45 (7.29)	33 (5.82)	78 (6.59)
6 – 6.11	**3**	76 (12.32)	64 (11.29)	140 (11.82)
7 – 7.11	**4**	67 (10.86)	56 (9.88)	123 (10.39)
8 – 8.11	**5**	86 (13.94)	74 (13.05)	160 (13.51)
9 – 9.11	**6**	81 (13.13)	81 (14.29)	180 (15.20)
10 – 10.11	**7**	87 (14.10)	93 (16.40)	162 (13.68)
11 – 11.11	**8**	76 (12.32)	93 (16.40)	169 (14.27)
≥ 12	**9**	86 (13.94)	59 (10.41)	145 (12.25)

**groups have been defined chronologically for convenience of expression in further tables. Values in parenthesis depicts percentage*

(Table 4.49) depicted the distribution of the enrolled children. There were N=1184 subjects enrolled from all the schools. Of which, n=617 boys and n=567 girls made the final population. Majority of the boys and girls aged between 8 to ≥ 12 years. This is a preadolescence period and there is a raised need for nutrients for achieving optimal growth and development both in terms of physical and cognitive. Therefore, deficiency of any nutrient to any extent can be detected easily. Hence, school children have been described as a vulnerable "Indicator Group" (Andersson M. et al 2007) to assess any nutritional deficiencies in the population.

4.4.1 NUTRITIONAL STATUS ASSESSMENT

Data on anthropometry was collected using standard weighing scales and height meters. Data given in **Table 4.50** reveals gender wise mean height for age and mean weight for age of the school children.

CDC standards (2000) have been used to determine the anthropometry indices, since WHO growth standards shows weight-for-age up to 10 years of age only.

Table 4.50: Anthropometric parameters of the subjects

Group	Weight (kg) (Mean±SD)			Height (cm) (Mean±SD)		
	Boys	**Girls**	**Total**	**Boys**	**Girls**	**Total**
1	13.23 ± 1.60	13.25± 1.54	13.25 ± 1.54	101.88 ± 4.38	101.28 ± 4.85	101.57 ± 4.55
2	14.34± 1.85	13.4 ± 1.87	13.34 ± 1.91	104.89 ± 5.31	104.21 ± 4.28	104.6 ± 4.89
3	15.78 ± 2.03	15.22 ± 2. 5	15.53 ± 2.27	109.44 ± 4.96	108.20 ± 5.49	108.87 ± 5.23
4	18.02 ± 2.70	17.39 ± 2.39	17.74 ± 2.57	115.11 ± 6.31	114.04 ± 6.01	114.63 ± 6.17
5	19.10 ± 3.63	18.77 ± 3.20	18.95 ± 3.43	118.92 ± 6.04	119.23 ± 6.60	119.07 ± 6.29
6	20.67 ± 2.91	21.17 ± 3.94	20.93 ± 3.44	123.36 ± 6.40	123.71 ± 6.80	123.54 ± 6.59
7	23.81 ± 4.13	23.67 ± 4.84	23.74 ± 4.50	129.99 ± 6.43	129.36 ± 6.46	129.67 ± 6.44
8	24.53 ± 4.03	26.85 ± 5.29	25.81 ± 4.90	132.32 ± 7.13	134.40 ± 7.11	133.47 ± 7.18
9	28.46 ± 5.87	30.67 ± 5.87	29.36 ± 5.95	139.03 ± 9.30	139.50 ± 6.27	139.23 ± 8.18
Total	**20.95 ± 5.88**	**21.50 ± 6.61**	**21.21 ± 6.20**	**122.56 ± 12.74**	**123.06 ± 12.54**	**122.80 ± 12.75**

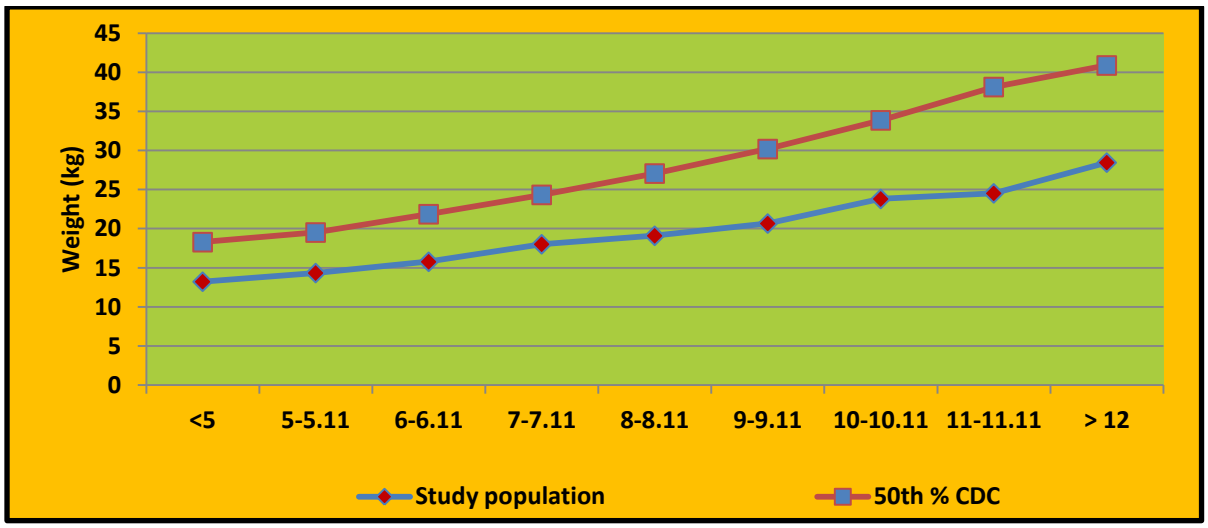
**groups have been defined chronologically for convenience of expression in further tables*

Observed mean weight of the boys was 20.95 ± 5.88 kg and 21.50 ± 6.61 kg for girls. Mean height of the boys was 122.56 ± 12.74 cm and 123.06 ± 12.54 cm in girls. There was non-significant difference between both the groups. Overall the mean weight was 21.21 ± 6.20 kg and mean height was 122.80 ± 12.75 cm for the population.

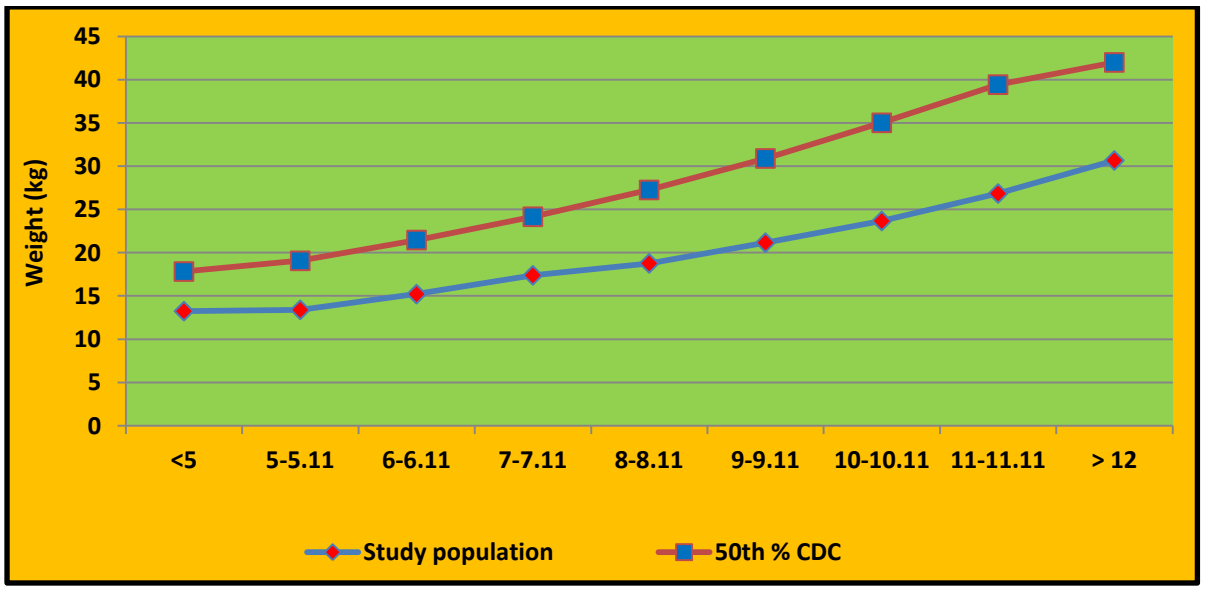
When data on mean weight of both the genders were compared with 50^{th} percentile values of the CDC standards, the difference was observed to be significant **Figure 4.11 (A) - Boys, (B)-girls**

Figure 4.11: Weight comparison of children with 50th percentile of CDC standards

(A)- Boys



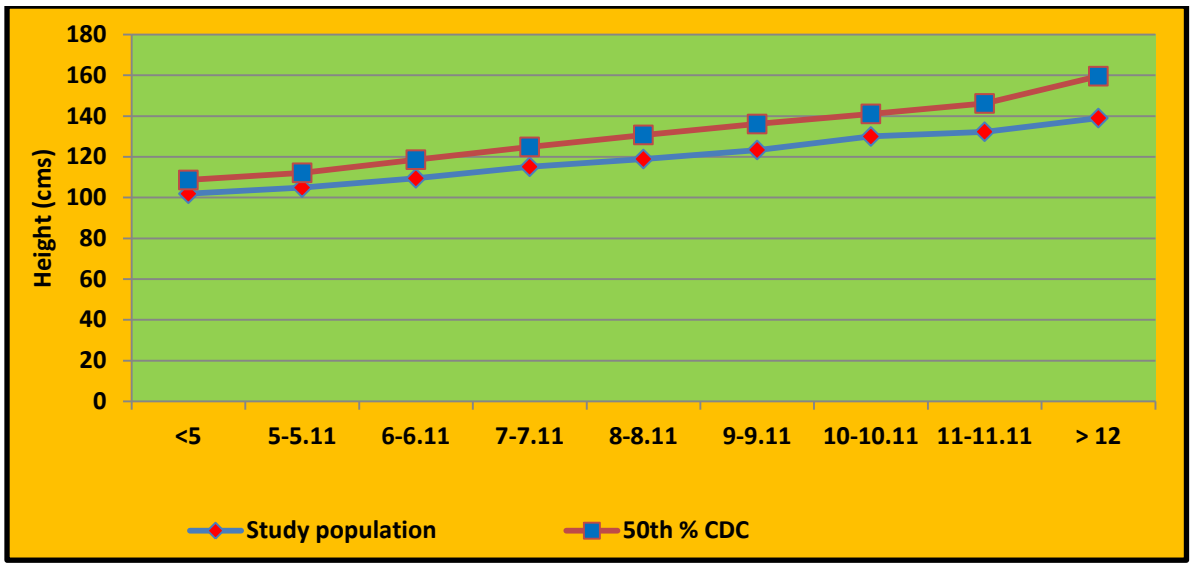
(B) – Girls



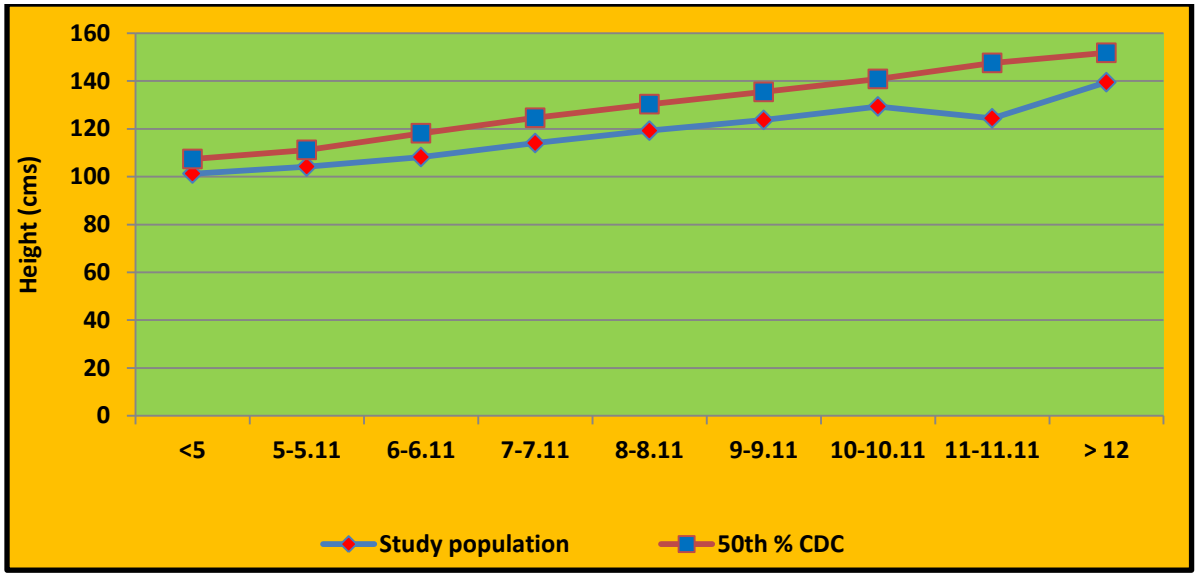
Further, data was also compared for height of the children with 50th percentile of CDC standards and the difference could be observed from the **Figure 4.12 (A)-Boys and (B)-Girls.**

Figure 4.12: Height comparison of children with 50th percentile of CDC standards

(A)- Boys



(B)- Girls



When, Z scores of the population, were compared with the CDC standard, it was also observed that, the prevalence of severe stunting (<-3 SD) was 13.60%, with 15% of the boys and 11.6% of the girls. There was a non significant difference between both the genders but the prevalence was higher amongst boys compared to girls. Overall 44.60% of the children were stunted (<-2SD). However, 20% of the children were falling into normal category **(Table 4.51).**

Table 4.51: Prevalence of undernutrition (CDC standards)

Nutritional Status (Z score)	Percent subjects			Chi square
	Boys	Girls	Total	
Height for Age (HAZ)				
-1 to >0	129 (20.9)	110 (19.4)	239 (20.19)	4.715^{NS}
2 to -1.01	211 (34.2)	206 (36.3)	417 (35.22)	
-3 to -2.01	182 (29.5)	185 (32.6)	367 (31.00)	
<-3	95 (15.4)	66 (11.6)	161 (13.60)	
Weight for Age (WAZ)				
-1 to >0	47 (7.6)	64 (11.3)	111 (9.38)	10.31**
-3 to -2.01	120 (19.4)	115 (20.3)	235 (19.85)	
-2 to -1.01	175 (28.4)	181 (31.9)	356 (30.07)	
<-3	275 (44.6)	207 (36.5)	482 (40.71)	
BMZ				
-1 to >0	171 (27.8)	120 (21.2)	209 (17.65)	17.012***
-2 to -1.01	89 (14.4)	167 (29.5)	338 (28.55)	
-3 to -2.01	149 (24.2)	143 (25.2)	292 (24.66)	
<-3	207 (33.6)	137 (24.2)	344 (29.05)	

*Values in parenthesis depicts percentage. **p<0.01,***p<0.001*

On comparing children for the weight for age indices, there was a significant difference between both the genders. Severe underweight was observed amongst 44.6% of the boys compared to 36.5% girls (p<0.01), thus depicted higher prevalence of thinness also amongst boys with 33.6% compared to 24.2% girls (p<0.001).This finding on gender wise comparison was supported by, the results of (Das et al 2009). They conducted a study in Kharagpur town. A total of N=930 (Boys-472 and Girls-458) were investigated for their anthropometry indices. While comparing the data between both the genders, a significant difference was observed upon undernutrition with higher prevalence amongst boys (35.59%) compared to the girls (19.43%) counterparts.

In our study, results on overall severe underweight and thinness were observed amongst 40% and 30% of the children respectively. However, 70.79% (<-2SD) of the children were categorized as underweight and 53.71% (<-2SD) of the children were falling under thinness indices. Using WHO growth standards 2007, percent distribution of weight-for-age classification was found to be slightly different than the CDC standards (2000). The distribution revealed 77.8% (<-2SD) children underweight, which was comprised of 71.1% boys and 65.1% girls. However, the

gender wise difference for underweight prevalence was visible using both the classifications.

While the difference on percent distribution for thinness (BMZ) and stunting (HAZ) was observed to be minute. Our data suggests the higher rate of malnutrition in current times amongst rural school children and adolescents respite existing various health programs in place.

The departmental studies conducted by (Iyer U and Daundhiyal G 2010; Iyer U and Jain V 2011; Bhoite R and Iyer U 2011; Sharma K and Dave S 2009) were also carried out in rural villages of Gujarat and majority of them depicted the nutritional scenario of rural Vadodara. These studies also demonstrated the similar status with respect to underweight, stunting and thinness.

A study conducted by Iyer U and Daundhiyal G (2010) in Gandhinagar amongst primary school children revealed a prevalence of underweight, stunting and thinness with ranges 65.5-71.8%, 35.1-59.5% and 60.2-67.5% respectively. However, another study by Iyer U and Jain V (2011) depicted the prevalence of undernutrition in rural Vadodara with 78.9%, 48% and 65.4% respectively, while a study by Bhoite R and Iyer U (2011) also supported these findings with 70%, 31.5% and 65.03% prevalence rate in rural villages of Vadodara. All these studies together support our study findings, since the total prevalence of underweight, stunting and thinness was 70.78%, 44% and 53.71% respectively (CDC standards).

4.4.2 MICRONUTRIENT STATUS ASSESSMENT

Assessment of micronutrient status included iron and iodine status of the children in the current phase. It is widely known that, micronutrient deficiencies like iodine, iron and Vitamin A strikes the children of rural and urban India, but the most prevalent are iodine and iron, which affects the physical and mental growth of the children in its hidden form which persists in majority of them. Hence, assessment of the status of these two micronutrients is very essential.

4.4.2.1 Iron status assessment

Iron status of the children was assessed by using hemoglobin levels as an indicator. Of the total N=1184 enrolled children, data on hemoglobin concentration availed were

for n=972 children, owing to the dropout rates due to refusal by the children for finger prick and few of the children were not attending the school regularly. Data on iron status revealed that, mean hemoglobin of the population ranged from 8.83 – 9.62 g/dl.

Table 4.52: Age group and gender wise iron status of the subjects

Age (Yrs)	Group*	Boys (N=617)	Girls (N=567)	Total (N=1184)	"t" value
<5	1	9.15 ± 1.02	10.05 ± 1.03	9.62 ± 1.10	2.26*
5- 5.11	2	9.25 ± 1.13	9.01 ± 1.11	9.15 ± 1.12	0.91
6- 6.11	3	9.08 ± 1.08	8.93 ± 1.27	9.02 ± 1.16	0.67
7- 7.11	4	9.46 ± 1.20	9.38 ± 1.24	9.43 ± 1.21	0.31
8- 8.11	5	9.06 ± 1.28	8.53 ± 1.34	8.83 ± 1.33	0.22*
9- 9.11	6	9.46 ± 1.21	9.34 ± 1.15	9.40 ± 1.18	0.57
10- 10.11	7	9.49 ± 1.21	9.16 ± 1.25	9.34 ± 1.23	1.67
11- 11.11	8	9.03 ± 1.34	8.83 ± 1.03	8.92 ± 1.18	0.91
≥ 12	9	9.33 ± 1.30	8.98 ± 1.24	9.18 ± 1.28	1.42
Total 95% CI		**9.27 ± 1.22 (9.17-9.37)**	**9.04 ± 1.23 (8.94-9.14)**	**9.17 ± 1.23 (9.10-9.24)**	**2.88****

groups have been defined chronologically for convenience in expression in further tables.*p<0.05,p<0.01.*

This value revealed that, the population was suffering from moderate levels of iron deficiency anemia **(Table 4.52).** Mean hemoglobin of the boys ranged from 9.03-9.49 g/dl for all age groups. Mean hemoglobin of the girls ranged from 8.83- 10.05 g/dl, which showed wider variations within the gender group, the mean value of the different age groups. Age wise mean hemoglobin values were non significantly different for both the genders, except <5 years ($p<0.05$) and 8-8.11 years ($p<0.05$).

Overall mean hemoglobin value of the boys was 9.27 ± 1.22 (95% CI: 9.17-9.37) g/dl and 9.04 ± 1.23 g/dl (95% CI: 8.94-9.14) for girls. The difference was observed to be significant ($p<0.01$).

Similar pattern of study conducted in Delhi in 2003 (Gomber et al 2003) to assess the prevalence of anemia in children of age group 5 -10.9 years. It revealed that mild anemia, among 5 -5.9 years old children was 28.9% and mild anemia among ≥6 years old was 42.4%. There was no significant difference in the mean Hb values among males (11.90 ±1.90g/dl) and girls (11.85 ± 1.20g/dl). In this study, the notable feature was the presence of Vitamin B12 deficiency as the second most common cause of

deficiency anemia. The higher prevalence of combined deficiency anemia (Iron and Vitamin B12) may be attributed to inadequate food intake, poor stores and other nutritional deficiencies among these children.

Another study conducted by (Goyle A and Prakash S 2009) in government schools of Jaipur city has revealed higher prevalence of anemia amongst adolescent girls (10-12 years) with mean hemoglobin 9.23 ± 1.36 g/dl. Percent prevalence observed was 31.2% mildly and 65.1% moderately anemic.

According to WHO classification for anemia 2001, children were classified with mild, moderate and severe anemic **(Table 4.53)**. There were average 2% of the children without anemia. However, of the total 98% anemic subjects, 69.7% were moderately and 24.1% were mildly anemic. Severe anemia was observed amongst 4.4% of the total population. Moderate anemia was more prevalent amongst girls (71.8%) and mild anemia was more prevalent amongst boys (29.5%) compared to their counterparts of the opposite gender. There was no significant difference in the prevalence of anemia between both the genders (γ^2-5.520, $p>0.05$). Thus, all the categories of anemia were observed to be prevalent at all ages and in both the genders.

Our study results were supported by the departmental study (Iyer U and Jain V 2011) with 98% anemia observed. There were 46% mildly anemic, 51% moderately anemic and 1% severely anemic. This in turn revealed lower prevalence of moderate and severe anemia, but comparatively higher prevalence of anemia compared to our study results.

Table 4.53: Distribution of subjects according to WHO classification for anemia

Age Group* (Yrs)	Mild (10-11.49 g/dl)			Moderate (7-9.99 g/dl)			Severe (<7 g/dl)			Normal(>11.5 g/dl)		
	Boys	**Girls**	**Total**	**Boys**	**Girls**	**Total**	**Boys**	**Girls**	**Total**	**Boys**	**Girls**	**Total**
1	3	6	9	10	7	17	--	--	--	--	1	1
	(23.1)	(42.9)	(33.3)	(76.9)	(50)	(63)	(0)	(0)	(0)	--	(7.1)	(3.7)
2	10	6	16	30	23	53	2	2	4	1	--	1
	(23.3)	(19.4)	(21.6)	(69.8)	(74.2)	(71.6)	(4.7)	(6.5)	(5.4)	(2.3)	--	(1.4)
3	14	10	24	48	35	83	3	2	5	--	--	--
	(21.5)	(21.3)	(21.4)	(73.8)	(74.5)	(74.1)	(4.6)	(4.3)	(4.5)	--	(0)	(0)
4	18	14	32	37	20	57	--	3	3	--	1	1
	(32.7)	(36.8)	(34.4)	(67.3)	(52.6)	(61.3)	(0)	(7.9)	(3.2)	--	(2.6)	(1.1)
5	16	9	25	54	42	96	3	5	8	1	--	1
	(21.6)	(16.1)	(19.2)	(73)	(75)	(73.8)	(4.1)	(8.9)	(6.2)	(1.4)	(0)	(0.8)
6	19	12	31	50	48	98	3	1	4	4	2	6
	(25)	(19)	(22.3)	(65.8)	(76.2)	(70.5)	(3.9)	(1.6)	(2.9)	(5.3)	(3.2)	(4.3)
7	25	19	44	51	50	101	2	4	6	3	--	3
	(30.9)	(26)	(28.6)	(63)	(68.6)	(65.6)	(2.5)	(5.5)	(3.9)	(3.7)	--	(1.9)
8	13	10	23	39	60	99	4	4	8	2	--	2
	(22.4)	(13.5)	(17.4)	(67.2)	(81.1)	(75)	(6.9)	(5.4)	(6.1)	(3.4)	--	(1.5)
9	19	11	30	40	33	73	3	2	5	2	1	3
	(29.7)	(23.4)	(27)	(62.5)	(70.2)	(65.8)	(4.7)	(4.3)	(4.5)	(3.1)	(2.1)	(2.7)
Total	**137**	**97**	**234**	**359**	**318**	**677**	**20**	**23**	**43**	**13**	**5**	**18**
	(29.5)	**(21.9)**	**(24.1)**	**(67.9)**	**(71.8)**	**(69.7)**	**(3.8)**	**(5.2)**	**(4.4)**	**(2.5)**	**(1.1)**	**(1.9)**

Values in parenthesis depicts percentage

4.4.2.2 Iodine status assessment

Iodine status of the children was assessed using urinary iodine excretion and thyroid hormones as indicators. Urinary iodine reflects the recent dietary intake of iodine and thus proves to be a good index for evaluating the degree of iodine deficiency. If sufficient numbers of specimens are collected, iodine concentration in casual urine samples of children can provide an adequate assessment of population's iodine nutrition. On collecting data of urine samples, of the total N=1184 enrolled children, data on urinary iodine concentration could be availed on n=1034 children. The dropout rates owed to refusal by the children for giving the sample and few of the children were not attending the school regularly. Median urinary iodine excretion of the children has been presented in the **Table 4.54** based on the gender and age classified groups.

Table 4.54: Iodine status of the subjects (Based on UIE)

Age (Yrs)	Group	Urinary iodine levels(µg/l) (Median+ SD)			't' value
		Boys	Girls	Total	
<5	1	101.40 ± 71.33	180.18 ± 127.43	122.60 ± 105.15	74*
5- 5.11	2	202.44 ± 94.74	204.24 ± 109.77	202.44 ± 101.82	610^{NS}
6- 6.11	3	140.91 ± 94.76	151.59 ± 116.46	144.57 ± 104.16	1673^{NS}
7- 7.11	4	182.75 ± 125.09	194.24 ± 110.85	188.76 ± 119.46	1193^{NS}
9- 9.11	5	136.14 ± 116.98	124.59 ± 84.23	129.77 ± 104.47	2156^{NS}
8- 8.11	6	131.91 ± 99.39	141.48 ± 115.14	138.71 ± 107.19	2560^{NS}
10- 10.11	7	179.04 ± 108.28	140.68 ± 121.39	180.00 ± 107.50	3411^{NS}
11- 11.11	8	122.68 ± 112.07	188.30 ± 107.32	126.99 ± 116.81	2323^{NS}
≥ 12	9	152.02 ± 95.02	134.16 ± 85.66	139.37 ± 91.62	1551^{NS}
Total		**147.43 ± 106.46**	**144.92 ± 109.54**	**146.33 ± 107.81**	**1347^{NS}**

**p<0.05*

Median urinary iodine excretion of the boys was 147.43 µg/L and 144.92 µg/L for girls. The overall median urinary iodine excretion was 146.33 µg/L for the population, which suggested iodine sufficiency with slightly higher levels of excreted iodine than the cutoffs. These results were encouraging enough to observe the difference within a span of 5 years, since a departmental study conducted by (Nair S and Chitre N 2007, unpublished) showed a median UIE to be 44 µg/L at baseline. However, in our study

the levels were observed to be higher. This could be due to availability of optimally iodized salt from locally produced iodized salt also. This was a result due to efforts made by (Nair S and Joshi K, 2007-2011, unpublished) on building up the capacity for iodized salt production amongst local salt producers. Further, it could also be due to the lifting of ban between 2004 to 2006 on consumption of iodized salt for all, but in May 2006 the ban was reinstated. Hence, the improvement on iodine nutrition was observed.

Our findings were supported by the study conducted by Biswas A et al (2002) in Malda district, West Bengal amongst school children. The data on UIE revealed median urinary iodine excretion to be 150 µg/L with 85.3% iodine sufficient and 14.7% mildly deficient children. The findings indicated the transition phase from iodine deficiency to iodine sufficiency with 85.1% iodized salt consumption in the district. Out study population also reflected the similar scenario with 29.7% iodine deficient children and 82.4% of the population was consuming iodized salt based on the findings of the data availed by assessing household salt samples for iodine content **(Table 4.55).**

Table 4.55: Availability of iodized salt in the study region

Iodine Levels (ppm)	Samples N (%)	Categories
0 ppm	4 (1.3)	No iodine
< 15 ppm	49 (16.2)	Inadequate
≥15 ppm	249 (82.4)	Adequate(Recommended)
Total	**302(100)**	

Values in parenthesis depicts percentage

According to epidemiological criteria based on WHO/ICCIDD/UNICEF (2007) classification for urinary iodine cutoffs towards mapping iodine deficiency disorder amongst the population, the distribution was skewed towards the right of the curve, indicating majority of the children with sufficient excretion of urinary iodine levels. It was observed that, average 70% of the subjects had sufficient urinary iodine excretion, which comprised of 69.8% boys and 70.9% of girls **(Table 4.56).** There were still 30% of the children striving to reach the desired urinary iodine excretion levels or can be stated that, these children were had insufficient iodine nutrition. Therefore, an intervention containing optimal iodine content is a must to reach those deficient children, who were compromising on their daily requirement of iodine.

Table 4.56: Distribution of subjects according to WHO classification for IDD by UIE

Age Group*	Normal (≥ 100 µg/L)			Mild (50- 99.99 µg/L)			Moderate (20-49.99 µg/L)			Severe (< 20 µg/L)		
	Boys	**Girls**	**Total**	**Boys**	**Girls**	**Total**	**Boys**	**Girls**	**Total**	**Boys**	**Girls**	**Total**
1	8	10	18	4	1	5	1	3	4	--	--	--
	(61.5)	(71.4)	(66.7)	(30.8)	(7.1)	(18.5)	(7.7)	(21.4)	(14.80)	--	--	--
2	36	27	64	5	2	7	4	1	5	--	1	1
	(80)	(87.5)	(83.1)	(11.)1	(6.3)	(9.1)	(8.9)	(3.1)	(6.5)	--	(3.1)	(1.3)
3	53	33	86	7	10	17	6	6	12	4	1	5
	(75.7)	(66)	(71.7)	(10)	(20)	(14.2)	(8.6)	(12)	(10)	(5.7)	(2)	(4.2)
4	43	27	70	10	8	18	6	3	9	4	4	8
	(68.3)	(64.3)	(66.7)	(15.9)	(19)	(17.1)	(9.5)	(7.1)	(8.6)	(6.3)	(9.5)	(7.6)
5	51	41	92	18	10	28	9	8	17	--	1	1
	(65.4)	(68.3)	(66.7)	(23.1)	(75)	(20.3)	(11.5)	(13.3)	(12.3)	--	(1.7)	(0.7)
6	51	51	102	24	11	35	3	7	10	2	1	3
	(63.8)	(72.9)	(68)	(30)	(15.7)	(23.3)	(3.8)	(10)	(6.7)	(2.5)	(1.4)	(2)
7	66	64	130	13	11	24	5	4	9	1	3	4
	(77.6)	(78)	(77.8)	(15.3)	(13.4)	(14.4)	(5.9)	(4.9)	(5.4)	(1.2)	(3.7)	(2.4)
8	40	50	90	17	13	30	4	6	10	3	6	9
	(62.5)	(66.7)	(64.7)	(26.6)	(17.3)	(21.6)	(6.3)	(8)	(7.2)	(4.7)	(8)	(6.5)
9	50	33	83	16	10	26	6	7	13	--	--	--
	(69.4)	(66)	(68)	(22.2)	(20)	(21.3)	(8.3)	(14)	(10.7)	--	--	--
Total	**398**	**337**	**735**	**114**	**76**	**190**	**44**	**45**	**89**	**14**	**17**	**31**
	(69.8)	**(70.9)**	**(70.3)**	**(20)**	**(16)**	**(18.2)**	**(7.7)**	**(9.5)**	**(8.5)**	**(2.5)**	**(3.6)**	**(3)**

Values in parenthesis depicts percentage

The results were also motivating by looking into the prevalence of IDD observed by Nair S and Chitre N (2007, unpublished). The researchers observed prevalence of IDD amongst boys with 17.8% normal, 28.9% mild and 42.2% were moderately deficient. However, in females 21.2% were normal, 23.1% were mild, 44.2% were moderately deficient. In our study results for boys showed 69.8% normal with 20% mild, 7.7% moderate and 2.5% were severely deficient. However, girls showed a percent prevalence distribution pattern with 70.9% normal, 16% mild, 9.5% moderate and 3.6% severely deficient.

4.4.2.3 Thyroid analytes

Thyroid hormone profile of a subsampled group (N=212) was carried out to have a picture of the prevalent status. The subsamples were selected on a random basis, irrespective of their age and gender.

Table 4.57: Thyroid analytes of the subjects at baseline (N=212)

Indicator	Mean	SD	Median	5th percentile	95th percentile
Boys (n=112)					
TSH	2.87	1.42	2.54	0.88	5.69
FT_4	1.46	0.21	1.46	1.15	1.81
TT_4	9.71	1.65	9.82	7.09	12.61
Ln_Tg	1.52	1.43	1.65	3.37	-0.93
Girls (n=100)					
TSH	2.87	1.23	2.50	1.34	5.06
FT_4	1.30	0.14	1.28	1.11	1.54
TT_4	9.75	1.66	9.95	6.62	12.21
Ln_Tg	1.48	1.36	1.69	-1.61	3.02
Total (n=212)					
TSH	2.87	1.33	2.54	1.08	5.36
FT_4	1.39	0.20	1.36	1.12	1.73
TT_4	9.73	1.65	9.87	7.00	12.34
Ln_Tg	1.50	1.39	1.65	-1.02	3.29

On an average 50 children from each school and 7-8 children from each standard (1^{st} to 6^{th}) were selected for availing serum aliquots for thyroid hormone assessment. Hence, final number for thyroid hormone analysis was n=212.

Data on thyroid analytes revealed that, mean TSH value of boys was 2.87 µIU/ml (Percentile range: 0.88-5.69) and 2.87µIU/ml (Percentile range: 1.34-5.06) of girls. The difference was non significant between both the genders. However, overall mean FT_4 concentration of the children was 1.39 ng/dl and it varied gender wise. Mean FT_4 value of the boys was 1.46 ng/dl, which was significantly higher compared to girls with 1.30 ng/dl ($p<0.001$). Mean TT_4 and Tg values of the population were 9.73 µg/dl and 1.50 µg/L, these values did not vary according to gender **(Table 4.57).**

Based on the classification mentioned by (Das et al 2011 and Marwah et al 2008), the children were classified as for thyroid dysfunctions. There were 204 (96.23%) children without any thyroid dysfunction-euthyroids. There were 5 (2.36%) children with subclinical hypothyroidism (TSH >97th percentile and normal TT_4), 3 (1.4%) with subclinical hyperthyroidism (TSH <0.27 µIU/ml and normal TT_4). However, there were no cases detected with hypothyroidism (TSH >97th percentile and TT_4<4.8 µg/l) and hyperthyroidism (TSH <0.27 µIU/ml and TT_4>12.7µg/l). This in turn suggests that, thyroid insufficiency was not prevalent amongst the population and indicated that, the children are on sufficient iodine nutrition.

The prevalence of subclinical hypothyroidism was resembled by the study conducted amongst school age children of Chattigarh, a case control study where the prevalence of subclinical hypothyroidism was 2.7% amongst children with goiter and 2.4% amongst normal children (Das et al 2011).

4.4.3 IQ AND COGNITIVE STATUS ASSESSMENT

Other than the biochemical parameters for assessing iodine and iron deficiencies, IQ and cognitive function tests were also performed to measure the intensity of the deficiencies and development of any neuronal malfunctioning. These tests were used as field indicators to measure IQ and cognitive development of the children **(Table 4.58).**

Baseline data on IQ and cognition could be collected for maximum n=864 children due to various limitation like inability to draw, read and write in a given time, lack of

interest, higher rate of absenteeism, unwillingness to participate. Hence, to maintain the authenticity of the data, such children were excluded.

Table 4.58: Gender and age wise IQ and cognition test scores of the subjects (N=1184)

Tests	Boys			Girls			't' value	
	<12 yrs	≥12 yrs	Total	<12 yrs	≥12 yrs	Total	<12 yrs	≥12 yrs
Draw-a-man Test (DMT) (N=823)								
	(n=374)	(n=40)	(n=414)	(n=368)	(n=41)	(n=409)		
Mean ± SD	85.82 ± 18.76	66.77 ± 12.33	83.97 ± 19.09	88.00 ± 19.43	64.60 ± 12.71	85.66 ± 20.13	1.56^{NS}	0.75^{NS}
Median	83.26	66.67	82.13	85.71	64.95	83.54		
't' value	$^{b}8.79***$			$^{c}10.49***$			$^{a}1.23^{NS}$	
Visual Memory Test (VMT) (N=864)								
	(n=402)	(n=46)	(n=448)	(n=371)	(n=45)	(n=416)		
Mean ± SD	0.38 ± 0.22	0.54 ± 0.45	0.40 ± 0.23	0.47 ± 0.28	0.61 ± 0.21	0.48 ± 0.27	$4.83***$	0.76^{NS}
Median	0.32	0.55	0.36	0.45	0.59	0.50		
't' value	$^{b}4.97***$			$^{c}7.31***$			$^{a}4.58***$	
Clerical Task (CT) (N=863)								
	(n=408)	(n=46)	(n=454)	(n=363)	(n=46)	(n=409)		
Mean ± SD	0.71 ± 0.27	0.80 ± 0.21	0.72 ± 0.26	0.76 ± 0.24	0.77 ± 0.26	0.76 ± 0.25	$4.97***$	1.09^{NS}
Median	0.81	0.88	0.81	0.81	0.88	0.81		
't' value	$^{b}5.03***$			$^{c}7.47***$			$^{a}4.75***$	

*^{a}'t' value for comparison between total scores of boys and girls, ^{b}'t' value for comparison between both the groups of boys, ^{c}'t' value for comparison between both the groups of girl.***p<0.001*

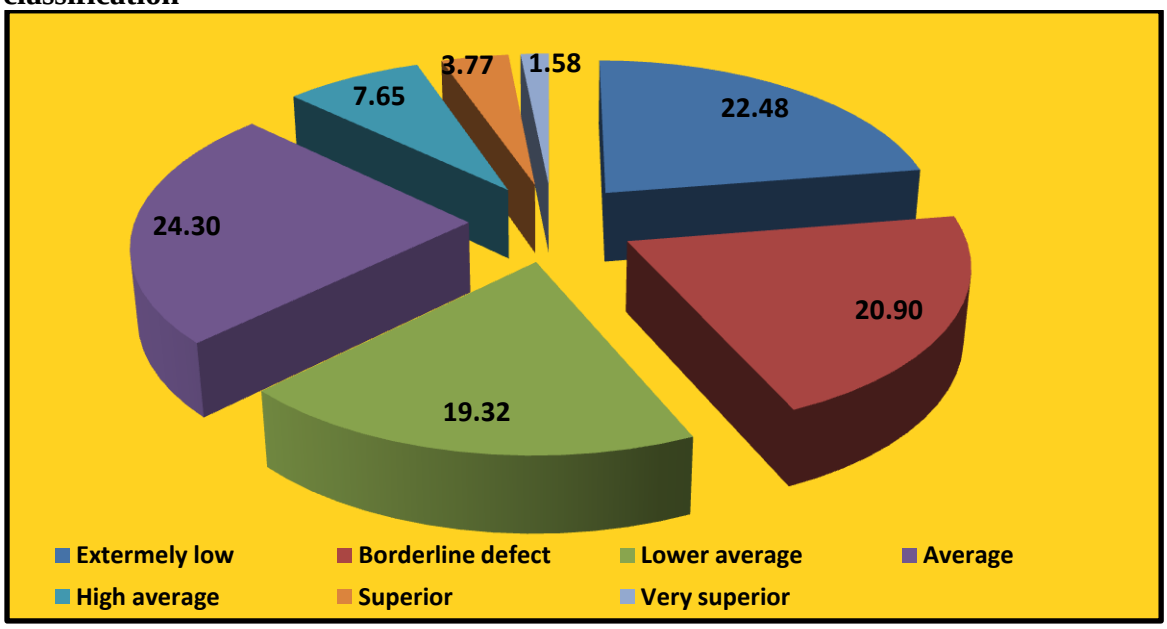
Draw-a-man test (DMT): This test is used to assess the intelligent quotient of the children and their brain development. Hence, this test was selected considering the effect of iodine deficiency on IQ of the children **(Table 4.58).**At baseline, this test could be performed on n=823 children. Mean score for the population was 84.80 ± 19.62, which comprised of the mean scores of 83.97 ± 19.09 for boys and 85.66 ± 20.13 of the girls. There was a significant difference (p<0.001) between scores within

group (age) for each gender. However, there was a non significant difference between group wise scores of both the genders.

In an earlier study in India by (Sheshadri S and Gopaldas T 1989), DMT scores were found to be 86.0 ± 2.5 and 85.0 ± 3.0 for experimental and control groups in boys respectively. These scores were of the urban school children of Vadodara and they have now been comparable to the scores of the rural school children. This could be due to increased awareness or adequate iodine nutrition amongst current generation compared to the previous one.

Further analysis on the final scores of both the genders did not show any significant difference (^{a}**1.23, p>0.05).** Based on the classification for IQ by Phatak P (2002) the children were on **lower average scores**. Thus, extending the distribution of the DMT scores as per the classification provided by Phatak P (2002) and Weschler's scales, our study children were divided into various categories of their IQ **(Figure 4.13).**

Figure 4.13: Percent distribution of the children based on the DMT scores classification



Overall distribution revealed majority of the subjects into average and lower average grades with 24.3% and 19.3 % respectively. However, there were 20.9% children observed to have borderline defect indicating achievement of lowest score to be classified as normal children compared to 22.5% with extremely lower percentages.

This difference could have been due to their surroundings, their limited exposure and imagination, perception and mental stability. In some cases, there could have been lack of interest in drawing and other confounding factors which would have obstructed their performance. Children from the same group were also there, who had achieved scores above average, superior and very superior with 7.7%, 3.8% and 1.6% respectively. This means that, they had a better perception for drawing a human figure and capabilities to express their imaginations on paper, which in turn depicts the level of IQ indirectly.

Hence, this test has demonstrated two extremes of IQ from a homogenous group of children derived from DMT. But if there is a battery of tests performed related to IQ for future research, then their average performance from all test scores may give a better idea of their actual IQ. However, in our study we found the distribution pattern of IQ scores skewed towards left side of the curve indicating lower performance of the children.

Visual memory test (VMT): This test is used to assess the short term memory power and identification-recall capability of the children. When there is predominant anemia or iodine deficiency, then the test may show variability compared to the normal subjects **(Table 4.58)**. At baseline, this test could be performed on n=864 children. Mean score for the population was 0.44 ± 0.25, which comprised of the mean scores of 0.40 ± 0.23 for boys and 0.48 ± 0.27 of the girls. There was a significant difference ($p<0.001$) **('t' value- ^{b}4.97 and ^{c}7.31)** between scores for age groups (<12 years and ≥ 12 years) of each gender. There was also a significant difference ('t' value- 4.83, $p<0.001$) observed between <12 years age group scores of both the genders, but it was non significant for ≥ 12 years age group. Further analysis on the final scores of both the gender showed a significant difference (^{a}**4.75, p<0.001).**This revealed that, the girls performed better than the boys and had a good concentration power.

On comparing our findings with a departmental study conducted by Sen A and Kanani S (2007), mean scores of VMT in the school girls belonged to urban Vadodara were higher compared to our study population. The observed mean score was 0.54 for younger girls (9-11 years) and 0.80 for older girls (12-13 years). However, these scores were 0.47 for <12 years girls and 0.61 for ≥ 12 years girls. This difference could be due to very high prevalence of anemia and under nutrition amongst our study

population. This also could be the result of lower exposure rate, attentiveness, lack of interest and lack of brain storming exercise during school learning.

***Clerical Test (CT)*:** This test is used to assess the concentration, discrimination and evaluation-recognition capability of the children. When there is predominant anemia or iodine deficiency, then the test may show variability compared to the normal subjects**(Table 4.58)**.At baseline, this test was performed on n=863 children. Mean score for the population was 0.73 ± 0.26, which comprised of the mean scores of 0.72 ± 0.26 for boys and 0.76 ± 0.25 of the girls. There was a significant difference ($p<0.001$) **('t' value: ^{b}5.03-boys and ^{c}7.47- girls)** between scores for age groups (<12 years and ≥ 12 years) within each gender. There was also a significant difference ($p<0.001$) observed between <12 years age group scores of both the genders, but it was non significant for ≥ 12 years age group. Further analysis on the final scores of both the gender showed a significant difference (**^{a}4.58, p<0.001).** This revealed that, the girls performed better than the boys and had a better concentration.

While comparing our findings for CT with the results obtained on younger and older girls in urban settings by (Sen A and Kanani S 2007), our study subjects (girls <12 years and ≥ 12 years) performed better with 0.76 and 0.77 respectively compared to 0.63 and 0.75 scores. This could be due to difference in defined groups based on the age category and also the provided time for the completion of CT, since in our study 5 minutes were given to each child compared to the 2 minutes provided in the quoted reference study. Hence, it can be stated that, if the children would have been matched for the time and age group, then our study children would have performed lower than in the referred study.

4.4.4 INTERRELATION BETWEEN PARAMETERS

This section included the cross tabulated data between different parameters assessments, to have a deep inside upon the factors associated for the deficient status of the children.

On correlating nutritional, biochemical and cognitive parameters of the children, (**Table 4.59)** was generated. There was a better correlation between different analytes

of the health status of the children, which helped to focus on further research in different aspects.

Table 4.59: Correlation between anthropometry, iodine and iron status of the subjects

	Sex	Age	HAZ	WAZ	BMZ	UIE	Hb	DMT	VMT	CT
Sex	**1**									
Age	**0.054**	**1**								
HAZ	**-0.010**	**-0.154****	**1**							
WAZ	**0.024**	**0.044**	**0.728*****	**1**						
BMZ	**0.038**	**0.187****	**0.063**	**0.708****	**1**					
UIE	**0.004**	**-0.025**	**0.032**	**0.041**	**0.037**	**1**				
Hb	**-0.091****	**-0.015**	**0.178****	**0.134****	**0.008**	**0.036**	**1**			
DMT	**0.204****	**-0.382****	**0.137****	**0.057**	**-0.045**	**0.013**	**-0.009**	**1**		
VMT	**0.222****	**0.472****	**0.014**	**0.101****	**0.134****	**0.004**	**-0.033**	**0.084****	**1**	
CT	**0.135****	**0.222****	**0.003**	**0.063**	**0.088****	**-0.025**	**0009**	**0.155****	**0.438****	**1**

**p<0.05, **p<0.01*

Table 4.59 revealed a significant correlation between age and all three tests, where DMT showed a negative correlation with age (r= -0.382, p<0.01). This suggested a lower IQ amongst children with older age compared to the children belonged to similar standards of the school where they belonged to lower age groups. However, VMT (r=0.472, p<0.01) and CT (r=0.135, p<0.01) showed a significant positive correlation. All the tests were significantly (p<0.01) correlating with each other. It was also observed that, all the three tests were significantly correlated with the nutritional status of the children (Z scores), where DMT correlated with HAZ (r=0.137, p<0.01) and CT correlated with BMZ (r=0.088,p<0.01). However, VMT correlated significantly with WAZ (r=0.101, p<0.01) and BMZ (r=0.134, p<0.01).

Hemoglobin concentration of the children also correlated significantly with nutritional status, HAZ (0.178,p<0.01) and WAZ (0.134, p<0.01). However, it correlated negatively with age (p<0.01).

Table 4.60: Cognitive parameters crosstabulated with iron deficiency anemia classification

Indicators (Anemia categories)	Boys		Girls		Total		't' value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Draw-a-man Test (DMT) (N=823)							
Normal + Mild	113	82.31 ± 17.97	99	85.99 ± 19.79	212	84.03 ± 18.89	**-0.60^{NS}**
Moderate + Severe	301	84.60 ± 19.48	310	85.55 ± 20.21	611	85.08 ± 19.88	**-1.41^{NS}**
't' value		**1.13^{NS}**		**-0.20^{NS}**		**0.78^{NS}**	
Visual Memory Test (VMT) (N=864)							
Normal + Mild	122	0.39 ± 0.23	101	0.48 ± 0.30	223	0.43 ± 0.27	**4.22*** **
Moderate + Severe	326	0.40 ± 0.23	315	0.49 ± 0.27	641	0.44 ± 0.25	**2.63*** **
't' value		**0.67^{NS}**		**0.09^{NS}**		**0.68^{NS}**	
Clerical Task (CT) (N=863)							
Normal + Mild	125	0.75 ± 0.26	96	0.76 ± 0.26	221	0.75 ± 0.26	**0.28^{NS}**
Moderate + Severe	329	0.71 ± 0.26	313	0.76 ± 0.24	642	0.75 ± 0.25	**2.54^{NS}**
't' value		**1.35^{NS}**		**0.11^{NS}**		**0.84^{NS}**	

****p<0.001*

Table 4.60 revealed that, there was a non-significant difference between two categories distributed based on iron deficiency anemia classification. When the children were classified into two groups based on the gender, a significant difference ($p<0.001$) between both the groups was observed for visual memory test (VMT) in both the categories of anemia. However, rest of the tests did not show any significant difference within or **between groups**. This could have been due to majority of the subjects (99%) being anemic, so clear difference between anemic and non-anemic could not be derived.

Table 4.61: Cognitive parameters crosstabulated with iodine deficiency disorders

Indicators (IDD categories)	Boys		Girls		Total		't' value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
Draw-a-man Test (DMT) (N=823)							
Normal	289	84.24 ± 19.69	289	85.62 ± 20.00	578	84.93 ± 19.85	**-0.83^{NS}**
Deficient	125	83.35 ± 17.68	120	85.76 ± 20.50	245	84.53 ± 19.11	**0.99^{NS}**
't' value		**0.43^{NS}**		**-0.20^{NS}**		**0.78^{NS}**	
Visual Memory Test (VMT) (N=864)							
Normal	317	0.39 ± 0.23	296	0.48 ± 0.28	613	0.44 ± 0.26	**4.40*** **
Deficient	131	0.41 ± 0.21	120	0.49 ± 0.25	251	0.45 ± 0.24	**2.37*** **
't' value		**0.95^{NS}**		**0.03^{NS}**		**0.61^{NS}**	
Clerical Task (CT) (N=863)							
Normal	317	0.71 ± 0.27	289	0.75 ± 0.26	606	0.73 ± 0.26	**1.67^{NS}**
Deficient	137	0.74 ± 0.25	120	0.79 ± 0.20	257	0.77 ± 0.23	**1.73^{NS}**
't' value		**1.35^{NS}**		**0.11^{NS}**		**0.84^{NS}**	

****p<0.001*

A departmental study conducted by Nair S and Patel A 2005 also used a battery of similar tests to our studies, which included cancellation test, Draw-a-man test and Maze test. The results of DMT revealed that, the subjects with deficient urinary iodine levels had lower performance compared to the subjects with sufficient levels. In our study there was no significant difference observed **(Table 4.61)**. The data revealed that, there was a non-significant difference between two groups distributed for iodine deficiency disorder classification based on urinary iodine levels. When the children were distributed for the gender classified groups, a significant difference ($p<0.001$) between both the groups was observed for visual memory test (VMT) in both the categories of anemia. However, rest of the tests did not show any significant difference within or **between groups**. This could have been due to majority of the subjects (70%) being iodine sufficient, so clear difference between iodine deficient and sufficient children could not be concluded.

While assessing the IQ and cognition by CT, the difference was observed to be non significant between normal and deficient subjects based on UIE. Our findings were supported by Nair S and Patel A 2005, indicating no variations between both the groups.

Table 4.62: Distribution of DMT categories cross tabulated with UIE classification

b / a	Extremely low	Borderline Defect	Lower average	Average	High average	Superior	Very superior	Total	Chi Square
Normal	131 (15.92)	119 (14.46)	104 (12.64)	149 (18.10)	45 (5.47)	23 (2.79)	7 (0.85)	578 (70.23)	**0.35** ^{NS}
Mild	41 (4.98)	31 (3.77)	28 (3.40)	34 (4.13)	8 (0.97)	4 (0.49)	3 (0.36)	149 (18.10)	
Moderate	9 (1.09)	16 (1.94)	18 (2.19)	10 (0.12)	7 (0.85)	4 (0.49)	2 (0.24)	66 (8.01)	
Severe	4 (0.49)	6 (0.73)	9 (10.04)	7 (0.85)	3 (0.36)	0 (0)	1 (0.12)	30 (36.45)	
Total	185 (22.48)	172 (20.90)	159 (19.32)	200 (24.30)	63 (7.65)	31 (3.77)	13 (1.58)	823 (100)	

a- Describes classification of UIE , b- Describes classification of DMT scores for IQ, Values in parenthesis depicts percentage

According to the classification defined by (Phatak P 2002) there are 18.10% and 5.47% of children among UIE sufficient group were average or above average compared to 4.13% and 0.97% of UIE deficient children respectively **(Table 4.62).**

However, when the children were classified into two categories (UIE- $\geq$ 100 µg/L and < 100 µg/L) according to urinary iodine sufficiency, there was a difference in the distribution of children above average IQ was observed. It was observed that, 38.75% of children in UIE sufficient group were above average compared to 33.87% in UIE deficient group (data not presented). The difference was non significant but still remarkable. A similar pattern was also derived by a departmental study conducted by (Nair S and Patel A 2005) in urban school children of Vadodara, with 30% of the UIE sufficient children with above average IQ and a notable difference between both the groups. This in turn proved DMT as more reliable in analyzing minute difference in IQ levels between the subjects.

Further, comparison between parameters was carried out for the assessment of inter reliance of the biochemical and nutritional indicators **(Table 4.63)**.

Table 4.63: Mean Hb cross tabulated with nutritional status of the subjects

Nutritional Grade	N	Hb level (g/dl) (Mean ± SD)	't' Value
HAZ			
Normal + Mild (>-2SD)	521	9.39 ± 1.20	3.081 ($p < 0.002$)
Mod + Severe (<-2SD)	451	8.92 ± 1.21	
WAZ			
Normal + Mild (>-2SD)	270	9.46 ± 1.23	3.838 ($p < 0.001$)
Mod + Severe (<-2SD)	702	9.06 ± 1.21	
BAZ			
Normal + Mild (>-2SD)	425	9.19 ± 1.29	0.373^{NS}
Mod + Severe (<-2SD)	547	9.16 ± 1.18	

As indicated in **Table 4.59,** there was a significant correlation between Hb and HAZ – WAZ. This could also be observed from the **Table 4.63**, where a significant difference between Hb concentration of the children with or without stunting and underweight. It was observed that, mean hemoglobin was significantly higher ($p<0.01$) amongst children with milder level of stunting compared to the moderately-severely stunted children. Similarly hemoglobin concentration was also observed to

be significantly higher ($p<0.001$) amongst children with normal to milder level of underweight compared to moderately-severely underweight children. There was no significant difference observed in mean hemoglobin concentration of both the categories for BMZ.

A similar pattern of the cross tabulation was assessed in a study by (Iyer U and Jain V 2011), but there was no significant difference in all the three indices for nutritional status when the mean Hb concentration was compared for both the categories (<-2SD and >-2SD). In our study the difference was observed for both WAZ and HAZ.

However, study by (Sharma and Vaidya S 2010) revealed significant variation ($p<0.01$) based on HAZ categories cross tabulated with mean Hb levels. This finding in turn supports the results of ours results, where difference was observed significant at ($p<0.01$) for HAZ. Another study conducted by (Bhoite R and Iyer U 2011) revealed a variations in percent prevalence of anemia crosstabulated with nutritional status categories with regards to HAZ and WAZ of the children. The findings revealed 25.3% anemic subjects in normal category of WAZ compared to 74.7% anemic in moderated and severe category. This in turn supports our findings on variations in mean hemoglobin levels based on the severity of underweight.

On comparing the data on nutritional status classification and distribution of median UIE level, a cross tabulation was carried out to compare variations based on their nutritional status grades in **Table 4.64.**

Table 4.64: Median UIC cross tabulated with nutritional status of the subjects

Nutritional Grade	N	UI level (µg/l) (Median ± SD)	't' Value
HAZ			
Normal + Mild (>-2SD)	570	154.22 ± 106.91	0.196^{NS}
Mod + Severe (<-2SD)	475	142.08 ± 108.65	
WAZ			
Normal + Mild (>-2SD)	297	155.90 ± 106.18	0.255^{NS}
Mod + Severe (<-2SD)	748	143.22 ± 108.47	
BAZ			
Normal + Mild (>-2SD)	469	146.96 ± 112.16	0.671^{NS}
Mod + Severe (<-2SD)	575	145.91 ± 104.23	

As indicated in **Table 4.64**, where there was non significant difference observed between median urinary iodine excretion of the children with or without stunting, wasting and thinness. This could have been due to majority of the children being iodine sufficient. This could also be observed from the **Table 4.59,** there was no significant correlation between UIE and any parameters of the growth standards.

On comparing hemoglobin concentration and urinary iodine with different parameters, it could be understood that, iodine deficiency does not affects the nutritional status of the children, where as anemia does hamper it to a great extent. Thus, it was necessary to understand the inter correlation between iodine and iron deficiency are amongst the population. On comparing classifications of anemia and iodine deficiency, no significant difference was observed **(Table 4.65)**.

Table 4.65: Iron status cross tabulated with iodine status of percent subjects

Hb Categories	**UI categories**					**Chi Sq**
	Normal	**Mild**	**Moderate**	**Severe**	**Total**	
Normal	10 (1.03)	5 (0.52)	3 (0.31)	0 (0)	18 (1.86)	**0.040^{NS}**
Mild	173 (17.87)	44 (4.55)	14 (1.45)	2 (0.20)	233 (24.07)	
Moderate	464 (47.93)	121 (12.5)	62 (6.40)	27 (2.79)	674(69.63)	
Severe	32 (3.31)	8 (0.83)	1 (0.10)	2 (0.21)	43 (4.44)	
Total	679 (70.14)	178 (18.39)	80 (8.26)	31 (3.20)	968 (100)	

Values in the parenthesis depicts percentage

This could be due to difference in the rate of prevalence. At one end, majority of the subjects are moderately anemic and on the other hand, majority of the subjects are iodine sufficient. Prevalence of both the micronutrient deficiency and sufficiency are at their extremes. Hence, there are lower possibilities to find any interrelation between both the classifications as depicted in **(Table 4.65)**.

After comparing micronutrient deficiencies with each other, it was essential to compare interrelation between growth standards with each and their reliance on each other.

Table 4.66: WAZ cross tabulated with HAZ and BMZ

HAZ	Weight for Age (WAZ)					
	Normal	**Mild**	**Moderate**	**Severe**	**Total**	**Chi Sq**
Normal	80 (6.76)	98 (8.28)	48 (4.05)	13 (1.10)	239 (20.19)	
Mild	27 (2.28)	110 (9.29)	183 (15.46)	97 (8.19)	417 (35.22)	
Moderate	4 (0.34)	25 (2.11)	115 (9.71)	223 (18.83)	367 (31)	**0.643*** **
Severe	0 (0)	2 (0.17)	10 (0.84)	149 (12.58)	161 (13.60)	
Total	111 (9.38)	235 (19.85)	356 (30.07)	482 (40.71)	1184 (100)	
BMZ						
Normal	73 (6.17)	56 (4.73)	49 (4.14)	61 (5.15)	239 (20.19)	
Mild	74 (6.25)	129 (10.90)	106 (8.95)	108 (9.12)	417 (35.22)	
Moderate	51 (4.31)	102 (8.61)	94 (7.94)	120 (10.14)	367 (31)	**0.144*** **
Severe	11 (0.93)	52 (4.39)	43 (3.63)	55 (4.65)	161 (13.60)	
Total	209 (17.65)	338 (28.55)	292 (24.66)	344 (29.05)	1184 (100)	

Values in parenthesis depicts percentage

From **Table 4.66,** it could be gathered that, all three indices are interlinked to each other. The percent prevalence of WAZ may vary according to the variations in BMZ ($p<0.001$) and HAZ ($p<0.001$). However, height and weight for age are two independent variables but their interrelation depicts the need for good nutrition towards achieving an optimal growth amongst the children at the verge of their growth spurt.

DISCUSSION

4.4 BASELINE SURVEY

4.4.1 NUTRITIONAL STATUS ASSESSMENT

Optimum growth and development of school age children presents a sound foundation in the areas of health, nutrition, language development, personality building, socio-

emotional adjustment and cognitive development. At this stage there is more emphasis on academic achievement and personality development. The learning process of children is conditioned by multiple factors such as characteristics of the child, his family and educational system (Ivanoic*et al.*, 1996 and Hutchison *et al.*, 1997). Optimal nutrition during school age has impact on physical, mental and behavioral development of the children. Early malnutrition during childhood causes some degrees of damage to brain and neuromuscular system, which leads to low IQ, poor motivation and poor academic performance and further lead to problems in learning.

The UNICEF report (2005) pointed out that over one billion children, half of the world's population of the children (640 million) has been denied of adequate shelter, 400 million have no access to safe drinking water, 270 million lack health care amenities, 140 million children have never been to school and more than 150 million children are malnourished worldwide. Indian children are equally deprived of their rights to survival, health, nutrition, education and safe drinking water. It is also reported that 63 % of them go to bed hungry and 53 % suffer from chronic malnutrition, 27 million are severely under weight and 33 million never attended the school. The report highlights that the brain damage due to iodine deficiency was 26 and 6.6 million children in world and India respectively. NFHS III (2005-2006) has revealed that over 70 % children suffer from iron deficiency, while 1.5 million children suffer from vitamin A deficiency. Thus in India nutritional deficiency due to low food intake, poverty and ignorance contribute to brain damage and low intelligence development among children. This has promoted on increased focus on the diverse needs of the school age children and reduces the heavy burden of malnutrition among them.

While conducting a baseline nutritional survey amongst our study population of school aged children (5-15 years) revealed that, the mean age of the children was 9.29 ±2.33 years. Our study reports higher rate of prevalence for rural school children of both the genders. Mean height and weight of the children were comparatively lower than the standards. Anthropometric indices as WAZ, HAZ and BMZ in turn represented undernutrition with 70%, 44% and 53% prevalence respectively.

This finding was supported by one of the largest studies of anthropometric status of rural school children in low income countries (Ghana,Tanzania, Indonesia, Vietnam and India) found the overall prevalence of stunting and underweight to be high in all five countries, ranging from 48 to 56% for stunting and from 34 to 62% for underweight (Partnership for Child Development 1998).In all countries there was a trend for Z-scores for height-for-age and weight-for-age to decrease with age, thus as children got older they became progressively shorter relative to the reference population.

It was also observed that, the boys in most countries tend to be more stunted than girls and in all countries boys were more underweight than girls. A similar trend was also observed amongst our study subjects also with significantly higher ($p<0.01$) prevalence of underweight compared to the girls.

Another report stated that, the severity and prevalence of stunting and underweight have been found to increase with age, with older children diverging further from the reference medians for height until puberty. The evidence suggests that boys are more likely to be stunted and underweight than girls, and in some countries, more likely to be wasted than girls. This may be due to a bias in the school population. It is also suggested that it could reflect delayed onset of puberty (Shahabuddin AKM, et al 2000; Partnership for Child Development1998)

The prevalent malnutrition also results in low self-esteem and poor social relationship leading to further behavioral changes. The behavioral problem such as scholastic backwardness and social maladjustment may force the children to dropout from the school. Malnourished children join the school but less than 50% are unable to complete their education (Udani, 1991). In our study population also this could have been one or the other confounding factor towards higher rate of absenteeism (>30%) and drop out (20%) till the end of the study. Thus, our final population reached to N=947 out of 1184 enrolled.

4.4.2 MICRONUTRIENT STATUS ASSESSMENT

4.4.2.1 Iron status assessment

Our study data reveals a very high prevalence of anemia amongst rural school children. The higher prevalence of moderate anemia was observed with mean

hemoglobin concentration 9.27 ± 1.27 g/dl for boys and 9.04 ± 1.23 g/dl among girls. This in turn presented the current status of the upcoming adult generation. This may affect the GDP of the country and medical cost: benefit ratio would be skewed towards the positive side with the increased expense on the treatment. As estimated by Micronutrient Initiative's "National Damage Assessment Reports" for 80 countries where micronutrient deficiencies are prevalent and estimated that, on average 1% of GDP is lost to iron deficiency anemia (Stein A and Qaim M 2007).

Further, the review studies from last 5 decades till date have revealed increased or similar prevalence of anemia, whether it is a study by Sheshadri S and Gopaldas T (1979) or a study by Bhoite R and Iyer U (2011), all have revealed a prevalence of anemia from 15-99 % in India. Latest NFHS III data (2005-2006) has also depicted the prevalence of anemia in Gujarat with 70-80%. Thus, the prevalence calls for an effective intervention to combat the level of deficiency at a sustained level.

4.2.2.2 Iodine status assessment

The results on the excretion of urinary iodine levels were very encouraging, indicated by the median urinary iodine excretion with 146.33 µg/L. It is essential to note that, there has been a great improvement in iodine nutrition after the implementation of universal salt iodization from last 6 years (May 2006- till date). This statement is also supported by another study conducted in Kutch district of the Gujarat state (Chudasama R et al 2010). The authors have indicated the iodine nutrition sufficiency amongst 81.4% of the children with UIE ≥ 100 µg/L, which also coincides our study results, since almost 70.3% of the children have been remarked as normal during baseline data survey of the current study.

Our study findings were also supported by the global data estimates of 2007 on UIE deficiency, which revealed the prevalence rate of iodine insufficiency amongst school children to be 31.5% in 47 WHO countries and 30.3% in 9 countries of South-East Asia region (Benoist B et al 2008).

Das S et al (2011) has carried out a survey to assess the prevalence of goitre in the post-iodization phase and the relationship of goitre with micronutrient status and thyroid autoimmunity in school children of the Union Territory of Chandigarh, a city of Northern India. The study revealed that, the median urinary iodine excretion to be

130-137 µg/L, yet the prevalence of goiter was observed to be 15.1% it was significantly ($p<0.05$) higher amongst adolescents compared to school children (17.7 v/s 13.9 %). These observations suggest that there might be other goitrogens or deficiency of other micronutrients responsible for the persistence of goiter despite adequate salt iodization.

4.2.2.3 Thyroid analytes assessment

However, while assessing the thyroid hormone estimations as confirmatory tests, there was no difference found between goitrous and nongoitrous children. In our study also the mean TSH, FT_4 and TT_4 were observed to be within normal ranges.

The prevalence of subclinical hypothyroidism was observed to be 2.36% and subclinical hyperthyroidism was observed amongst 1.4% school children. This suggested a sufficient functioning and availability of adequate iodine pool amongst the study population.

Thus, the data reflects that, our study area is in a transition phase from iodine deficiency to sufficiency with 82.1% iodized salt consumption and 71% urinary iodine excretion sufficiency was observed.

4.4.3 IQ AND COGNITIVE STATUS ASSESSMENT

Data on cognition and IQ tests revealed that, the IQ of the rural children is below average. This could be due to their lower accessibility to the resources. In support of our data, it is reported by a number of observational studies, school aged children living in iodine deficient villages were found to have poorer levels of IQ, cognitive and motor function than school children in iodine-sufficient villages (Azizi et al 1993 and 1995, Bleichorodt et al 1987, Boyages et al 1989, Fenzi et al 1990). However, villages differ in many other factors that may affect children's development. These include accessibility and quality of education, economic development and remoteness. Although researchers have often taken into account macro measures of socioeconomic status of the children's families, other factor that may also affect their development such as stimulation in the home and children's nutritional status etc. (PAHO, TMRU and World Bank 1998, Sameroff et al 1993).

With respect to iron deficiency anemia, cognition tests (CT and VMT) were performed and the results revealed significant difference among gender and age groups. However, with the increase in age, the difference by gender was not observed. This in turn is indicating the termination of their cognitive development towards the initiation of the puberty or it could be due to indifferent improvement in both the genders after 12 years. In support of our discussion, a study looked at the possible connection between iron deficiency and cognitive test scores in national representative samples of 5,398 children 6-16 years of age in United States. Four standardized tests were used. It was observed that 49% of children with normal iron had below average score which were significantly better than the non-anemic iron deficient children (71%), and also the anemic iron deficient children (72%) (Halterman et al 2001).

Thus, if iron and iodine deficiencies are not corrected, they may lead to IDA and any of the consequences of IDD, which are associated with an impaired development of mental and physical coordination. Once afflicted, this impairment is not eradicated even after the anemia has been treated, impairing school achievements in older children.

The study observations call for a regular and well monitored school health program. All the indicators throw light on the real picture of nutritional status of rural Gujarat after six decades of our independence. The results reflect that the prevalence of malnutrition and micronutrient deficiencies is very high despite the ongoing mid day meal program. Since, most of the subjects are falling into mild and moderate categories of micronutrient deficiencies, dietary diversifications and awareness campaigning from ground level to the beneficiaries is required. There is a need to introduce a fortified food vehicle which can be used by all in same amounts and more or less in equal quantities. The cost of the product should be feasible for all and it should require minimum technical integrations, thus can be produced at a local level. Double fortified salt can be used as such a tool to supplement micronutrients to the vulnerable population at a feasible cost. [(21)] The study widens our views and application to achieve the MDGs by combating both micronutrient deficiencies, when they are encompassed with a higher enormity of malnutrition.

After considering all the baseline results on micronutrient status and bearing in mind those 99% iron deficient and 30% iodine deficient subjects, our study was extended to second section with an interventional strategy to combat both these deficiencies, in the form of a dietary ingredient- salt- doubly fortified with iodine and iron.

4.4.4 INTERRELATION BETWEEN PARAMETERS

Data on correlation amongst different parameters revealed a significant correlation between hemoglobin and nutritional status, indicating effect of better nutrition on hemoglobin concentration in the body. Hemoglobin also correlated negatively with age of the children, indicating increased requirement of iron with age and if not satisfied may lead to decreased hemoglobin concentration in the circulation. Correlation also revealed, lowered IQ and increased concentration - memory with age amongst the school children.

4.5 INTERVENTIONAL STRATEGIES AND BASELINE SURVEY OF SES STATUS OF THE POPULATION

After complete data collection at baseline, children were subjected to division in different groups implementing different interventional strategies.

4.5.1 DISTRIBUTION OF THE SUBJECTS INTO DIFFERENT GROUPS

1. ***Double Fortified Salt (DFS) supplementation:*** Subjects were divided into supplemented and non-supplemented groups. Supplemented group (DFS Group) was provided free supply of DFS for 9 months and non-supplemented groups (IS Group) were provided with educational inputs to consume adequately iodized salt.

2. ***Deworming:*** Subjects were further divided into dewormed groups (DW Group) and non-dewormed group (NDW Group). Hence, the subjects were divided into total four groups.
    - **Supplemented and dewormed group (Experimental + DW group= E+DW)** – This group comprised of one out of two experimental/supplemented schools. Children, who were supplemented with DFS, were dewormed with Albendazole 400 mg dose, twice during the phase (2^{nd} month and 7^{th} month). Since, worm infestation is a very common problem in school aged children

living in rural areas; this group was formed to assess the efficacy of DFS as a preventive measure in controlling IDD and IDA together in the absence of worm infestations in our subjects.

- **Supplemented and non dewormed group (Experimental+ Non DW group= E) –** The group comprised of one out of two experimental/supplemented schools. Children, who were supplemented with DFS, were not dewormed with Albendazole 400 mg dose. This group was formed to assess the efficacy of DFS as a preventive measure in controlling IDD and IDA together in the absence of any worm eradication treatment or in general circumstances.
- **Non supplemented and dewormed group (Control + DW group= C+DW) –** This group comprised of one out of two control/non supplemented schools. Children, who were provided NHE on iodine- iron nutrition and ensured with iodized salt consumption, were dewormed with Albendazole 400 mg dose, twice during the phase (2^{nd} month and 7^{th} month). Since, worm infestation is a very common problem in school aged children living in rural areas; this group was formed to assess the impact of deworming tablet supplementation on nutritional status of the subjects in the absence of any worm infestations in our subjects.
- **Non supplemented and non dewormed group (Control + Non DW group = C) –** This group comprised of one out of two control/non supplemented schools. Children, whose mothers/caretakers were provided NHE on iodine-iron nutrition and ensured with iodized salt consumption, were not dewormed with Albendazole 400 mg dose. This group was formed to assess the impact of NHE alone on the nutritional status of the subjects.

3. ***Nutrition Health Education:*** All the children and their parents were subjected to nutrition, health education based on their data collected with reference to knowledge, attitude and practices. This strategy was implemented were creating awareness on iodine and iron nutrition; need for adequate iodine and iron nutrition during childhood and adolescence; storage and cooking practices upon iodized/Double fortified salt etc. Impact was assessed by collecting KAP, 24 hour dietary recall and FFQ questionnaires.

4.5.2 SOCIO ECONOMIC STATUS SURVEY OF THE POPULATION

Table 4.67 reveals a socio-economic status of the population. Data from N=212 was collected. There were n=116 subjects from experimental and n=96 from control group.

Religion and type of families: on comparing distribution amongst children, 62.5% and 94.8% of the children were Hindus, who belonged to experimental and control group respectively. There were 37.5% and 5.2% Muslims amongst these groups.

Parental Literacy: While comparing the data on parental education, a large chunk of the mothers of the children were illiterate and educated till primary school. However, majority of the fathers of the children were educated till primary and secondary school amongst both the groups. There was almost 17% difference amongst both the group with illiteracy rate of the mothers. This in turn plays a vital role in health status and dietary practices of the children. There were 15% of the fathers in control group, who completed their higher secondary education. This proportion was significantly higher compared to the fathers of the children belonged to experimental group and mothers of both the groups. This might lead to variation in the income of the family also, since higher education might be paying them more due to better opportunities compared to the rest.

Occupation: While comparing parental occupation, majority of the fathers of the children were laborers in both the groups. However, one third (35%) of them belonged to the experimental group were farmers and one fourth (25%) of them belonged to control group had their own shops. One fifth (14-20%) of the fathers belonged to both the groups were into industry/other jobs.

While looking into the data distribution for maternal occupation, three forth (≥ 75%) of mothers were housewives. However, few of them were laborers also.

Type and size of the families: While observing the data on type of family, there were 68.97% and 46.88% of the families belonged to Joint/extended type belonged to experimental and control group respectively. The rest were nuclear families amongst the groups. There was almost 20% difference in both the groups.

Table 4.67: Socio economic status of the families of the study children

Characteristic	Experimental	Control		Characteristic	Experimental	Control
Religion				**Family Type**		
Hindu	110 (94.8)	60 (62.5)		Joint/Extended	80(68.97)	45(46.88)
Muslim	6 (5.2)	36 (37.5)		Nuclear	36(31.03)	51(53.13)
Father's Education				**Mother's Education**		
Illiterate	7 (6.0)	9 (9.4)		Illiterate	46 (39.7)	22(22.9)
Primary	47 (40.5)	45 (46.9)		Primary	52 (44.8)	59 (61.5)
Secondary	56 (48.3)	28 (29.2)		Secondary	12 (10.3)	8 (8.3)
High Secondary	5 (4.3)	14 (14.6)		High secondary	6 (5.2)	7 (7.3)
Graduate	1 (0.9)	0 (0)		Graduate	0 (0)	0 (0)
Father's Occupation				**Mother's Occupation**		
Job	17 (14.7)	20 (20.8)		Job	1 (0.9)	2 (2.1)
Labor	50 (43.1)	47 (49)		Labor	20 (17.2)	6 (6.3)
Farmer	41 (35.3)	4 (4.2)		Farmer	0 (0)	0 (0)
Business	4 (3.4)	24 (25)		Business	4 (3.4)	1 (1.0)
Vendor	2 (1.7)	1 (1.0)		Vendor	2 (1.7)	1 (1.0)
Nothing	1 (0.9)	0 (0)		Housewife	88 (75.9)	85 (88.5)
Family Size				**Family Income**		
2-5	36 (31)	51(53.1)		<1000	3 (2.6)	2(2.1)
6-10	68 (58.6)	44(45.8)		1000-2000	74 (63.8)	37(38.5)
>10	12 (10.3)	3 (3.1)		>2000-3000	25 (21.6)	17(17.7)
				>3000	14 (12.1)	40(41.7)
No. of family members						
Adults				**Children**		
1	0 (0)	0 (0)		1	0 (0)	5 (5.2)
2	41 (35.3)	52(54.2)		2	47 (40.5)	32(33.3)
3	15 (12.9)	28(29.2)		3	34 (29.3)	43(44.8)
4	40 (34.5)	12(12.5)		4	21 (18.1)	6 (6.3)
≥ 5	20 (17.24)	4 (4.2)		≥ 5	14 (12.06)	10(10.42)

Values in parenthesis depicts percentage

There were 31% and 53.1% of the children belonged to experimental and control group had small families with 2-5 members. However, almost half or more than half of the families in both the groups, had number of family members more than 6-10 depicting joint/extended family culture in the rural communities. Data also revealed that, majority of the families had more than two children and more than two adults in the families.

Family Income and per capita income: Majority of the families has monthly income between Rs. 1000-2000 amongst experimental group and there were one fifth (21.6%) of the families with monthly income Rs. >2000-3000 in experimental group. However, this distribution was more extended towards the higher income (Rs. >3000) with 41.7% amongst control group.

4.5.3 CONTENT ESTIMATION AND STABILITY OF DFS

Data on salt iodine and iron content is provided in **Table 4.68**. It was observed that, the iodine and iron content of DFS remained stable after 1 year also, in lab condition at room temperature and dry storage. During rainy season, the salt formed plaques and were hardened during storage, which was broken, homogenized and then estimated for the storage stability and content losses after 1 year of production. At baseline mean iodine and iron content of DFS was observed to be 40 ppm and 1050 ppm, which showed a negligible variation in the contents with 37.5 ppm and 979 ppm respectively.

Table 4.68: Iron and Iodine content of DFS

Content	Period	Mean value
Iodine (ppm)	Baseline	36 ppm
	1 year	37.5 ppm
Iron (ppm)	Baseline	1050 ppm
	1 year	979 ppm

A series of studies including a large scale multi-centric study conducted by NIN on storage stability and content retention by modified method of titration (also used in our study). This showed an excellent stability of iodine and iron longer than 1 year. Such data were confirmed in different laboratories and showed narrowed range of variations in iodine content (Ranganathan et al 2007). Iron content was found to be stable at all instances as observed by our laboratory findings.

4.5 INTERVENTIONAL STRATEGIES

4.5.1 DISTRIBUTION OF THE SUBJECTS INTO DIFFERENT GROUPS

There are many studies on impact assessment of double fortified salt supplementation, which has been carried out among school children, in different parts of the world. However, NIN-DFS has been proven to be the most cost-effective and stable formula for the Indian climatic condition. Hence, the mentioned formula was chosen as a tool to reach the target population.

Many a studies conducted using DFS and deworming provides independent results. Few studies have used deworming and showed improvement in the hemoglobin concentration of both experimental and control groups (Malvika V and Rajgopalan 2000, Andersson et al 2008). Thus to ensure efficacy of DFS along with deworming and without deworming, the experimental group was sub divided into two groups and thus the control group, to observe the effect of deworming in non supplemented group and a comparable group without any intervention.

To improve the acceptability and efficacy of DFS in the presence of Vitamin C rich foods in Experimental groups and to improve iron and iodine nutrition in both the groups, NHE was imparted intermittently and BCC was provided to develop best cooking and dietary practices with maximum nutritional gain.

4.5.2 SOCIO ECONOMIC STATUS SURVEY OF THE POPULATION

Summing up with this section, the current scenario of the socio- economic status of the rural poor population was observed. It was observed that, the majority of the families were BPL and it is assumed that the per capita income would be $\leq$ 500 Rs.

The nutritional status, micronutrient deficiencies and cognition – IQ test performances could be attributed to the homely environment, parental ignorance, food insecurity and other environmental factors only.

4.5.3 CONTENT ESTIMATION AND STABILITY OF DFS

Data on iodine and iron content of DFS at baseline and after 1 year showed an excellent stability using standard methods.

4.6 IMPACT ASSESSMENT ON DIFFERENT PARAMETERS IMPLEMENTING INTERVENTIONAL STRATEGIES

Owing to different reasons for drop outs, final number of the children included in the study was N=947, who could complete the study including all the groups. In this section, impact of interventional strategies on the overall health status of the children, has been assessed.

4.6.1 IMPACT ON NUTRITIONAL STATUS

Bearing in mind the study objective of assessing the efficacy of double fortified salt supplementation on nutritional status of the children, section**4.6.1** presents data related to height-for-age, weight-for-age and BMI of the children in the various groups.

4.6.1.1 Impact on weight

Table 4.69: Impact assessment with gender wise mean weight of the children

	Weights of the subjects (kg) (Mean + SD)				**'F' Value**
BOYS	**E+DW(n=129)**	**E (n=108)**	**C+DW(n=133)**	**C (n=146)**	
Initial	19.79 + 4.78	20.44 + 5.98	20.45 + 4.86	21.37 + 5.65	**2.067^{NS}**
Final	22.91 + 5.28	24.08 + 6.97	23.36 + 5.34	24.71 + 6.51	**2.332^{NS}**
Difference	3.12 + 1.35	3.64 + 1.80	2.91 + 1.80	3.33 + 1.70	
't' Value	**25.452*** **	**18.170*** **	**14.114*** **	**21.291*** **	
GIRLS	**(n=110)**	**(n=95)**	**(n=81)**	**(n=146)**	
Initial	20.42+5.44	19.37+4.86	23.02+7.68	21.92+7.21	**5.865*** **
Final	24.20+6.54	23.11+ 6.12	26.32+8.08	25.44+7.61	**3.665** **
Difference	3.78+ 1.56	3.74 + 2.00	3.30+2.11	3.53 +2.00	
't' Value	**26.312*** **	**21.064*** **	**18.618*** **	**23.703*** **	
TOTAL	**(n=239)**	**(n=203)**	**(n=214)**	**(n=291)**	
Initial	20.08+ 5.09	19.93+ 5.49	21.42+6.19	21.64+6.47	**5.441** **
Final	23.50+ 5.91	23.62+ 6.58	24.48+6.65	25.08+ 7.08	**3.219***
Difference	3.42 + 1.48	3.69 + 1.89	3.06+ 1.93	3.43 + 1.85	
't' Value	**35.727*** **	**27.749*** **	**23.213*** **	**31.595*** **	

Table 4.69 reveals that, overall initial mean weight was 20.83 ± 5.92 kg (95% CI: 20.45-21.20), which increased to 24.24 ± 6.62(95% CI: 23.82-24.66) kg at the end. There was 3.40 ± 1.80 kg (95% CI: 3.51-3.29) increase in total population. The difference ranged from 3.42- 3.69 kg for each study group. On comparing mean weight gain before and after interventions, there was a non significant difference between all the groups when assessed for boys. However, mean weight before and after intervention varied significantly ($p<0.001$) amongst girls belonging to all four groups. Overall mean weight gain was highly significant for all four groups and it was also very significant for both the divisions of gender (Boys and girls).

Post hoc analysis (Bonferroni) revealed significant difference between mean weight at baseline and at the end of the study between girls for all four groups. The difference at baseline varied significantly between E+DW *v/s* C+DW ($p<0.05$), E v/s C+DW ($p<0.001$) and C ($p<0.01$). However, the final weight varied with the significance level at ($p<0.05$) between E v/s C+DW. The highest mean weight increase was observed amongst the girls belonging to E+DW group, but it was found non significantly higher compared to rest of the groups.

While comparing the mean weight of the boys belonging to all four groups, post hoc analysis revealed that, a non significant difference was observed at baseline and at the end. Thus, it can be interpreted that, DFS – iron did not give any specific impact on the weight gain in the children, when compared to the controls.

4.6.1.2 Impact on height

Overall mean height of the children was 122.06 ± 12.45 cm (95% CI: 122.85-121.27) initially, which improved up to 128.42 ± 13.03 cm (95% CI: 129.25-127.59) towards the end. Hence, there was 6.36 ± 1.87 cm (95% CI: 6.48-6.24) increase in total population. The difference ranged from 6.15- 6.85cm for each study group **(Table 4.70)**.

Table 4.70: Impact assessment with gender wise mean height of the children

	Height of the subjects (cms) (Mean ± SD)				'F' Value
Boys	**E+DW(n=129)**	**E (n=108)**	**C+DW(n=133)**	**C (n=146)**	
Initial	119.81±11.47	120.98 ± 3.51	121.09 ±10.86	123.73 ± 12.39	**2.632***
Final	126.54 ± 1.63	27.06 ± 14.26	127.36 ± 11.74	130.07 ± 13.12	**2.162^{NS}**
Difference	6.73 ± 1.62	6.08 ± 1.86	6.91 ± 2.35	6.34 ± 1.59	
't' Value	**32.992*** **	**29.961*** **	**36.615*** **	**54.051*** **	
Girls	**(n=110)**	**(n=95)**	**(n=81)**	**(n=146)**	
Initial	122.89 ±13.04	119.17 ±11.23	124.86±13.47	123.78±12.98	**3.565*** **
Final	129.89 ±13.30	125.71 ±12.22	130.89±14.03	129.74 ±13.53	**2.782***
Difference	7.00 ±2.22	6.53± 2.12	6.00 ±1.47	5.96 ±1.33	
't' Value	**47.044*** **	**33.922*** **	**30.724*** **	**48.245*** **	
Total	**(n=239)**	**(n=203)**	**(n=214)**	**(n=291)**	
Initial	121.22 ±12.28	120.13 ±12.49	122.52 ±12.03	123.75±12.67	**3.906** **
Final	128.08 ±12.51	126.42 ±13.33	128.68 ±12.73	129.90±13.30	**2.951***
Difference	6.85 ± 1.92	6.29 ± 2.00	6.16 ±2.06	6.15± 1.47	
't' Value	**55.04*** **	**44.87*** **	**43.69*** **	**71.17*** **	

**p<0.05, **p<0.01, ***p<0.001*

As depicted in **Table 4.70**, while comparing difference in height with before and after intervention period amongst boys, it was observed that, there was a weak significant difference ($p<0.05$) between all four groups, but towards the end there was no difference observed between these groups. *Post hoc analysis* (Bonferroni) revealed the baseline difference to be significant between E+DW *v/s* C ($p<0.05$).

However, difference in height was significant ($p<0.001$) amongst girls at the initial stage, which remained at ($p<0.05$) towards the end. *Post hoc* analysis (Bonferroni) revealed this difference as significant between E *v/s* C+DW and C groups ($p<0.05$) at baseline and towards the end the difference was non significant between all four groups.

The difference between each group and **within groups** has been presented in **Table 4.71.** It was observed that, there was a significant improvement in weight and height, thus on BMI of the children, irrespective of group. There was also a significant difference observed **between groups**.

Table 4.71: Mean change in anthropometry parameters

Study group	N	Difference in Anthropometry (Mean± SD)		
		Height (cm)	**Weight (kg)**	**BMI(kg/m^2)**
E+DW	239	6.85 ± 1.92	3.42 ± 1.48	0.63 ± 1.04
E	203	6.29 ± 2.00	3.69 ± 1.89	0.78 ± 0.98
C+DW	214	6.16 ± 2.06	3.06 ± 1.93	0.75 ± 0.91
C	291	6.15 ± 1.47	3.43 ± 1.85	0.76 ± 1.01
'F'value		**7.83*** **	**4.42** **	**1.06^{NS}**
't' value				
E *v/s* C		0.85^{NS}	1.52^{NS}	0.25^{NS}
E+DW *v/s* C		4.64***	0.05^{NS}	1.40^{NS}
C+DW *v/s* C		0.08^{NS}	2.18*	0.09^{NS}
E *v/s* E+DW		3.00**	1.64^{NS}	1.55^{NS}
E *v/s* C+DW		0.64^{NS}	3.38***	3.23^{NS}
E+DW *v/s* C+DW		3.67***	2.23*	0.14^{NS}

**p<0.05, **p<0.01, ***p<0.001*

Height:

Data on difference in height revealed that, highest difference was achieved by E+DW group compared to all other groups. When difference in mean height was compared **between groups**, E+DW group showed a significant difference compared to all three groups- E (p<0.01), C *v/s* C+DW (p<0.001).*Post hoc* analysis (Bonferroni) also revealed similar results. However, on comparing E group *v/s* control (C and C+DW) groups, non significant difference was observed **(Table 4.71)**.

This depicts that, DFS with deworming (E+DW) has been most effective strategy compared to DFS supplementation alone (E group) *and* deworming alone (C+DW).

Weight:

Data on difference in weight revealed that, highest difference was achieved by E group and lowest by C+DW group compared to rest of the groups. When difference in mean weight was compared **between groups**, it was non significant between E, E+DW and C groups. However, these three groups showed significant difference- E (p<0.001), C and E+DW (p<0.05) - compared to C+DW group. *Post hoc* analysis (Bonferroni) also revealed similar results **(Table 4.71)**. Thus, it revealed non supportive role of deworming in gaining weight amongst the children.

BMI:

Data on difference in BMI revealed a non significant difference between any of the groups. However, it was highest amongst E group compared to all other groups.

4.6.1.3 Impact on the prevalence of malnutrition

In terms of gain in weight and height parameters, to interpret the data meaningfully, an attempt was made to assess gender wise difference in percent prevalence of malnutrition; following tables were generated, so as to interpret the data meaningfully.

Table 4.72 and **Table 4.73** are indicating gender wise distribution of Z scores depicting the prevalence of malnutrition among children before and after intervention.

Table 4.72: Impact assessment with prevalence of malnutrition (Boys)

Z Score	Percent Subjects								Chi square	
	E+ DW		E		C+DW		C			
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
WAZ										
≥-2SD	17 (13.2)	61 (47.3)	20 (18.5)	79 (73.1)	58 (43.6)	91 (68.3)	44 (30.1)	92 (63)	**35.84*** **	**19.99*** **
<-2SD	112 (86.8)	68 (52.7)	88 (81.5)	29 (26.9)	75 (56.4)	42 (31.6)	102 (69.9)	54 (37)		
Chi Sq	**35.58*** **		**64.91*** **		**16.62*** **		**31.71*** **			
HAZ										
≥-2SD	43 (33.3)	104 (80.6)	51 (50.8)	100 (92.6)	92 (69.2)	120 (90.2)	85 (58.2)	120 (82.2)	**36.95*** **	**10.76** **
<-2SD	86 (66.7)	25 (19.4)	57 (52.8)	8 (7.9)	41 (30.8)	13 (9.8)	61 (41.8)	26 (17.8)		
Chi Sq	**58.84*** **		**52.84*** **		**18.22*** **		**20.06*** **			
BMZ										
≥-2SD	53 (41.1)	68 (52.7)	47 (43.5)	75 (69.4)	63 (47.4)	83 (62.4)	54 (37)	90 (61.6)	**3.23^{NS}**	**7.10^{NS}**
<-2SD	76 (58.9)	61 (47.3)	61 (56.5)	33 (30.6)	70 (52.6)	50 (37.6)	92 (63)	56 (38.4)		
Chi Sq	**3.50^{NS}**		**14.77*** **		**6.07** **		**17.76*** **			

*Values in parenthesis depicts percentage. **p<0.01, ***p<0.001*

***Weight- for-age* (WAZ):**

As demonstrated in **Table 4.72,** it can be stated that, there was a significant difference in the prevalence of underweight in boys between all four study groups at baseline.

However, the improvement in the Z score towards the end was also, highly significant ($p<0.001$) within and **between groups**. This could be due to growth spurt of the children.

***Height-for-age* (HAZ):**

On observing Z scores for height, it was observed that the prevalence of stunting varied highly significant ($p<0.001$) between study groups of boys at baseline. Improvement in height brought significant difference **within groups** ($p<0.001$) in each study group. However, at the end of the study the prevalence decreased **betweengroups**to a lesser level of significance ($p<0.01$) compared to baseline.

***BMI- for- age* (BMZ):**

Data on BMZ revealed non significant difference **between groups** at baseline and towards the end. There was a non significant reduction in the prevalence of thinness for before and after intervention in E+DW group. This difference was significant at ($p<0.01$) for C+DW compared to E and C groups, where the improvement at higher level of significance **within groups** ($p<0.001$).

Similar pattern of analysis was also carried out for the girls. It revealed Z scores for each anthropometry parameter are mentioned below in **Table 4.73**.

***Weight- for-age* (WAZ):**

Table 4.73, reveals data on prevalence of malnutrition in girls, it can be stated that, there was a significant reduction in the prevalence of underweight in girls between all four study groups at baseline. However, the improvement in the Z score towards the end was also, highly significant ($p<0.001$) within and **between groups**. This could be due to growth spurt of the children, since they are on various growth phases of life.

***Height-for-age* (HAZ):**

On observing Z scores for height, it was observed that, the prevalence of stunting varied significantly at ($p<0.001$) between study groups of girls at baseline. Improvement in height brought significant difference **within groups** ($p<0.001$) in each study group. However, at the end of the study the prevalence differed **between groups** to a lesser level of significance at ($p<0.01$) compared to baseline.

Table 4.73: Impact assessment with prevalence of malnutrition (Girls)

Z Score	Percent Subjects								Chi square	
	E+ DW		E		C +DW		C			
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
WAZ										
≥-2SD	12 (10.9)	60 (54.5)	20 (21.1)	63 (66.3)	38 (46.9)	68 (84)	52 (35.9)	101 (69.7)	**36.77*** **	**18.84*** **
<-2SD	98 (89.1)	50 (45.5)	75 (78.9)	32 (33.7)	43 (53.1)	13 (16)	93 (64.1)	44 (30.3)		
Chi Sq	**47.57*** **		**39.56*** **		**24.56*** **		**33.22*** **			
HAZ										
≥-2SD	50 (45.5)	88 (80)	43 (45.3)	83 (87.4)	57 (70.4)	77 (95.1)	85 (58.6)	132 (91)	**16.19*** **	**11.92** **
<-2SD	60 (54.5)	22 (20)	52 (54.7)	12 (12.6)	24 (29.6)	4 (4.9)	60 (41.4)	13 (9)		
Chi Sq	**28.07*** **		**37.70*** **		**17.27*** **		**40.44*** **			
BMZ										
≥-2SD	35 (31.8)	72 (65.5)	44 (46.3)	68 (71.6)	46 (56.8)	58 (71.6)	70 (48.3)	102 (70.3)	**12.94** **	**1.26^{NS}**
<-2SD	75 (68.2)	38 (34.5)	51 (53.7)	27 (28.4)	35 (43.2)	23 (28.4)	75 (51.7)	43 (29.7)		
Chi Sq	**24.91*** **		**12.53*** **		**3.87***		**14.63*** **			

*Values in parenthesis depicts percentage. *p<0.05, **p<0.01, ***p<0.001*

***BMI- for- age* (BMZ):**

Data on BMZ revealed significant difference **between groups** at baseline (p<0.01), but it was non significant difference towards the end. There was a significant reduction at(p<0.001) in prevalence of thinness for before and after intervention in E+DW, E and C group. However, there was a significant reduction (p<0.05) in C+DW group compared to all other study groups.

Table 4.74 reveals an overall scenario of the nutritional status distribution of the population. The anthropometry indices (Z scores) were divided into 2 categories as normal and malnourished base on ± 2SD cutoffs. While doing this, a significant improvement (p<0.001) in nutritional status- Z scores of the children was observed in each group.

Table 4.74: Overview of the impact assessment on prevalence of under nutrition

Z Score	Percent Subjects								Chi square	
	E+ DW		E		C +DW		C			
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
WAZ										
Normal	4 (1.7)	45 (18.8)	9 (4.4)	56 (27.6)	34 (15.9)	89 (41.6)	29 (10)	97 (33.3)	**36.02*****	**29.77*****
UW	235 (98.3)	194 (81.2)	194 (95.6)	147 (72.4)	180 (84.1)	125 (58.4)	262 (90)	194 (66.7)		
Chi Sq	**17.54*****		**24.72*****		**36.20*****		**57.93*****			
HAZ										
Normal	25 (10.5)	100 (41.8)	38 (18.7)	104 (51.2)	62 (29)	148 (69.2)	61 (21)	169 (58.1)	**24.99*****	**36.33*****
Stunted	214 (89.5)	139 (58.2)	165 (81.3)	99 (48.8)	152 (71)	66 (30.8)	230 (79)	122 (41.9)		
Chi Sq	**38.81*****		**44.50*****		**34.96*****		**55.71*****			
BMZ										
Normal	16 (6.7)	46 (19.2)	30 (14.8)	72 (35.5)	53 (24.8)	82 (38.3)	58 (19.9)	91 (31.3)	**30.10*****	**22.77*****
Thinness	223 (93.3)	193 (80.8)	173 (85.2)	131 (64.5)	161 (75.2)	132 (61.7)	233 (80.1)	200 (68.7)		
Chi Sq	**42.41*****		**40.32*****		**45.30*****		**61.93*****			

*Values in parenthesis depicts percentage. ***p<0.001*

4.6.2 IMPACT ON MICRONUTRIENT STATUS

4.6.2.1 Impact assessment on iron status

DFS being fortified with iodine and iron, efficacy of DFS in improving iron and iodine status was assessed in this section.

Total population was broadly distributed in experimental and control for DFS supplementation and further to dewormed and non dewormed subgroups in both study groups ending up with four major groups (E+DW, E, C+DW and C group).

Data on comparison between experimental group and control group for before and after impact of intervention is depicted in **Table 4.75**. It was observed that, at baseline an overall significant difference (1 g/dl) between experimental and control groups ($p<0.001$) and both the groups were non comparable. This was more visible for gender wise differences. However, the difference reduced to non significant levels towards the end, since experimental group showed higher level of significant ($p<0.001$) improvement in hemoglobin levels with 0.42 g/dl, whereas control group showed significant ($p<0.001$) reduction of hemoglobin levels with -0.54 g/dl. Mean

improvement in hemoglobin was 0.38 g/dl for boys and 0.47 g/dl for girls in experimental group and in control group the reduction was -0.52 g/dl for boys and -0.57 for girls. Thus, towards the end of the study the difference between both the study groups was 0.02 g/dl, which was found non significant.

Table 4.75: Impact of intervention on mean hemoglobin concentration of the children

	Hemoglobin Level(g/dl) (Mean±SD)				**'t' value**	
	Experimental		**Control**			
	Boys	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**
Initial	8.80 ± 1.23	8.52 ± 1.23	9.69 ± 1.05	9.54 ± 1.01	**8.73*** **	**9.305*** **
Final	9.18 ± 0.87	8.99 ± 0.86	9.16 ± 0.89	8.96 ± 0.90	**0.22^{NS}**	**0.34^{NS}**
Difference	0.38 ± 1.04	0.47 ± 1.14	-0.52 ± 0.87	-0.57 ± 0.89	**10.62*** **	**10.56*** **
Paired 't'	**5.66*** **	**5.91*** **	**10.02*** **	**9.75*** **		
	Total		**Total**		**Total**	
Initial	8.67 ± 1.24		9.62 ± 1.03		**12.71*** **	
Final	9.09 ± 0.87		9.07 ± 0.90		**0.336^{NS}**	
Difference	0.42 ± 1.08		-0.54 ± 0.88		**14.97*** **	
Paired 't'	**8.18*** **		**13.98*** **			

****p<0.001*

On comparing hemoglobin concentration based on gender wise variations **within groups**, it was observed that, there was a significant difference among boys and girls at baseline ($p<0.01$) and towards the end ($p<0.05$) in experimental group. However, the improvement in hemoglobin concentration varied non significantly. When hemoglobin concentration of boys and girls belonged to control group was compared, significant ($p<0.01$) was observed at the end; however baseline concentration and difference varied non significantly.

A study conducted by NIN (Sivakumar B et al 2001) revealed a significant reduction in the Hb concentration of the residential school children, in and around Hyderabad after 2 years of supplementation. When the children were matched with respect to gender, age and school, showed that the mean increment of hemoglobin at the end of the school study, after adjusting for the differences in initial hemoglobin, were significantly higher in the DFS group ($p<0.01$) as compared to the iodized salt group.

A comparison of data has clearly shown that NIN- DFS is the only formulation that has been proved to be stable, acceptable and had the desired impact (Sivakumar B and Nair M 2002). The efficacy of NIN-DFS in controlling anemia resulted in mean (95%CI) rise in hemoglobin of 0.72 (0.43-1.01) g/dl in comparison to iodized salt, which is well within the range of global experience (Bhan committee 2006). Another study conducted by (Malvika V and Rajgopalan S 2006) reported the mean improvement in the hemoglobin concentration with 0.65 g/dl compared to the reduction in control group with -0.30 g/dl after the period of one year. This finding supports our study results with increased hemoglobin in experimental group and reduction in control group. **Table 4.76** reveals the evidence for the same, indicating mean increase upto 0.60 g/dl in E+DW group ($p<0.001$) and 0.21 g/dl in E group ($p<0.01$), which are experimental groups, compared to significant reduction ($p<0.001$) in both the control groups (C+DW and C group) with -0.54 g/dl and -0.56 g/dl respectively.

Table 4.76: Mean hemoglobin concentration of each group before and after intervention

	E+DW	**E**	**C+DW**	**C**	**'F' Value**
Initial	8.46± 1.24	8.91±1.19	9.69±1.05	9.51±1.01	**62.55*** **
Final	9.06±0.86	9.12±0.88	9.15±0.94	9.01±0.87	**1.24^{NS}**
Difference	0.60±1.09	0.21±1.04	-0.54±0.85	-0.56±0.90	**83.91*** **
Paired 't'	**8.47*** **	**2.92** **	**9.23*** **	**10.49*** **	

***p<0.01, ***p<0.001*

This could be due to incorporation of multiple strategies as additional benefit along with DFS supplementation, which is been proven highly evident from the improvement in E+DW group.

In support to our statement a study conducted by (Thi Lee H 2007) in Vietnamese school children revealed that, 10.7 mg iron through fortified noodles supplementation along with deworming (Mabendazole 500 mg) 2 doses within a span of 6 months showed an increase in mean Hb with 1.78 g/dl. This was comparatively a remarkable improvement which is significantly higher than our results. However, this improvement could have been due to very lower prevalence of IDA as 0.9% compared to our 99% anemic subjects at baseline.

On comparing data on gender wise mean hemoglobin levels in all four study groups **(Table 4.77)**, the observed variation was highly significant **between all four groups** ('t' value- $p<0.001$) at baseline and it varied non significantly towards the end for both the genders. However, the difference at the end was highly significant ('t' 47.04- $p<0.001$) between all four groups, ranged from -0.55 - +0.63 g/dl for boys and -0.61 - +0.56 for girls.

When each group was divided based on gender and pre-post comparison for mean hemoglobin conc. was done, higher level of significant difference ($p<0.001$) was observed among both genders; except E group where the change remained non significant in boys towards end compared to baseline.

Mean hemoglobin concentration variations in boys at baseline and at the end of the study **between groups** revealed that, E+DW group showed significant difference at ($p<0.001$) compared to rest of the three groups. The difference was also significant for girls category ($p<0.001$) compared to C+DW and C group, but it varied non significantly when compared to E group.

Similarly, baseline and final mean hemoglobin concentrations of boys and girls belonged to E group also varied significantly ($p<0.001$) compared to C+DW and C group. There was no significant difference between C+DW and C groups was observed amongst the boys, however the difference was significant ('t' -1.96, $p<0.05$) at the end amongst the girls.

Table 4.77: Impact on gender wise mean hemoglobin levels of the children

Study group	Hemoglobin levels (g/dl) (Mean ± SD)								Paired 't' value	
	Boys (n=516)				Girls (n=431)					
	N	Initial	Final	Difference	N	Initial	Final	Difference	Boys	Girls
E + DW	**129**	8.50 ± 1.18 (8.29-8.71)	9.14 ± 0.81 (8.99-9.28)	0.63 ± 1.05 (0.45-0.81)	**110**	8.41± 1.31 (8.16-8.66)	8.97± 0.90 (8.80-9.14)	0.56 ± 1.15 (0.34-0.78)	**6.87*** **	**5.10*** **
E	**108**	9.14 ± 1.21 (8.92-9.38)	9.23 ± 0.94 (9.04- 9.40)	0.08 ± 0.94 (0.94-0.10)	**95**	8.65 ± 1.11 (8.42-8.88)	9.01 ± 0.80 (8.85-9.18)	0.37 ± 1.12 (0.14-0.59)	**0.86^{NS}**	**0.31*** **
C+DW	**133**	9.73 ± 1.02 (9.55-9.90)	9.17 ± 0.93 (9.01-9.33)	-0.55 ± 0.82 (-0.70- -0.42)	**81**	9.63 ± 1.11 (9.38-9.87)	9.12 ± 0.95 (8.94-9.33)	-0.50 ± 0.91 (-0.70- -0.30)	**7.86*** **	**4.99*** **
C	**146**	9.65 ± 1.07 (9.47-9.82)	9.15 ± 0.86 (9.01-9.29)	-0.50 ± 0.93 (-0.65- -0.34)	**145**	9.49 ± 0.96 (9.33-9.65)	8.87 ± 0.86 (8.73-9.01)	-0.61 ± 0.88 (-0.76- -0.47)	**6.45*** **	**8.45*** **
'F' value		**33.57*** **	**0.20^{NS}**	**47.04*** **		**30.51*** **	**1.49^{NS}**	**38.91*** **		
't' value										
E+DW v/s E		4.108***	0.747^{NS}	4.297***		1.406^{NS}	0.377^{NS}	1.217^{NS}		
E+DW v/s C		8.353***	0.144^{NS}	9.414***		7.259***	0.871^{NS}	8.936***		
E+DW v/s C+DW		8.921***	0.286^{NS}	10.243***		6.904***	1.114^{NS}	7.143***		
E v/s C		3.446***	0.622^{NS}	4.838***		6.021***	1.313^{NS}	7.197***		
E v/s C+DW		3.972***	0.453^{NS}	5.525***		5.799***	0.803^{NS}	5.677***		
C v/s C+DW		0.612^{NS}	0.512^{NS}	0.577^{NS}		0.904^{NS}	1.960*	0.898^{NS}		

p<0.05,*P<0.001*

After categorizing the children gender wise and then based on their age into two categories (<12 years and ≥ 12 years), mean hemoglobin concentration was observed to vary significantly **(Table 4.78)**. On comparing data **between groups,** significant difference (p<0.001) was observed amongst both the genders belonged to each age group (<12 years and ≥12 years).

Table 4.78: Change in mean hemoglobin levels in the subjects after the intervention: Level of significance between the groups

Study group	**Boys (N=516)**				**Girls (N=431)**			
	N	**<12 yrs**	**N**	**≥12 yrs**	**N**	**<12 yrs**	**N**	**≥ 12 yrs**
E + DW	116	0.64 ± 1.04	13	0.55 ±1.11	98	0.51 ± 1.15	12	0.93 ± 1.06
E	95	0.02 ± 0.95	13	0.49 ±0.75	86	0.33 ± 1.15	9	0.65 ± 0.80
C + DW	128	-0.55 ±0.82	5	-0.74±0.59	71	-0.50±0.92	10	-0.51 ± 0.83
C	118	-0.47 ±0.93	28	-0.62±0.93	131	-0.65±0.88	14	-0.33 ± 0.85
'F' value		**40.53*** **		**7.95*** **		**32.96*** **		**6.98*** **
't' value								
E *v/s* C		3.75***		4.17***		6.74***		2.75*
E+DW *v/s* C		8.58***		3.29**		8.31***		3.30**
C+DW *v/s* C		0.73^{NS}		0.42^{NS}		1.07^{NS}		0.52^{NS}
E *v/s* E+DW		4.50***		0.17^{NS}		1.04^{NS}		0.72^{NS}
E *v/s* C+DW		4.67***		4.04**		5.08***		3.04**
E+DW *v/s* C+DW		9.94***		3.35**		6.36***		3.55**

P<0.05,p<0.01, ***p<0.001*

***Difference in Boys*:**

Individual group comparison for the difference in hemoglobin concentration amongst boys at the end of the study, revealed a higher level of significant variation between experimental groups (E *v/s* E+DW) and control groups (C *v/s* C+DW) (p<0.001) for <12 years age group. However, both control groups (C *v/s* C+DW) showed non-significant difference in this category.

When the individual groups were compared for ≥ 12 years category, significant (p<0.001) difference between E and C group was observed, whereas the significance was at (p<0.01) difference between E+DW *v/s* C and C+DW, E *v/s* C+DW. On comparing **within group**, C *v/s* C+DW group did not show any significant difference for this category. Similarly E *v/s* E+DW groups showed difference at non significant levels.

***Difference in Girls*:**

Individual group comparison for the difference in hemoglobin concentration amongst girls at the end of the study, revealed a higher level of significant variation between experimental groups (E *v/s* E+DW) and control groups (C *v/s* C+DW) ($p<0.001$) for <12 years age group. However, **within group** comparison for both control group (C *v/s* C+DW) and experimental group (E *v/s* E+DW) showed non-significant difference in this category.

When the individual groups were compared for ≥ 12 years category, significant difference between experimental and control groups was observed, E+DW *v/s*C ($p<0.01$) and C+DW ($p<0.01$), E *v/s* C+DW ($p<0.01$) and C ($p<0.05$) was observed. On assessment for **within group** comparisons, C *v/s* C+DW group did not show any significant difference for this category also. Similarly E *v/s* E+DW groups showed difference at the non significant level.

A study conducted by (Vinodkumar M and Rajgopalan S 2007) also supported our findings by indicating, a significant increase ($p<0.05$) in mean hemoglobin concentration of the children with 0.65 g/dl compared to 0.3 g/dl decline ($p<0.05$) amongst control group within a span of one year. These children were also dewormed twice to enhance the efficacy of fortified salt during the study period among the school children of Chennai city.

Table 4.79: Impact on intervention on percent prevalence of anemia

Categories Hb (g/dl)	Percent prevalence of anemia								Chi square	
	E+DW		E		C+DW		C		Pre	Post
	Pre	Post	Pre	Post	Pre	Post	Pre	Post		
<7	27 (11.3)	2 (0.8)	9 (4.4)	-	4 (1.9)	4 (1.9)	2 (0.7)	1 (0.3)	**100.25*** **	**10.16^{NS}**
7-9.99	190 (79.5)	200 (83.7)	155 (76.4)	167 (82.3)	126 (58.9)	174 (81.3)	188 (64.6)	250 (85.9)		
10-11.49	19 (7.9)	36 (15.1)	37 (18.2)	34 (16.7)	78 (36.4)	32 (15)	94 (32.3)	38 (13.1)		
≥ 11.5	3 (1.3)	1 (0.4)	2 (1.0)	2 (1.0)	6 (2.8)	4 (1.9)	7 (2.4)	2 (0.7)		
Chi sq	**152.52 *****		**61.80*****		**222.16*****		**203.14*****			

*Values in parenthesis depicts percentage. ***P<0.001*

Table 4.79 and **Figure 4.14** reveals that, there was a significant shift in each category of the classification for anemia (WHO 2001). On depicting the results of each group, it can be stated that, each group has shown positive or negative shift in percent prevalence.

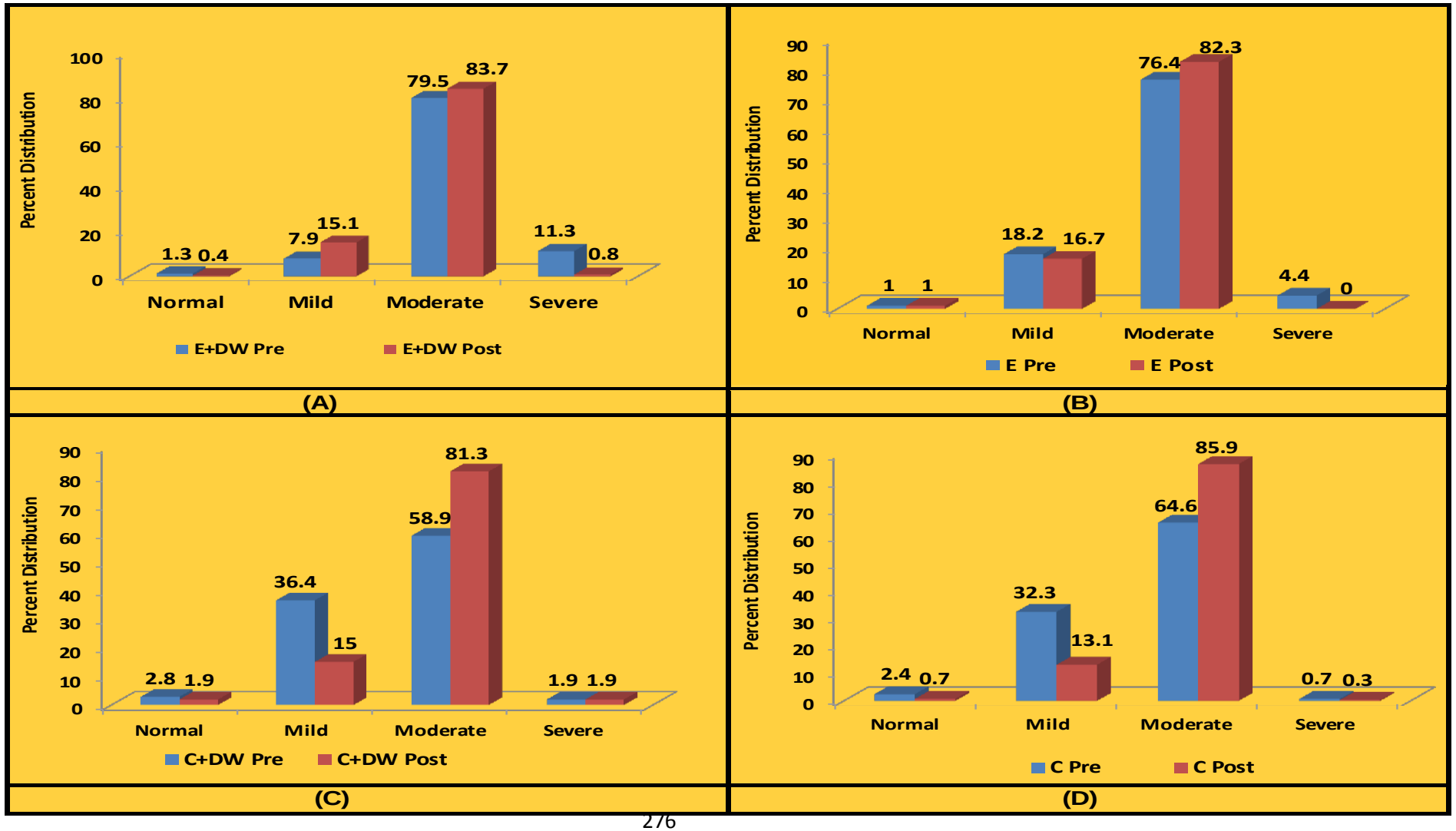
***E+DW Group*: Figure 4.14 (A)** revealed a positive shift in the distribution curve with significant ($p<0.001$) reduction in the prevalence of severe anemia (11.3% to 0.8%). However, it also showed a negative shift with the increased prevalence of mild anemia (7.9 to 15.1%) and moderate anemia (79.5 to 83.7%). This suggests that, there has been a positive impact of DFS supplementation along with deworming on the percent prevalence of anemia.

***E Group*: Figure 4.14 (B)** in this supplemented group, effect of DFS supplementation solely on percent prevalence of anemia was observed. There was a rise in hemoglobin with increase in the percent population of moderately anemic subjects (76.4 to 82.3%) and positive shift with reduction in severely anemic (4.4 to 0%). However, reduced percentages of mildly anemic subjects (18.2 to 16.7%) showed a negative shift.

***C+DW Group:* Figure 4.14 (C)** in this dewormed and no supplemented group, effect of deworming alone on controlling percent prevalence of anemia was assessed. There was 23% rise in the prevalence of moderate anemia (58.9 to 81.3%) and prevalence of mildly anemic subjects decreased from 36.4 to 15%. So there was a negative shift.

***C Group*: Figure 4.14 (D)** In this non dewormed and non supplemented group, prevalence of anemia over a period of one year during the growth spurt of the children without intervention was observed. There was an increase in moderate anemia by 21% (64.6 to 85.9%) and decrease in mild anemia by 20% (32.3 to 13.1%). Hence, it can be stated that, 20% of the mildly anemic subjects negatively shifted to moderate category of anemia.

Figure4.14: Distribution of anemia classification before and after intervention in children



Difference in percent prevalence amongst each group with the level of significance has been depicted in **Table 4.80.** This indicates a positive impact of supplementation with or without deworming over a controlled condition with or without deworming amongst our study groups.

Table 4.80 revealed that, there was a 6.3% reduction in anemia amongst E+DW group which was highly significant ($p<0.001$). However, E group showed a non significant increase in the category **(Table 4.79)** with reduced percentages of mildly anemic (McNemar's test) subjects.

Table 4.80: Change in prevalence of anemia in all groups

Study Group	N	Percent prevalence of moderate + severe anemia					Chi square (A *v/s* B)
		N	Initial % (A)	n	Final % (B)	% difference	
E+DW	239	217	90.8	202	84.5	- 6.3	**60.69*** **
E	203	164	80.8	167	82.3	+1.5	**0.711**^{NS}
C+DW	214	130	60.7	178	83.2	+22.5	**30.97*** **
C	291	190	65.3	251	86.3	+21.0	**46.74*** **

****p<0.001*

Control groups (C and C+DW) showed higher level of significance ($p<0.001$) with increase in the prevalence of moderate anemia and revealed no significant impact of deworming in controlling anemia (**Table 4.80)**. Thus, it can be stated that, E+DW has been proven to be the best strategy to bring about reduction in the prevalence of anemia within a desired period of study tenure. However, DFS supplementation alone also was proven to be the better strategy compared to the control groups, which maintained the status and resisted the drastic reduction in the hemoglobin status during the growth spurt of the children at preadolescent and adolescent age.

Table 4.81: Impact of intervention on gender wise and group wise distribution of the children

	Urinary iodine excretion (µg/L) (Median ± SD)												‘U’ test	
	Experimental				Control				Total					
	N	Boys	N	Girls	N	Boys	N	Girls	N	Boys	N	Girls	Boys	Girls
Initial	237	131.85 ± 106.73	205	133.61 ± 105.29	279	158.83 ± 104.29	226	155.70 ± 109.33	516	147.43± 106.19	431	144.83± 108.67	**3.35*** **	**3.39*** **
Final		186.08 ± 102.75		171.93 ± 113.93		278.67 ± 148.38		196.55 ± 126.81		229.76 ± 137.68		188.40 ± 121.90	**7.36*** **	**1.98***
‘Z’ test		**5.56*** **		**4.90*** **		**9.83*** **		**3.67*** **		**11.17*** **		**6.06*** **		
		Total				**Total**				**Total**			**‘U’ test**	
Initial		132.31 ± 105.96				158.18 ± 106.49				145.91 ±107.27			**4.72*** **	
Final		177.02 ± 10.7.96				238.22 ± 143.66				204.03 ± 131.84			**6.72*** **	
‘Z’ test		**7.45*** **				**10.04*** **				**12.47*** **				

**p<0.05, ***p<0.001*

Our study findings have also been supported by the conclusions discussed in the NIN reports, indicating the efficacy of NIN-DFS in reducing anemia prevalence significantly ($p<0.001$) by improving hemoglobin concentration amongst anemic children (Rangnathan S and Sesikeren B 2008).

4.6.2.2 Impact assessment on iodine status

Table 4.81 reveals data on impact of intervention on median urinary iodine excretion (UIE) on school children belonging to both experimental and control groups. In experimental group median UIE at baseline was observed to be 132.31µg/L, which was a representation of the UIE levels of the boys with 131.85 µg/L and girls with 133.61 µg/L.After intervention (DFS supplementation and NHE) amongst experimental group, the median UIE increased significantly ($p<0.001$) to 177.02 µg/L. The improvement was also significant ($p<0.001$) amongst the boys (186 µg/L) and girls (171.93 µg/L).

However, in control group median UIE was 158.18 µg/L at baseline, which represented the median UIE levels of the boys with 158.83 µg/L and girls with 155.70 µg/L. After intervention (NHE) amongst control group, the median UIE increased significantly ($p<0.001$) to 238.22 µg/L. The improvement was also significant ($p<0.001$) amongst the boys (278.67 µg/L) and girls (196.55 µg/L)**(Table 4.81)**.

The study results reported by (Zimmerman et al 2002) on impact of DFS and IS on the iodine status of Moroccan school children supports our findings on the impact of DFS. It revealed a significant increase ($p<0.01$) in UIE of both the groups after 10 months intervention, indicating compatibility of DFS in supplying iodine same as an iodized salt. Another study conducted by (Zimmerman et al 2004) in West and North African children, reported no difference observed in median urinary iodine excretion between both the groups.

On comparing median UIE **between groups**(Experimental *v/s* Control) at baseline and at the end, a significant ($p<0.001$) difference was observed, which was also represented by comparison between boys belonged to both the groups and so as with girls. The difference was found to be significant ($p<0.001$) at baseline between boys and girls. Towards the end, this difference remained with similar level of significance

between boys (p<0.001), but the significance level reduced to p<0.05 on comparison between UIE of the girls.

Overall median UIE of the population was 145.91 µg/L at baseline, which increased significantly (p<0.001) to 204.03 µg/L towards the end. This suggests the increased iodine nutrition amongst the children. When the baseline median values for individual groups were compared without gender wise differentiation, the improvement in UIE levels was again found significant at higher level (p<0.001) at the end, similar to the results at gender wise variations. When the comparison was made between groups, (all the study groups) showed significant difference (p<0.001) at baseline and at the end, which also supports the above findings.

Table 4.82: Median urinary iodine excretion of each group before and after intervention

	E+DW	**E**	**C+DW**	**C**	**'H' Value**
Initial	118.92	148.62	156.28	158.83	**35.51*** **
Final	165.30	201.44	261.54	227.60	**61.87*** **
Difference	41.08	45.62	103.10	43.98	**23.63*** **
'Z' test	**5.91*** **	**4.61*** **	**8.16*** **	**5.96*** **	

***p<0.001

Sufficient iodine nutrition at baseline gave a clue to availability of iodized salt in the region. However, an action of two essential micronutrients through single vehicles was to be assessed as an objective of the study. Hence, continuing with the sufficient population, half of the children were provided replaced supply of iodized salt with DFS to assess the efficacy of iron and iodine together.

Further classification of experimental and control groups based on deworming was carried out. All the four groups were compared for the variations in UIE within and between both the genders. On comparing all groups for baseline and final UIE levels, a significant (p<0.001) difference between all four groups was observed- indicated by H values **(Table 4.83)**.

Table 4.83: Impact of intervention on all four groups

Study group	Urinary iodine excretion (µg/L) (Median ± SD)								'U' test Boys *v/s* Girls	
	Boys (n=516)				Girls (n=431)				Initial	Final
	N	Initial	Final	'z' test	N	Initial	Final	'z' test		
E+DW	**129**	111.50± 95.35	162.11± 95.20	4.63***	**110**	122.90± 101.35	165.50± 109.64	3.74***	**0.28^{NS}**	**0.38^{NS}**
E	**108**	150.99± 115.02	210.98± 108.83	3.11***	**95**	145.23± 108.81	193.00± 117.39	3.26***	**0.69^{NS}**	**0.54^{NS}**
C+DW	**133**	157.83± 103.73	311.10± 137.11	7.88***	**81**	147.23± 108.84	214.93± 125.71	2.82**	**0.11^{NS}**	**4.52*** **
C	**146**	158.83± 104.82	249.71± 157.65	5.89***	**145**	156.70± 109.66	195.49± 127.77	2.33*	**0.84^{NS}**	**3.59*** **
'H' test		**20.86*** **	**64.06*** **			**15.50*** **	**8.30*** **			

**p<0.05, **p<0.01, ***p<0.001*

On comparing UIE values before and after intervention **within groups ('Z' test)** and **genders**, there was a higher level of significant difference ($p<0.001$) amongst boys was observed for all four groups. However, the level of significance reduced for non supplemented groups (C+DW-$p<0.01$ and C-$p<0.05$) while comparing UIE amongst girls. Our study results were supported by the (Sivakumar B et al 2001) NIN with significant increase ($p<0.05$) in UIE levels amongst both experimental and control groups after intervention period **(Table 4.83)**.

When data on UIE **within groups** and **between genders ('U' test)** was compared at baseline and end, a non significant difference was observed for E+DW and E group. However, the difference showed a higher level of significance ($p<0.001$) between boys and girls at the end for C+DW and C group **(Table 4.83)**.

Table 4.84 reveals a comparison **between groups** and **age groups**. On comparing baseline and final UIE between all four groups, a significant ($p<0.001$) difference was observed for children aged <12 years. However, this difference was non significant for children aged $\geq$ 12 years.

Table 4.84: Change in median UIE levels in the subjects after the intervention: Level of significance between the groups

Study Group	Urinary iodine excretion (µg/L) (Median ± SD)							
	<12 years				≥12 years			
	N	Initial	Final	'z' test	N	Initial	Final	'z' test
E+DW	214	111.81± 100.16	156.44 ± 103.36	**5.12*** **	25	133.61 ± 78.72	200.50 ± 83.93	**3.22*** **
E	181	153.12 ± 112.31	196.85 ± 112.20	**3.84*** **	22	139.37 ± 111.29	235.06 ± 117.06	**2.94** **
C+DW	198	156.28 ± 106.49	273.79 ± 138.83	**8.01*** **	16	158.52 ± 92.17	187.46 ± 113.95	**1.40**^{NS}
C	248	159.87 ± 109.01	228.64 ± 148.27	**5.23*** **	43	158.13 ± 94.25	219.30 ± 139.89	**2.95** **
'H' test		**34.67*** **	**69.01*** **			**2.11**^{NS}	**1.12**^{NS}	
'U' test								
E *v/s* C		1.82^{NS}	2.03*			0.85^{NS}	0.56^{NS}	
E+DW *v/s* C		5.78***	5.36***			1.39^{NS}	0.25^{NS}	
C+DW *v/s* C		1.37^{NS}	2.89**			0.44^{NS}	0.87^{NS}	
E *v/s* E+DW		3.26***	3.08**			0.33^{NS}	0.68^{NS}	
E *v/s* C+DW		0.47^{NS}	4.94***			0.36*	0.89*	
E+DW *v/s* C+DW		4.16***	7.98***			0.75*	0.72*	

**p<0.05, **p<0.01, ***p<0.001*

Within groups comparison for baseline and final UIE levels showed a significant (p<0.001 and p<0.01) difference amongst all groups (E+DW, E and C) belonged to both the age groups (<12 years and ≥ 12 years). However, the difference was non significant for C+DW in ≥ 12 years age group.

Between group comparisons showed significant difference for majority of the comparisons in baseline UIE for <12 years age group, whereas E *v/s* C, E ***v/s*** C+DW and C ***v/s*** C+DW did not show significant difference. However, at the end of the study, all the groups showed significant improvement in UIE in this age group. On the other hand, when data was compared for >12 years age group, a non significant difference **between groups** at baseline and end UIE levels was observed. Only the comparison between experimental groups (E and E+DW) and C+DW showed a significant (p<0.05) difference in UIE levels at baseline and end in this age group.

Table 4.85: Impact of intervention on iodine deficiency disorder classification of the subjects

Categories UIE (µg/L)	Percent prevalence of IDD								Chi square	
	E+DW		E		C+DW		C			
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
<20	14 (5.9)	2 (0.8)	13 (6.4)	-	3 (1.4)	-	1 (0.3)	-	**37.34*** **	**18.86*** **
20-49.99	27 (11.3)	3 (1.3)	18 (8.9)	5 (2.5)	16 (7.5)	4 (1.9)	15 (5.2)	3 (1.0)		
50-99.99	55 (23)	31 (13)	35 (17.2)	10 (4.7)	39 (18.2)	10 (4.7)	48 (16.5)	21 (7.2)		
≥ 100	143 (59.8)	203 (84.9)	137 (67.5)	179 (88.2)	156 (72.9)	200 (93.5)	227 (78)	267 (91.8)		
Chi sq	**36.60*** **		**24.30*** **		**78.42*** **		**56.33*** **			

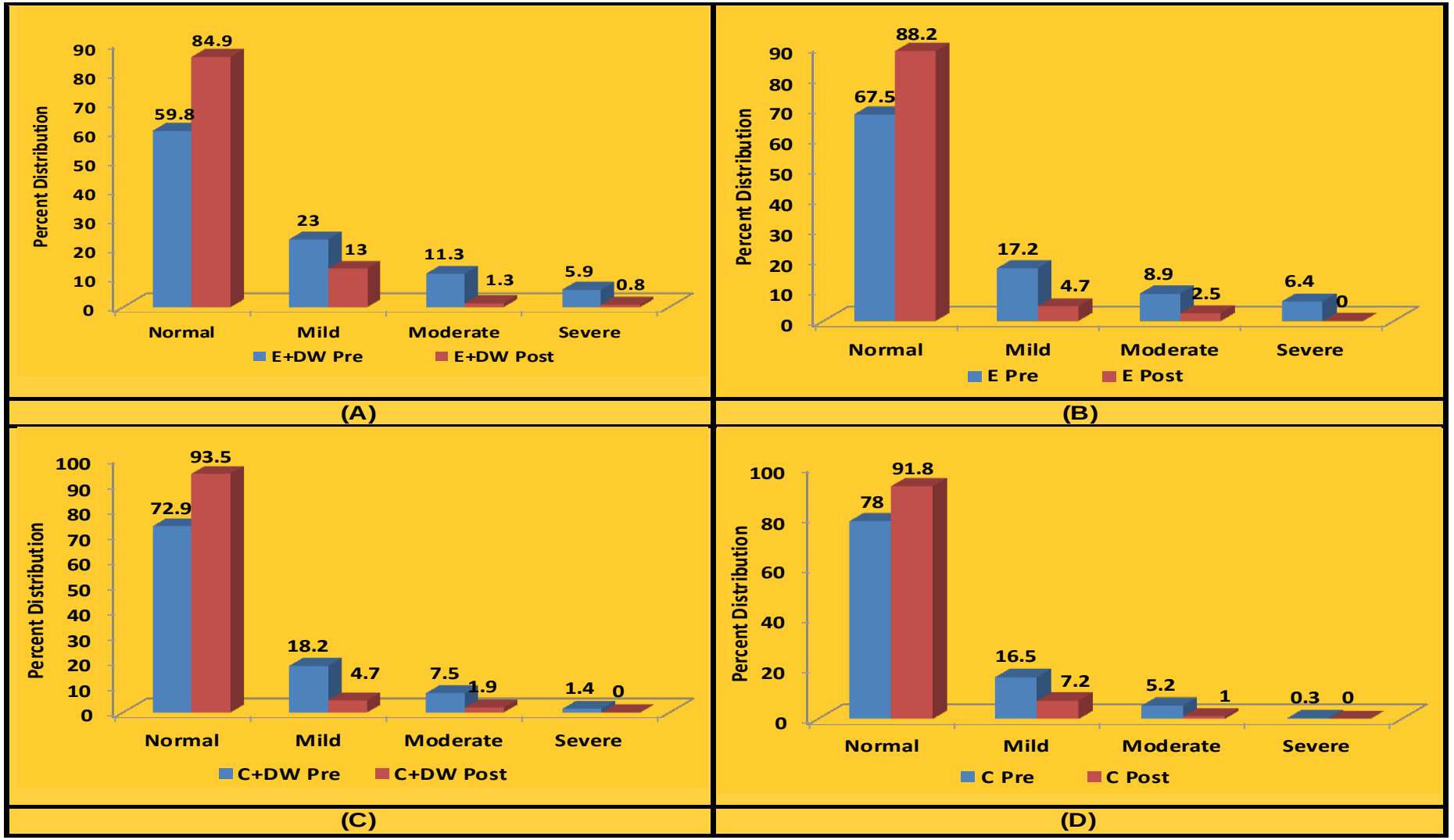
*Values in parenthesis depicts percentage. ***p<0.001*

Table 4.85 and **Figure 4.15** reveals that, there was a significant shift in each category of the classification for IDD (WHO/ICCIDD/UNICEF 2007). On depicting the results of each group, it can be stated that, ***E+DW Group*: Figure 4.15 (A)** revealed a positive shift in the distribution curve with significant ($p<0.001$) reduction in the prevalence of severe iodine deficiency (5.9% to 0.8%). It also showed a positive shift with the decreased prevalence of mild iodine deficiency (23 to 13%) and moderate iodine deficiency (79.5 to 83.7%). Proportion of normal subjects increased significantly ($p<0.001$) in the groups compared to the baseline (59.8 to 84.9%) indicating the successful acceptability and usage of DFS by the community **(Table 4.85)**.

While comparing percentages of normal subjects **(Table 4.85)** at baseline with E group and control groups (C+DW and C group), there was non significant difference between both experimental groups (E+DW *v/s* E group). However, this difference was significant ($p<0.01$) comparing E+DW group *v/s* control groups (C+DW and C) at baseline and at the end.

This suggests that, there has been a positive impact of DFS supplementation along with deworming on the percent prevalence of iodine deficiency.

Figure4.15: Distribution of ID classification before and after intervention in children



***E Group*: Figure 4.15 (B)** in this supplemented group, effect of DFS supplementation solely on percent prevalence of iodine deficiency was assessed. There was a shift in urinary iodine content with decrease in the percent population of moderately iodine deficient subjects (8.9 to 2.5%) and positive shift with reduction in severe iodine deficiency (6.4 to 0%). There was also a reduction in mild iodine deficiency (18.2 to 16.7%), which showed a negative shift.

Proportion of normal subjects increased significantly ($p<0.001$) in the groups compared to the baseline (57.5 to 88.2%) indicating the successful acceptability and usage of DFS by the community **(Table 4.85).**

While comparing percentages of normal subjects **(Table 4.85)** at baseline with control groups (C+DW and C group), there was non significant difference between E *v/s* C+DW groups. However, this difference was significant ($p<0.01$) comparing E group *v/s* C group at baseline. Towards the end, with a significant improvement in the proportion of normal subjects, the difference between E group and control groups (C+DW and C) remained non-significant.

***C+DW Group*: Figure 4.15 (C)** In this dewormed and non supplemented group, effect of deworming alone on controlling percent prevalence of iodine deficiency was assessed. There was 21% rise in the percent of normal subjects (72.9 to 93.5%) and prevalence of different categories of iodine deficiency decreased significantly ($p<0.001$). However, the improvement in the percent prevalence could have been due to NHE provided towards implementing best practices on consumption, cooking and storage of iodized salt. This may be an achievement in itself, with more than 90% of the children with normal iodine levels, indicating success of the program and their adequate iodine nutrition.

***C Group*: Figure 4.15 (D)** In this non dewormed and non supplemented group, prevalence of iodine deficiency over a period of one year during the growth spurt of the children without intervention was observed. There was a 14% increase in normal levels (78 to 91.8%) and decrease in all categories of iodine deficiency. This group also achieved significantly higher levels of normal subjects within the group ($p<0.001$).

Difference in percent prevalence amongst each group with the level of significance has been depicted in **(Table 4.86).** This indicates a positive impact of supplementation with or without deworming over a controlled condition with or without deworming amongst our study groups.

Table 4.86 revealed that, there was a reduction in prevalence of iodine deficiency amongst all groups. This reduction was at higher level of significance ($p<0.001$) amongst E group with 20.3%. However, the level of significance was ($p<0.01$) for E+DW and C groups. C+ DW group showed 20.6% reduction. Thus, overall there was a positive effect of DFS supplementation and NHE on both the groups.

Table 4.86: Change in the prevalence of iodine deficiency in all groups

Study Group	N	Percent prevalence of iodine deficiency based on UIE					Chi square (A *v/s* B)
		n	Initial % (A)	n	Final % (B)	% difference	
E+DW	239	96	40.2	38	15.9	- 24.3	**7.79****
E	203	67	33.0	25	12.3	- 20.3	**12.38*****
C+DW	214	58	27.1	14	6.5	- 20.6	**3.97***
C	291	64	22.0	24	8.2	- 13.8	**8.66****

**p<0.05, **p<0.01, ***p<0.001*

4.6.2.3 Impact assessment on thyroid analytes

Thyroid hormone analysis revealed that, the hormones of the study groups are described in **Table 4.87** at baseline and towards the end. The mean levels of each hormone and their ranges have also been quoted for each group. In our study population the mean values of thyroid hormones varied within normal ranges amongst majority of the study subjects.

The study results availed by Zimmerman et al (2002) on impact of DFS and IS on the iodine status of Moroccan school children revealed a similar pattern of research with analysis of thyroid hormone profile along with UIE. The study reported a non significant decrease in median TSH in both groups over the course of the study. Median TSH was within the normal range in both the groups throughout the study. Mean serum T_4 increased significantly from baseline in the DFS group and was significantly greater than in the IS group. The prevalence of hypothyroidism was significantly reduced in the DFS group compared with the IS group ($p<0.001$).

Table 4.87: Thyroid hormone analyses of the subjects (n=189)

Study group	Stage	Thyroid hormone profile of the subjects					'H' Value	
		Mean	SD	Median	5th percentile	95th percentile	Initial	Final
TSH (µIU/ml)								
E+DW	**Initial**	3.04	1.67	2.76	0.45	7.55	**1.56^{NS}**	**1.75^{NS}**
(n=48)	**Final**	2.33	1.04	2.24***	0.89	3.99		
E	**Initial**	2.91	1.11	2.70	1.53	5.50		
(n=41)	**Final**	2.46	0.82	2.22**	1.36	4.15		
C+DW	**Initial**	2.84	1.33	2.35	1.34	5.06		
(n=59)	**Final**	2.67	1.25	2.57^{NS}	1.21	4.79		
C	**Initial**	2.73	1.11	2.47	1.02	5.27		
(n=41)	**Final**	2.66	1.41	2.23^{NS}	1.02	5.75		
FT_4 (ng/dl)								
E+DW	**Initial**	1.43	0.13	1.44	1.20	1.65	**63.91*** **	**8.55***
(n=48)	**Final**	1.31	0.13	1.33***	1.07	1.55		
E	**Initial**	1.55	0.26	1.53	1.17	1.91		
(n=41)	**Final**	1.27	0.14	1.28***	0.96	1.48		
C+DW	**Initial**	1.27	0.12	1.26	1.08	1.48		
(n=59)	**Final**	1.23	0.17	1.24*	0.95	1.49		
C	**Initial**	1.31	0.15	1.29	1.09	1.56		
(n=41)	**Final**	1.24	0.16	1.27*	0.98	1.54		
TT_4 (µg/dl)								
E+DW	**Initial**	9.14	1.25	9.22	6.96	11.22	**9.95****	**16.31*****
(n=48)	**Final**	8.52	1.53	8.70**	5.93	11.80		
E	**Initial**	10.05	1.59	10.21	7.39	13.20		
(n=41)	**Final**	9.63	1.56	9.48^{NS}	7.16	12.46		
C+DW	**Initial**	9.89	1.65	10.23	6.98	12.40		
(n=59)	**Final**	9.45	1.42	9.44*	7.03	11.66		
C	**Initial**	9.74	1.78	9.60	6.40	13.38		
(n=41)	**Final**	9.78	2.02	9.75^{NS}	7.06	13.66		
Ln_Tg								
E+DW	**Initial**	1.64	1.64	1.68	-1.86	4.37	**3.98^{NS}**	**23.37*****
(n=48)	**Final**	1.23	1.10	1.20^{NS}	-0.51	3.25		
E	**Initial**	1.87	1.21	2.21	-0.96	3.68		
(n=41)	**Final**	0.51	1.68	0.54**	-2.60	2.98		
C+DW	**Initial**	1.18	1.63	1.69	-3.07	3.16		
(n=59)	**Final**	0.87	1.32	0.82**	-1.57	3.21		
C	**Initial**	1.44	1.15	1.48	-1.26	2.97		
(n=41)	**Final**	1.11	1.18	0.98**	-1.28	2.67		

p<0.05, **p<0.01,*p<0.001, ^{NS} Non significant reduction compared to baseline*

Serum TSH: Mean TSH values of E+DW, E, C+DW and C groups were 3.04 µIU/ml, 2.91µIU/ml, 2.84 µIU/ml and 2.67 µIU/ml respectively at baseline. These

values decreased to 2.33, 2.46, 2.67 and 2.66 µIU/ml respectively. On comparing median TSH of each group, the values were non significantly different between all the groups ('H' test). There was also a non significant difference observed, when groups were compared individually with each other ('U' test). At the end of the study also the difference was observed to be non significant **between groups** using both the test ('H' test and 'U' test) **(Table 4.87)**.

When within group comparison for baseline and end values was made, median TSH decreased significantly amongst experimental groups – E+DW ($p<0.001$) and E (0.01). However, the difference was observed to be non significant for control groups.

***Serum FT_4*:** Mean FT_4 values of E+DW, E, C+DW and C groups were 1.43, 1.55, 1.27 and 1.31 ng/dl respectively at baseline. These values decreased to 1.31, 1.27, 1.23 and 1.24 ng/dl respectively towards the end. While comparing median values of each group, the values varied significantly between all the groups ($p<0.001$) at baseline and towards the end ($p<0.05$). This difference was observed to be significant ('U' test) between all the groups, except C v/s C+DW at baseline. However, towards the end the difference remained significant between E+DW *v/s* C+DW ($p<0.01$) and C (0.05), rest of the groups showed non significant difference on compared with each other.

When groups were compared for baseline and end values individually, significant reduction was observed in each group. Experimental groups (E+DW and E) showed higher level of significant reduction ($p<0.001$) in end value compared to the baseline value. However, control groups (C+DW and C) showed reduction at ($p<0.05$) **(Table 4.87)**.

***Serum TT_4*:** Mean TT_4 values of E+DW, E, C+DW and C groups were 9.14, 10.05, 9.89 and 9.74 µg/dl respectively at baseline. These values decreased to 8.52, 9.63, 9.45 and 9.78 µg/dl respectively towards the end. While comparing median values of each group, the values varied significantly between all the groups ($p<0.01$) at baseline and towards completion of the study ($p<0.001$). This difference was observed to be significant ('U' test) between E+DW v/s E ($p<0.01$) and C+DW (0.01), rest of the groups showed non significant difference at baseline. However, towards the end of the study period, the degree of significant difference increased between E+DW *v/s* E

($p<0.001$) and control groups (C and C+DW) ($p<0.001$), rest of the groups showed non significant difference when compared with each other **(Table 4.87)**.

When individual groups were compared for baseline and end values, significant reduction was observed for each group. E+DW showed significant reduction at ($p<0.01$) and C showed reduction at ($p<0.05$) towards the end, compared to the baseline values. However, both non dewormed groups (E and C) did not show significant reduction compared to the baseline values.

Thus it is suggestive that, E+DW group showed highest level of significant reduction compared to the rest of the groups.

***Serum Tg*:** Mean Tg values of E+DW, E, C+DW and C groups were 1.64, 1.87, 1.18 and 1.44 µg/ml respectively at baseline. These values decreased to 1.23, 0.51, 0.87 and 1.11 µg/ml respectively towards the end. While comparing median values of each group, the values varied non significantly between all the groups at baseline, except C *v/s* E group ($p<0.05$) and the values varied significantly towards the end ($p<0.001$). However, this difference was observed to be significant ('U' test) between E+DW v/s E ($p<0.001$) and control groups C and C+DW (0.001), rest of the group comparisons showed non significant towards completion of the study.

When groups were compared for baseline and end values individually, significant reduction was observed for in each group ($p<0.01$), except E+DW group ($p>0.05$) **(Table 4.87)**.

4.6.3 IMPACT ON IQ AND COGNITION OF THE SUBJECTS

Towards the end of the study, difference in hemoglobin and UIE levels was observed compared to baseline. Thus, possible change in IQ and cognition scores was assessed in this section.

Data analysis on IQ and cognitive tests could be performed for N=700 children (Owing to drop outs) towards the end of the study.

At baseline there was a significant difference between all four groups for each test. However, the minimum difference was observed for Clerical Test (CT), since majority of the children were anemic and CT depicts concentration amongst children, which is affected due to anemia.

Table 4.88: Mean IQ and cognition score comparison between all groups

	Study Groups (N=700)				'F' value
	E+DW	**E**	**C+DW**	**C**	
	(n=195)	**(n=169)**	**(n=154)**	**(n=182)**	
Draw-a-man Test (DMT)					
Initial	81.46±18.53	94.25±19.08	85.55 ±21.11	81.65 ± 17.93	**17.16*** **
Final	89.14±22.26	96.90±19.75	87.51 ±19.08	86.34 ± 20.10	**9.24*** **
Difference	7.68 ± 16.91	2.65 ± 12.12	1.95 ± 18.30	4.69 ± 15.56	**4.69** **
Paired 't'	**6.34*** **	**2.84** **	**1.33**^{NS}	**4.07*** **	
Visual Memory Test (VMT)					
Initial	0.52 ± 0.25	0.37 ± 0.22	0.41 ± 0.27	0.50 ± 0.29	**14.35*** **
Final	0.63 ± 0.25	0.51 ± 0.26	0.48 ± 0.28	0.53 ± 0.27	**11.04*** **
Difference	0.11 ± 0.23	0.15 ± 0.19	0.07 ± 0.22	0.03 ± 0.26	**8.31*** **
Paired 't'	**6.58*** **	**9.89*** **	**3.81*** **	**1.77**^{NS}	
Clerical Test (CT)					
Initial	0.69 ± 0.24	0.75 ± 0.25	0.76 ± 0.24	0.75 ± 0.28	**2.74***
Final	0.79 ± 0.21	0.83 ± 0.19	0.83 ± 0.19	0.82 ± 0.21	**1.57** ^{NS}
Difference	0.10 ± 0.25	0.08 ± 0.22	0.07 ± 0.25	0.07 ± 0.26	**0.66** ^{NS}
Paired 't'	**5.93*** **	**4.89*** **	**3.96*** **	**3.74*** **	
't' test					
	DMT		**VMT**		**CT**	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
E *v/s* C	6.36***	4.96***	4.75***	0.56^{NS}	0.19^{NS}	0.83^{NS}
E+DW *v/s* C	0.10^{NS}	1.28^{NS}	1.06^{NS}	3.77***	2.08*	1.02^{NS}
C+DW *v/s* C	1.80^{NS}	0.55^{NS}	2.72**	1.65^{NS}	0.35^{NS}	0.78^{NS}
E *v/s* E+DW	6.46***	3.52***	6.53***	4.41***	2.38**	1.89*
E *v/s* C+DW	3.87***	4.34***	1.69^{NS}	1.10^{NS}	0.160^{NS}	0.04^{NS}
E+DW *v/s* C+DW	1.90*	0.74^{NS}	3.99***	5.31***	2.52**	1.83^{NS}
	Difference		**Difference**		**Difference**	
E *v/s* C	1.37^{NS}		4.75***		0.48^{NS}	
E+DW *v/s* C	1.79^{NS}		2.93**		1.29^{NS}	
C+DW *v/s* C	1.46^{NS}		1.33^{NS}		0.26^{NS}	
E *v/s* E+DW	3.29***		1.88^{NS}		0.86^{NS}	
E *v/s* C+DW	0.40^{NS}		3.41***		0.20^{NS}	
E+DW *v/s* C+DW	2.99**		1.59^{NS}		0.99^{NS}	

**p<0.05, **p<0.01, ***p<0.001*

DMT: Overall increment in IQ scores in E+DW group was observed to be highest compared to rest of the groups ($p<0.01$). The difference was observed to be significant compared to E group ($p<0.001$) and C+DW ($p<0.01$) *(Post hoc test and independent't')*. However, E group showed a non significant difference compared to both control groups (C and C+DW) and there was a non significant difference **between groups** (C *v/s* C+DW). This suggests that, DFS along with deworming showed better impact on IQ **(Table 4.88).**

As supportive evidence to our study results on DMT, a departmental study conducted by (Nair S and Patel A 2005) reported a higher mean score increase after intervention in the study group. In our study also, significant improvement was observed for experimental groups (E+DW) compared to the rest of the groups ($p<0.01$).

VMT: While comparing increment in VMT scores, E group showed a highest increment compared to other groups and there was a significant difference between all four groups at baseline and at the end of the study period ($p<0.001$). When compared for the differences in mean values **between groups,** significant difference was observed between E *v/s* C ($p<0.01$) and C+DW ($p<0.05$). However, E+DW also showed a significant difference compared to C group ($p<0.01$) and non significant difference compared to C+DW group. Comparisons **within** experimental groups (E *v/s*E+DW) and control groups (C *v/s* C+DW) showed a non significant difference**(Table 4.88)**.

CT: On comparing increment in CT scores, E+DW showed a highest increment compared to rest of the groups. However, when compared for **within groups** and **between groups** comparisons revealed non significant difference. This also proved a positive impact of DFS and deworming both on the concentration level of the children.

Further, **Table 4.89** reveals the mean score comparison **between groups** and genders of the study children.

Table 4.89: Mean change in IQ/cognitive scores in the subjects after intervention

Study group	Boys (N=348) values (Mean ± SD)				Girls (N=352) values (Mean ± SD)				't' test (Boys *v/s* Girls)			
	N	Initial	Final	Diff.	N	Initial	Final	Diff.	N	Initial	Final	Diff.
Draw-a-man test (DMT) (N=700)												
E+DW	**104**	79.98 ± 18.26	88.27 ± 19.41	8.29±15.71	**91**	83.15 ± 18.79	90.13 ± 25.20	6.98 ± 18.25	**195**	**1.19^{NS}**	**0.57^{NS}**	**0.54^{NS}**
E	**86**	92.10 ± 17.82	94.17 ± 17.90	2.07±12.50	**83**	94.47 ± 20.18	99.72 ± 21.25	3.25 ± 11.78	**169**	**1.49^{NS}**	**1.83^{NS}**	**0.63^{NS}**
C+DW	**85**	87.00 ± 21.08	85.71 ± 18.10	-1.29±17.33	**69**	83.76 ± 17.67	89.71 ± 20.14	5.95 ± 12.79	**154**	**0.95^{NS}**	**1.28^{NS}**	**2.46****
C	**73**	79.55 ± 18.24	81.00 ± 17.64	1.45 ± 16.26	**109**	83.06 ± 20.00	89.91 ± 20.91	6.86 ± 14.75	**182**	**1.29^{NS}**	**3.10****	**2.28***
'F'value		**8.80*****	**7.16*****	**6.53*****		**9.93*****	**4.17****	**1.03^{NS}**				
Visual memory test (VMT)(N=700)												
E+DW	**104**	0.49 ±0.22	0.61± 0.26	0.11±0.21	**91**	0.56±0.26	0.66±0.26	0.10±0.24	**195**	**1.66^{NS}**	**1.32^{NS}**	**0.38^{NS}**
E	**86**	0.36 ±0.22	0.48± 0.25	0.11 ±0.18	**83**	0.37±0.21	0.55±0.26	0.18±0.21	**169**	**0.08^{NS}**	**1.87^{NS}**	**2.43***
C+DW	**85**	0.35 ±0.23	0.36±0.19	0.01 ±0.20	**69**	0.49±0.30	0.62±0.30	0.13±0.23	**154**	**3.02****	**6.06*****	**3.04*****
C	**73**	0.41 ±0.28	0.38± 0.20	-0.03±0.3	**109**	0.55±0.29	0.63±0.27	0.08±0.22	**182**	**3.05****	**6.98*****	**2.89****
'F' value		**7.21*****	**21.65*****	**9.28*****		**9.31*****	**2.42^{NS}**	**3.83***				
Clerical test (CT) (N=700)												
E+DW	**104**	0.67±0.24	0.81±0.20	0.14± .23	**91**	0.71±0.23	0.77±0.21	0.06±0.26	**195**	**1.24^{NS}**	**1.24^{NS}**	**2.28***
E	**86**	0.70±0.27	0.80± 0.22	0.10± 0.24	**83**	0.80±0.21	0.86 ±0.15	0.06±0.20	**169**	**2.68****	**2.05***	**1.21^{NS}**
C+DW	**85**	0.74 ±0.26	0.82± 0.19	0.08± 0.24	**69**	0.77±0.21	0.85 ±0.18	0.07±0.25	**154**	**0.86^{NS}**	**0.89^{NS}**	**0.15^{NS}**
C	**73**	0.70 ±0.29	0.80± 0.21	0.11± 0.28	**109**	0.78±0.26	0.83 ±0.21	0.05±0.24	**182**	**1.90***	**0.70^{NS}**	**1.44^{NS}**
'F' value		**1.11^{NS}**	**0.12^{NS}**	**0.99^{NS}**		**2.33^{NS}**	**3.59***	**0.19^{NS}**				

**p<0.05, **p<0.01, ***p<0.001*

DMT: At baseline when scores of DMT were compared between genders within all groups, a non significant difference was observed for all four groups. However, this mean score difference **between groups** for both the genders was differing with higher degree of significance ($p<0.001$). Towards the end of the study, the difference remained significant for boys, but the degree of significant difference decreased amongst the girls towards the end. Hence, the difference remained non significant for girls between all four groups at the completion of the study **(Table 4.89)**.

Mean scores of both the genders **within groups**, towards the end and final difference was also compared. It revealed a significant difference ($p<0.05$) amongst C group and ($p<0.01$) for C+DW group for final scores at comparison between both the genders.

VMT: At baseline, a significant difference observed **within** C and C+DW **group** when compared for both the genders. The difference was non significant among both experimental groups (E and E+DW). The difference between both the genders of control groups remained significant towards the end also. When all the groups were compared with each other (between group comparison), a significance ($p<0.001$) (F value - 21.65) was observed in mean scores for boys at baseline and the end. However, the difference was non significant for mean scores of girls at the end (F value - 2.42), which was significant ($p<0.001$) at baseline (F value-9.31) **(Table 4.89)**.

A study conducted by (Seshardi S and Gopaldas T 1989) also reported the similar pattern with iron supplemented groups using different dosages amongst urban primary school children of both the genders. The findings revealed a significant improvement ($p<0.05$) in mean VMT scores in 30 mg and 40 mg groups compared to placebo group in the anemic boys and girls. However, the results for girls belonged to both supplemented groups also revealed significant difference at ($p<0.05$) compared to their counterparts of placebo groups.

CT: Comparison of both the genders **between groups** revealed non significant difference at baseline and at the end for the boys, it differed significantly with lower degree ($p<0.05$) for the girls. When the initial scores were compared between both the genders within individual groups, the difference was significant for E ($p<0.01$) and C ($p<0.05$), which turned to be non significant towards the end in C group and with lower degree of significance ($p<0.05$) for E group **(Table 4.89)**.

Table 4.90: Cross tabulation between anthropometry parameters, biochemical analytes and IQ/cognition scores

	HAZ	WAZ	BMZ	UIE	Hb	TSH	FT4	TT4	Tg	DMT	CT	VMT
HAZ	1											
WAZ	0.766**	1										
BMZ	0.131**	0.695**	1									
UIE	0.142**	0.132**	0.049	1								
Hb	0.094**	0.072*	0.006	0.088**	1							
TSH	0.113	0.109	0.079	0.053	0.103	1						
FT4	-0.124	-0.079	-0.033	0.007	0.002	-0.068	1					
TT4	0.067	0.118	0.102	-0.091	-0.011	-0.001	0.251**	1				
Tg	0.023	-0.060	-0.110	0.075	0.121	0.003	-0.020	-0.184*	1			
DMT	0.151**	0.139**	0.068*	-0.091**	-0.063	0.022	-0.029	0.121	-0.172*	1		
CT	0.081*	0.037	-0.019	0.040	-0.016	0.022	-0.050	0.004	-0.004	0.221**	1	
VMT	0.022	0.045	0.090*	-0.086**	0.032	0.085	0.066	0.067	-0.012	0.219**	0.478**	1

p<0.05, **p<0.01

A study by (Sheshadri S and Gopaldas T 1989) quoted above also reported a significant improvement ($p<0.05$) in mean CT scores amongst both control and experimental group compared to baseline data. However, they also reported a significant improvement in experimental group ($p<0.05$) compared to the control group in the groups of anemic boys. While data on girl participants who were anemic revealed significant improvement ($p<0.01$) compared to control group. However, in our study, there was no significant difference observed for both the genders specifically.

4.6.4 INTERRELATION BETWEEN PARAMETERS

Table 4.90 reveals a correlation data between different parameters of the children towards the end of the study. On correlating parameters with iodine status, a significant correlation ($p<0.01$) between 'Z' scores *v/s* UIE of the children was observed. This correlation was also significant within Z scores (WAZ *v/s* HAZ). Towards the completion time, Z scores correlated positively ($p<0.01$) and UIE correlated ($p<0.01$) negatively with DMT. However, CT correlated with HAZ ($p<0.05$) and VMT with BMZ ($p<0.01$). The UIE also correlated negatively with VMT. However, there was no significant correlation observed between IQ/Cognition tests and thyroid analytes, except a negative correlating between DMT and Tg ($p<0.05$).

While correlating parameters with iron status, there was a positive correlation between anthropometry indices – HAZ ($p<0.01$) and BMZ ($p<0.05$) - and Hb was observed. There was also a positive correlation observed between UIE and Hb ($p<0.01$).

Towards the end of the study, data on IQ/cognitive scores was collected and cross tabulated with the mean change in hemoglobin concentration of the children. The difference was tried to be extracted from the two populations of the children based on increase or decrease in hemoglobin concentration at the end (**Table 4.91**) for each study group. There was a non significant difference **within each study group** between both the categories **(a and b)** for all the three tests. This means that, DFS could not give remarkable impact on IQ or cognitive scores of the children at the end.

This could be because it might have been too early to measure the effect on IQ or cognition within a short period of time of supplementation (9 months).

Table 4.91: Mean change in IQ and cognition test scores in children cross tabulated with change in hemoglobin concentration at the end

Change in Hb	**Study Groups**								**'F' value**
	N	**E+DW**	**N**	**E**	**N**	**C+DW**	**N**	**C**	
DMT									
0.01->1[a]	146	7.31±16.32	103	1.76±10.19	46	2.18±22.28	49	2.06±13.42	**3.45***
0-<-1[b]	49	8.76±18.70	66	4.04±14.61	108	1.86±16.43	133	5.66±16.21	**2.27**^{NS}
't' test		0.48^{NS}		1.11^{NS}		0.09^{NS}		1.51^{NS}	
VMT									
0.01->1 [a]	146	0.12±0.21	103	0.15±0.18	46	0.06±0.22	49	0.05±0.16	**4.35****
0-<-1 [b]	49	0.07±0.27	66	0.13±0.22	108	0.07±0.23	133	0.03±0.28	**2.64***
't' test		1.06 ^{NS}		0.73 ^{NS}		0.11 ^{NS}		0.626 ^{NS}	
CT									
0.01->1 [a]	146	0.10±0.25	103	0.07±0.21	46	0.07±0.20	49	0.05±0.24	**1.07**^{NS}
0-<-1 [b]	49	0.09±0.25	66	0.11±0.23	108	0.08±0.26	133	0.08±0.26	**0.25**^{NS}
't' test		0.35 ^{NS}		1.15 ^{NS}		0.12 ^{NS}		0.65 ^{NS}	

**p<0.05, **p<0.01, ***p<0.001*

However, a significant difference between all four groups was observed for DMT and CT amongst the children with increased hemoglobin concentration. *Post hoc* analysis (Bonferroni) revealed a significant difference between E *v/s* E+DW ($p<0.05$) for DMT, indicating higher increase in E+DW group compared to E group in mean score. For CT no significant difference was observed between any of the four groups. While comparing the mean difference for VMT **between all four groups**, a significant difference was observed ($p<0.01$) and *post hoc* analysis (Bonferroni) revealed this difference between E group and both control groups- C ($p<0.05$) and C+DW ($p<0.01$).

When the study groups were compared for the second category of the children, who showed reduction in hemoglobin concentration towards the end of the study, no significant difference for DMT and CT was observed. However, a significant difference **between groups** was observed for VMT ($p<0.05$). This difference was noted significant using *post hoc* analysis (Bonferroni) between E *v/s* C ($p<0.05$).

Table 4.92: Mean change in IQ and cognition test scores in children cross tabulated with urinary iodine concentration at the end

Final UIE	Study Groups								'F' value
	N	E+DW	N	E	N	C+DW	N	C	
DMT									
<100	30	8.45±17.95	21	-0.59±11.7	8	10.38±14.82	19	7.58±15.12	**1.81^{NS}**
>100	165	7.53±16.77	148	3.11±12.15	146	1.47±18.40	163	4.35±15.62	**4.07****
't' test		**0.26^{NS}**		**1.35^{NS}**		**1.65^{NS}**		**0.88^{NS}**	
VMT									
<100	30	0.10±0.20	21	0.13±0.19	8	0.11±0.16	19	-0.02±0.42	**0.99^{NS}**
>100	165	0.11±0.23	148	0.15±0.20	146	0.07±0.23	163	0.04±0.23	**7.57*****
't' test		**0.10^{NS}**		**0.29^{NS}**		**0.83^{NS}**		**0.40^{NS}**	
CT									
<100	30	0.11±.0.25	21	0.12±0.25	8	0.02±0.10	19	0.08±0.22	**0.43^{NS}**
>100	165	0.10±0.25	148	0.08±0.22	146	0.08±0.25	163	0.07±0.26	**0.60^{NS}**
't' test		**0.02^{NS}**		**0.76^{NS}**		**1.58^{NS}**		**0.22^{NS}**	

**p<0.05, **p<0.01, ***p<0.001*

Towards the end of the study, data on IQ/cognitive scores was collected and cross tabulated with urinary iodine excretion of the children. The comparison between change in IQ and cognition scores between two classes of the children based on urinary iodine sufficiency (≥100 µg/L) and insufficiency (<100 µg/L) at the end was carried out (**Table 4.92**) for each study group. There was a non significant difference **within each study group** between both the categories **(a and b)** for all the three tests. This means that, DFS could not give remarkable impact on IQ or cognitive scores of the children at the end. This could be because it is too early to measure the effect on IQ or cognition within a short period of time of supplementation (9 months) and availability of adequately iodized salt among control groups also. Thus, the better performance based on iodine intake could not be compared.

However, a significant difference between all four groups was observed for DMT and VMT amongst the children with sufficient levels of urinary iodine excretion ($p<0.01$). *Post hoc* analysis (Bonferroni) revealed a significant difference between E+DW *v/s* C+DW ($p<0.01$) group for DMT, indicating higher increase in E+DW group compared to C+DW grouping mean score. For CT no significant differences were

observed between any of the four groups. While comparing the mean difference for VMT **between all four groups**, higher level of significant difference was observed ($p<0.001$).*Post hoc* analysis (Bonferroni) revealed this difference between C groups *v/s* both experimental groups -E ($p<0.05$) and E+DW ($p<0.001$). However, E group also showed a significant difference compared to C+DW ($p<0.01$) group.

When the study groups were compared for the second category of the children, who were at insufficient levels of urinary iodine concentration towards the end of the study, no significant difference for all three tests was observed.

4.6.5 IMPACT OF SUPPLEMENTATION, NHE AND BCC ON DIETARY INTAKE

The dietary intake of the various nutrients amongst school children was derived using two standard methods: (1) 24 hour dietary recall and (2) Food frequency. Both the methods depicted the scenario of nutritional intake of the study children belonging to all the groups, before and after intervention.

4.6.5.1 Dietary recall -24 hours

Nutritional intake of the school children was calculated for major nutrients based on their group, age and gender wise variations. **Table 4.93 and 4.94** reveals the complete scenario of the situation, where the pattern observed that, younger children were comparatively on a higher percentage of meeting the RDA compared to the elder children in all the groups.

Table 4.93: Mean dietary intake of the children belonged to experimental group- before and after intervention

Age group/ Nutrients	Stage	E+DW group				E Group			
		Boys		Girls		Boys		Girls	
		Mean± SD	% RDA	Mean± SD	% RDA	Mean± SD	% RDA	Mean± SD	% RDA
≤9 yrs		**(N=22)**		**(N=21)**		**(N=14)**		**(N=11)**	
Energy (Kcal)	**Pre**	1150.27±182.90	**75.65**	1023.85±230.09	**67.30**	945.86±158.65	**62.17**	964.55±146.30	**63.42**
	Post	1087.09±236.40	**71.52**	1098.14±325.81	**72.20**	1226.00±104.87	**80.65**	1253.27±114.84	**82.43**
	't'	**0.707^{NS}**		**1.22^{NS}**		**9.37*** **		**7.00*** **	
Protein (gm)	**Pre**	17.05±4.14	**68.75**	17.31±3.74	**69.79**	18.32±3.58	**73.87**	18.41±2.53	**75.14**
	Post	20.74±4.53	**83.62**	19.69±3.90	**79.40**	21.20±3.18	**86.53**	23.32±3.55	**95.18**
	't'	**3.18****		**2.39****		**2.04^{NS}**		**3.42****	
Fat (gm)	**Pre**	28.22±5.76	**102.62**	28.62±8.54	**104.07**	25.03±4.18	**91.01**	25.41±7.01	**92.4**
	Post	31.20±8.55	**113.45**	32.69±11.16	**118.87**	36.50±7.67	**132.72**	35.04±8.32	**127.42**
	't'	**1.52^{NS}**		**2.02***		**6.08*** **		**3.50*** **	
Fe (mg)	**Pre**	6.80±2.76	**46.90**	6.78±3.03	**46.76**	6.09±3.40	**42**	5.75±1.66	**39.65**
	Post	7.53±2.09 (Diet) + 10.00±1.92(DFS) =17.53±3.56	**120.89**	7.34±2.15(Diet) + 10.47±1.88(DFS) =17.80±3.41	**122.75**	8.90±3.88(Diet) + 10.50±1.28(DFS) =19.30±4.68	**133.10**	9.86±4.01(Diet) + 11.09±1.30(DFS) =20.95±4.62	**137.58**
	't'	**24.33*** **		**25.43*** **		**30.55*** **		**28.28*** **	
10-12 yrs		**(N=22)**		**(N=11)**		**(N=5)**		**(N=5)**	
Energy (Kcal)	**Pre**	1011.13±144.37	**46.16**	1029.18±190.36	**51.21**	950.80±204.53	**43.42**	1042.00±108.14	**51.80**
	Post	1099.14±162.76	**50.18**	1080.27±195.80	**53.74**	1337.00±76.99	**61.05**	1371.20±89.36	**68.22**
	't'	**2.05***		**0.95^{NS}**		**4.33****		**4.97****	
Protein	**Pre**	16.19±3.47	**40.58**	17.45±4.22	**43.19**	18.20±2.41	**45.61**	19.82±2.65	**49.06**

(gm)	**Post**	21.50±3.86	**53.88**	20.54±3.82	**50.84**	27.05±3.88	**67.79**	26.42±3.23	**65.39**
	't'	**6.58*** **		**3.28** **		**4.96** **		**2.60^{NS}**	
Fat (gm)	**Pre**	27.80±5.28	**79.43**	28.62±8.54	**81.77**	25.00±4.47	**71.43**	26.90±4.61	**76.86**
	Post	27.58±5.85	**78.8**	29.59±6.80	**84.54**	37.60±5.35	**107.42**	35.90±2.92	**102.57**
	't'	**0.16^{NS}**		**0.88^{NS}**		**3.38***		**7.60** **	
Fe (mg)	**Pre**	6.90±3.13	**32.85**	6.88±2.59	**25.48**	6.28±3.25	**29.90**	6.38±1.59	**23.62**
	Post	8.71±3.15(Diet) + 10.36±1.59(DFS) =19.07±3.98	**90.80**	7.58±1.94(Diet) + 9.81±1.40(DFS) =17.39±2.76	**64.40**	8.04±1.29(Diet) + 11.60±0.89(DFS) =19.64±0.99	**88.76**	8.77±1.33(Diet) + 11.80±1.64(DFS) =20.57±0.47	**72.48**
	't'	**30.57*** **		**23.23*** **		**29.00*** **		**16.05*** **	
≥12 yrs		**(N=3)**		**(N=2)**		**(N=0)**		**(N=0)**	
Energy (Kcal)	**Pre**	983.67±111.33	**35.76**	1061.00±125.87	**45.53**	-		-	
	Post	996.33±78.91	**36.23**	1379.50±170.41	**59.21**	-		-	
	't'	**0.63^{NS}**		**10.11^{NS}**		-		-	
Protein (gm)	**Pre**	16.02±2.10	**29.39**	15.80±2.40	**30.44**	-		-	
	Post	21.60±3.29	**39.63**	25.20±1.70	**48.55**	-		-	
	't'	**8.05** **		**18.80***		-		-	
Fat (gm)	**Pre**	25.67±5.48	**57.04**	25.00±0.00	**62.5**	-		-	
	Post	23.67±2.89	**52.6**	38.00±1.41	**95**	-		-	
	't'	**0.84^{NS}**		**13.00***		-		-	
Fe (mg)	**Pre**	5.47±1.33	**17.09**	9.50±3.54	**35.18**	-		-	
	Post	8.15±4.92(Diet) + 9.00(DFS) =17.15±4.92	**53.59**	14.35±2.05(Diet) + 12.00(DFS) =26.35±2.05	**97.59**	-		-	
	't'	**17.26*** **		**22.87*** **		-		-	

Table 4.94: Mean dietary intake of the children belonged to control group- before and after intervention

Age group/ Nutrients	Stage	C+DW group				C Group			
		Boys		Girls		Boys		Girls	
		Mean± SD	% RDA	Mean± SD	% RDA	Mean± SD	% RDA	Mean± SD	% RDA
≤ 9 yrs		(N=17)		(N=15)		(N=13)		(N=15)	
Energy (Kcal)	Pre	1109.47±221.16	72.99	981.93±146.60	64.60	1087.00±175.55	71.51	937.86±149.90	61.70
	Post	1078.82±205.46	70.97	1072.67±145.24	70.57	1195.08±176.92	78.62	1096.40±165.04	72.13
	't'	0.50^{NS}		1.70^{NS}		2.60*		3.10**	
Protein (gm)	Pre	20.32±4.41	81.94	20.28±5.26	81.77	19.77±3.16	80.69	18.22±4.00	74.37
	Post	23.04±6.76	92.90	19.89±4.86	81.18	23.22±6.65	94.77	21.55±3.46	87.96
	't'	1.48^{NS}		0.23^{NS}		2.00^{NS}		2.72**	
Fat (gm)	Pre	30.56±9.40	111.12	27.19±8.06	98.87	31.00±6.99	112.72	26.97±6.13	98.07
	Post	26.26±5.53	95.49	27.03±7.36	98.29	28.92±6.07	105.16	28.17±8.07	102.4
	't'	1.60^{NS}		0.05^{NS}		1.01^{NS}		0.55^{NS}	
Fe (mg)	Pre	5.03±2.37	34.68	4.42±1.59	30.48	5.38±1.83	37.10	4.05±1.44	27.93
	Post	5.48±1.77	37.79	5.98±1.74	41.24	5.24±1.81	36.14	5.98±1.42	41.24
	't'	0.75^{NS}		2.47*		0.26^{NS}		5.44***	
10-12 yrs		(N=12)		(N=5)		(N=6)		(N=11)	
Energy (Kcal)	Pre	1050.50±172.36	47.96	959.00±317.69	47.71	1261.83±246.79	57.61	953.91±147.02	47.45
	Post	1196.91±208.45	54.65	1138.00±162.06	56.61	1237.17±107.97	56.49	1141.00±150.42	56.76
	't'	2.57*		1.30^{NS}		0.35^{NS}		2.37*	

Protein (gm)	**Pre**	20.26±4.33	**50.77**	16.20±4.62	**40.09**	20.57±5.08	**51.55**	18.46±3.81	**45.69**
	Post	22.85±4.94	**57.27**	21.80±3.98	**53.96**	22.91±2.13	**57.42**	19.94±4.05	**49.36**
	't'	**2.18***		**2.39^{NS}**		**1.65^{NS}**		**0.84^{NS}**	
Fat (gm)	**Pre**	29.38±7.83	**83.94**	26.30±9.24	**75.14**	33.42±9.13	**95.48**	24.23±4.69	**69.22**
	Post	31.33±5.69	**89.57**	31.80±5.48	**90.85**	34.00±5.59	**97.14**	28.22±3.07	**80.62**
	't'	**0.74^{NS}**		**1.09^{NS}**		**0.11^{NS}**		**1.90^{NS}**	
Fe (mg)	**Pre**	4.38±1.25	**20.85**	4.66±2.15	**17.25**	6.36±1.90	**30.28**	4.39±1.48	**16.25**
	Post	6.89±2.66	**32.80**	6.64±1.57	**24.59**	7.07±1.98	**33.66**	6.32±2.11	**23.40**
	't'	**2.46***		**2.72***		**0.85^{NS}**		**2.01^{NS}**	
≥12 yrs		**(N=0)**		**(N=1)**		**(N=0)**		**(N=1)**	
Energy (Kcal)	**Pre**	-		1320.27	**48.00**	-		1057.00	**45.36**
	Post	-		1526.00	**55.49**	-		1304.00	**55.96**
	't'	-		**2.1****		-		**3.5****	
Protein (gm)	**Pre**	-		27.70	**53.37**	-		18.60	**35.83**
	Post	-		24.30	**46.82**	-		20.40	**39.30**
	't'	-		**3.1***		-		**4.9****	
Fat (gm)	**Pre**	-		47	**104.44**	-		24.50	**61.25**
	Post	-		47	**104.44**	-		37.00	**92.5**
	't'	-		**0.78^{NS}**		-		**11.87*****	
Fe (mg)	**Pre**	-		7	**25.92**	-		4.80	**17.77**
	Post	-		6.90	**25.55**	-		6.15	**22.77**
	't'	**-**		**0.45^{NS}**		**-**		**9.67*****	

**p<0.05, **p<0.01, ***p<0.001*

The results of the nutrient intake have been discussed according to their age groups, since all the age groups have their own RDA and the variation are also based on their genders.

≤ *9 years*: In this age group, there is no gender bias for the standard RDA. It was observed that, the majority of the selected children in all study groups belonged to this age group. While comparing data on baseline and end values of mean energy intake, a significant improvement in E and C groups was observed ($p<0.01$). However, E+DW and C+DW groups did not show significant improvement. Protein intake improved significantly ($p<0.01$) in both experimental groups (E+DW and E), except boys in E group did not show significant results. However, control groups (C+DW and C) did not show significant improvement in mean protein intake, except girls with improvement at ($p<0.01$) significance. Mean fat intake in E group was observed ($p<0.01$), whereas it was significant at ($p<0.05$) in boys who belonged to E+DW group and non significant in girls who belonged to the same group (E+DW). However, there was no significant improvement in control groups (C+DW and C) observed for any of the gender.

Mean iron intake improved at a higher level of significance ($p<0.001$) in both experimental groups (E+DW and E) among both the genders due to supplemented DFS along with the diet. However, it was observed to be significant amongst girls belonging to control groups (C+DW and C) at significance ($p<0.05$); it remained non significant for boys.

***10-12 years*:** In this age group, there are different standard RDA for both the genders. Dietary intakes were calculated based on the recommended allowances for each gender. It was observed that, after previous age group major chunk of the rest of the children were representatives of this age category from all the study groups. While comparing data on baseline and end values of mean energy intake, a significant improvement in experimental groups (E+DW and E) was observed ($p<0.01$).However, control groups did not show significant improvement, except boys from C+DW group and girls from C group ($p<0.05$). Protein intake improved significantly ($p<0.01$) in both experimental groups (E+DW and E), except girls in E group did not show significant results. However, control groups (C+DW and C) did not show significant improvement in mean protein intake, except boys with

improvement at ($p<0.05$) significance. Mean fat intake in E group improved significantly ($p<0.01$), whereas rest of the groups did not show significant improvement.

Mean iron intake improved with higher level of significance ($p<0.001$) in both experimental groups (E+DW and E) among both the genders due to supplemented DFS along with the diet. However, it was observed to be significant amongst children who belonged to C+DW group with significance ($p<0.05$) and it remained non significant for C group

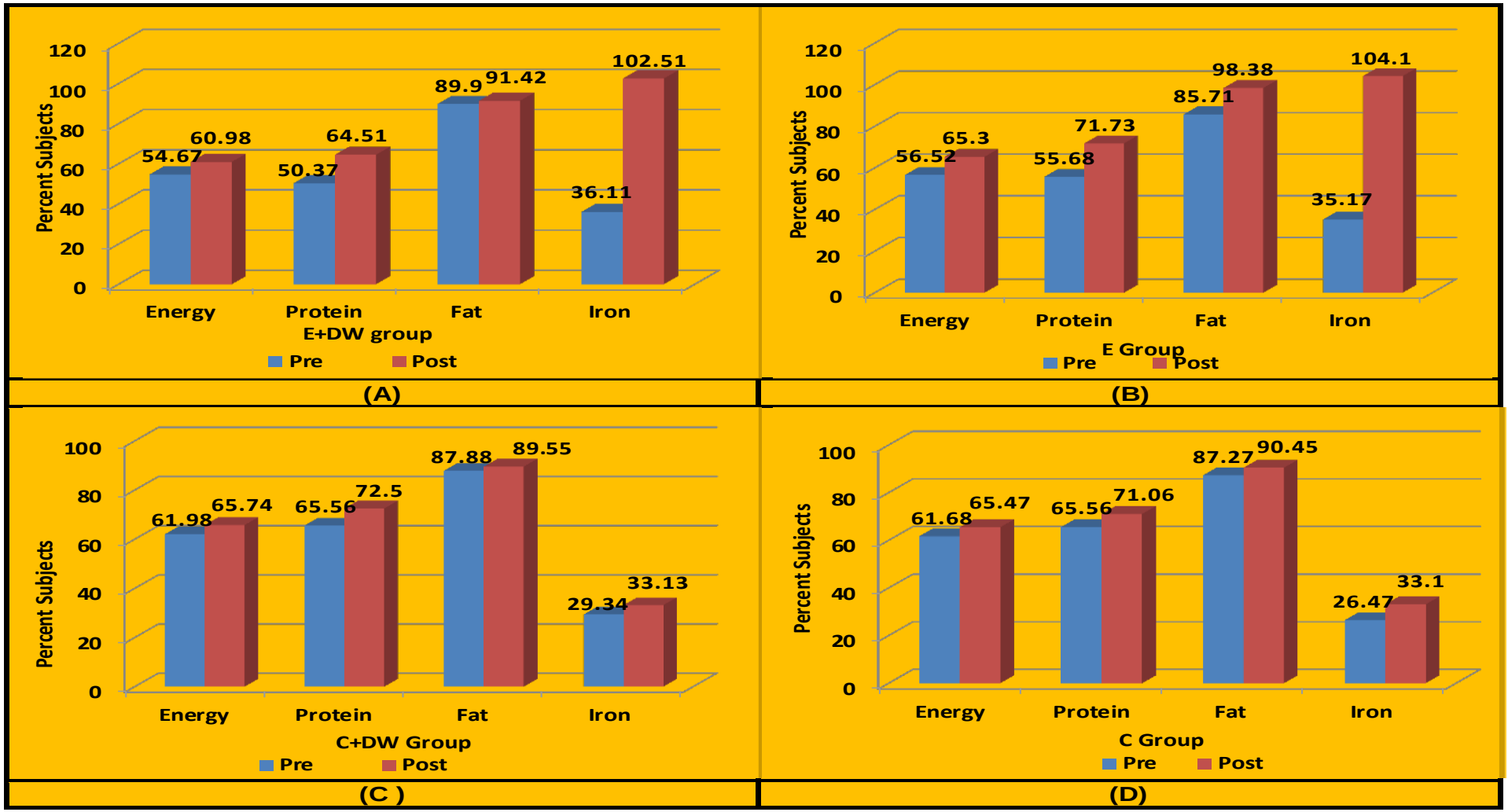
***≥ 12 years*:** Dietary intake of a very few children from this age group were collected, though it was unbiased. Hence, it would have been statistically incorrect to compare the groups based on this class. However, it can be stated that, the pattern of improved nutrition also was observed in this age group at more or less level of significance.

Due to varied RDA classifications, gender wise and group wise classification did not give a specific trend on the impact of intake of the children. Hence, further impact analysis of NHE on median RDA and dietary intake was obtained with reference to before and after intervention stage **(Figure 4.16 A,B,C,D)**. The cumulative data on distribution based on meeting the percent RDA was generated to have a pattern of difference within all the groups.

The figure on percent RDA revealed a pattern of improved dietary intake amongst all the groups.

***E+DW group*: Figure 4.16 (A)** depicts the change in median percent RDA of the nutrients in E+DW group. It was observed that, median intake of energy, protein and fat were 54.67%, 50.37% and 89.9% respectively, which improved to 60.98%, 64.51% and 91.42%. The data revealed a positive impact of NHE amongst mothers or the caretakers of the children. Median iron intake of the children was 36.11% at baseline, which improved to 102.51% with significance level at ($p<0.001$). The improvement in iron intake has shown a success of a minute but essential amount of iron in DFS.

Figure4.16: Median percentage of RDA for major nutrients of all the groups



***E group*: Figure 4.16 (B)** depicts the change in median percent RDA of the nutrients in E group. It was observed that, median intake of energy, protein and fat were 56.52%, 55.68% and 85.71% respectively, which improved to 65.30%, 71.73% and 98.38%. This represented a positive impact of NHE amongst mothers or the caretakers of the children. Median iron intake of the children was 35.17% at baseline, which improved to 104.1% with significance level at ($p<0.001$). The improvement was due to median iron intake through diet and DFS in the diet.

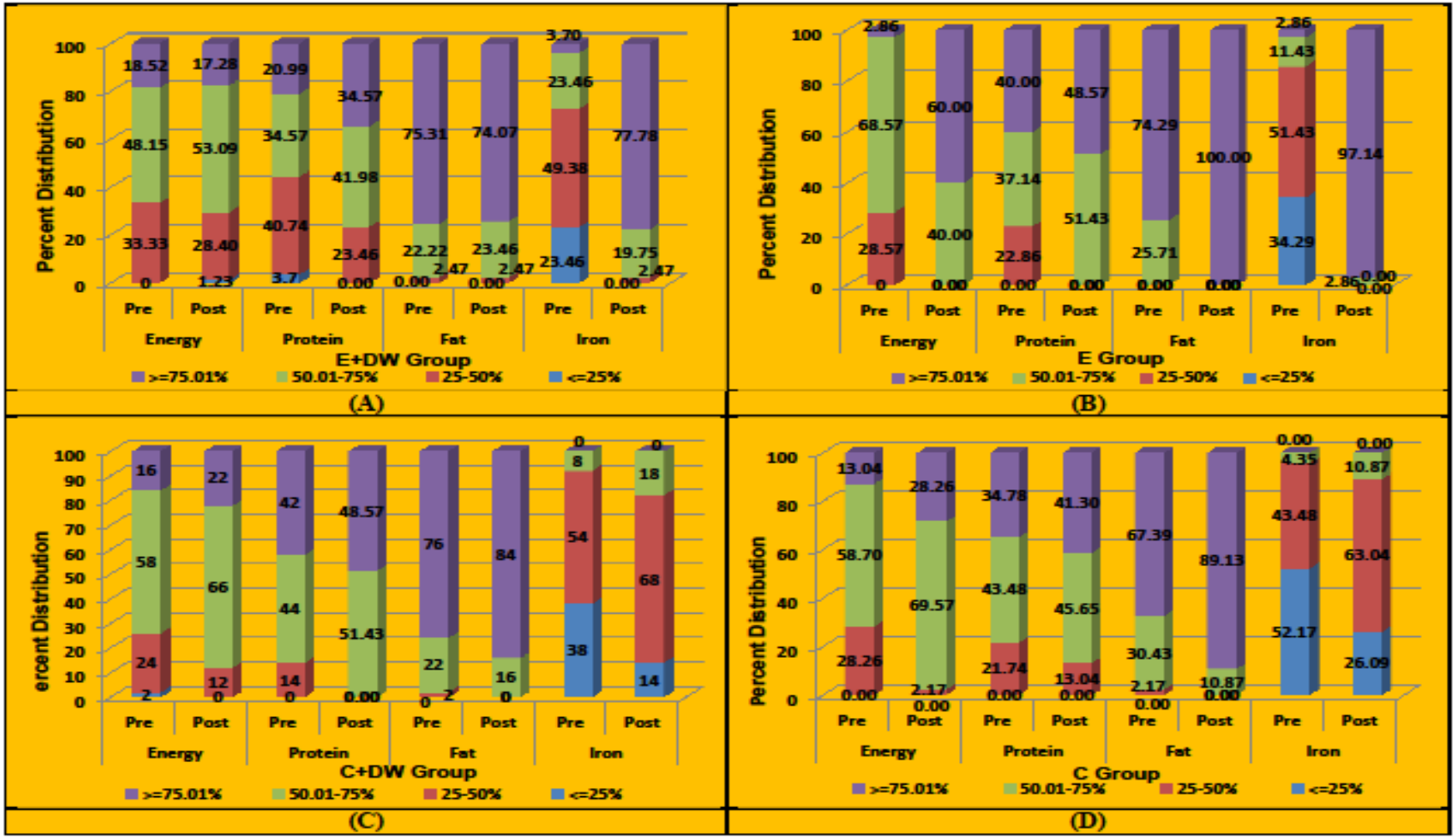
***C+DW group*: Figure 4.16 (C)** depicts the change in median percent RDA of the nutrients in C+DW group. It was observed that, median intake of energy, protein and fat were 61.8%, 65.56% and 87.88% respectively, which improved to 65.74%, 72.5% and 89.55%. This represented a positive impact of NHE amongst mothers or the caretakers of the children. Median iron intake of the children was 29.34% at baseline, which improved non significantly to 33.13%.

***C group*: Figure 4.16 (D)** depicts the change in median percent RDA of the nutrients in C group. It was observed that, median intake of energy, protein and fat were 61.8%, 65.56% and 87.27% respectively, which improved to 65.47%, 71.06% and 90.45%. This in turn presented a positive impact of NHE amongst mothers or the caretakers of the children. Median iron intake of the children was 26.47% at baseline, which improved significantly ($p<0.05$) to 33.1%.

Further, data on improvement in meeting the percent RDA classification is revealed in **Figure 4.17**. The data of all four groups showed a positive shift with improvement in achieving RDA of each nutrient, $\geq$ 50-75% or $\geq$ 76%.Results on the distribution for each nutrient in all the study groups have been mentioned below:

***E+DW group:* Figure 4.17 (A)** reveals that, initially there were 66.67%, 55.56%, 97.53% and 27.16% of the children were meeting their RDA >50% for energy, protein fat and iron respectively. Later after intervention provided, these percentages improved to 70.37%, 76.54%, 97.53% and 97.53% respectively. The improvement in dietary intake of iron through diet and DFS, improved at higher level of significance ($p<0.001$). There were 77.78% children, who were on $\geq$ 75% RDA for iron, reflected in the post data. Percent consumption of other nutrients also improved remarkably.

Figure 4.17 :Change in percent distribution of RDA classification



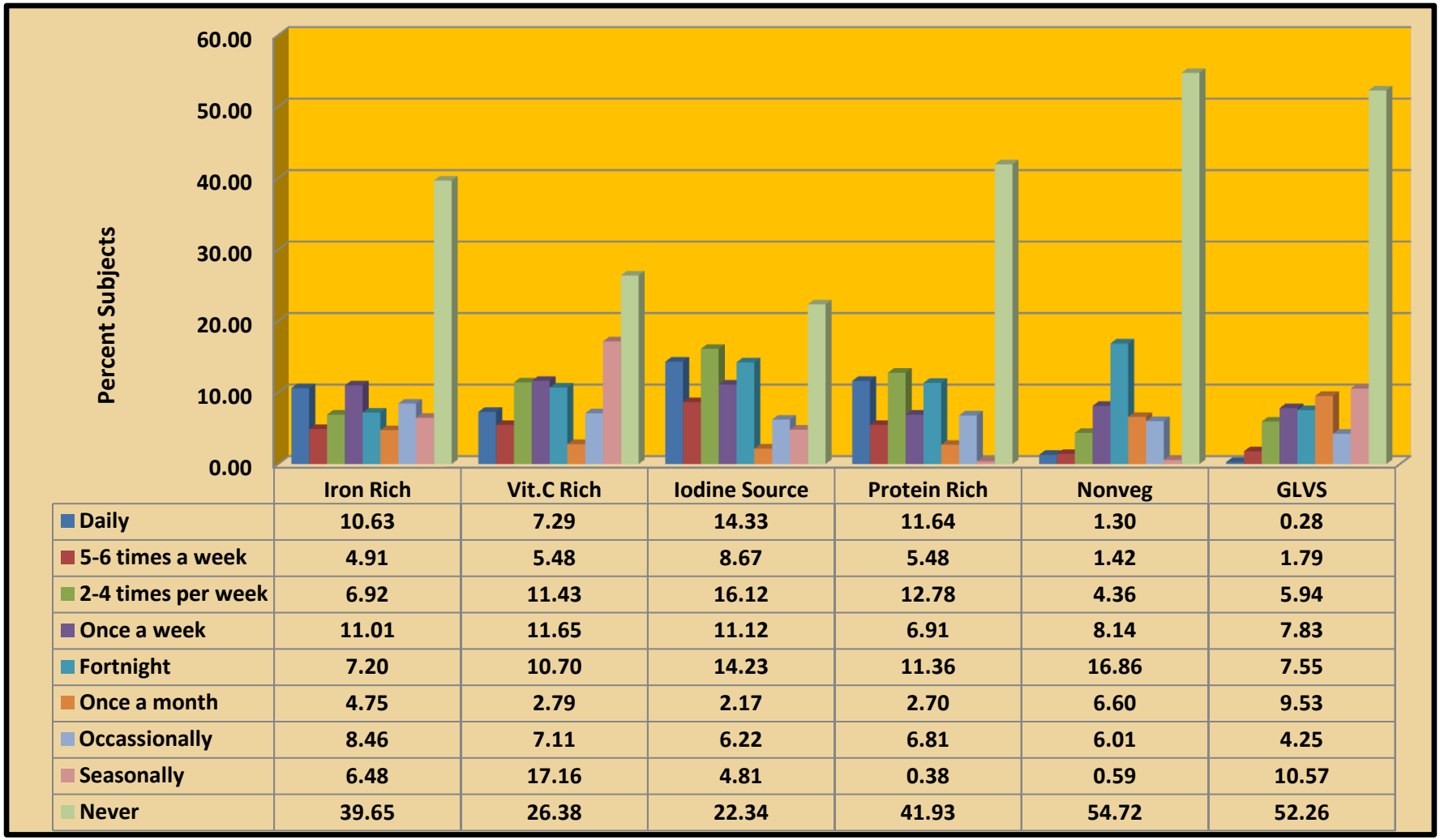
However, there were still 28% and 23% children falling into <50% RDA intake category, who were still to increase 50% intake to meet their energy and protein RDA.

E group: **Figure 4.17 (B)** reveals that, initially there were 71.43%, 77.14%, 100% and 14.29% of the children met their RDA >50% for energy, protein, fat and iron respectively. Later after intervention provided, these percentages improved to 100% for each nutrient respectively. The improvement in dietary intake of iron through diet and DFS, improved at higher level of significance ($p<0.001$). There were 97.14% children, who were on $\geq$ 75% RDA for iron, reflected in the data. Percent consumption of other nutrients also improved remarkably. However, there were still 40% and 50% children were meeting <75% of RDA for energy and protein respectively, but the scenario was better than the rest of the groups.

C+DW group**: Figure 4.17 (C)** reveals that, initially there were 74%, 86%, 99% and 8% of the children who were meeting their RDA >50% for energy, protein fat and iron respectively. Later after intervention provided, these percentages improved to 88%, 100%, 100% and 18% for each nutrient respectively. The improvement in dietary intake of iron through diet did not suffice their need, which is reflected in the data with 10% improvement compared to baseline. None of the children were on $\geq$ 75% RDA for iron, reflected in the data. It showed a significant difference compared to experimental groups. Percent consumption of other nutrients improved remarkably likewise experimental groups, whereas iron intake was the major concern. However, there were 12% of the children lacking 50% intake to meet their RDA for energy. This reflected a positive impact of NHE on the dietary intake of the children.

C group: **Figure 4.17 (D)** reveals that, initially there were 71.74%, 78.26%, 97.83% and 4.35% of the children who were meeting their RDA >50% for energy, protein fat and iron respectively. Later after intervention provided, these percentages improved to 97.83%, 86.96%, 100% and 10.87% for each nutrient respectively. The improvement in dietary intake of iron through diet did not suffice their need, which is reflected in the data with 6% improvement compared to baseline. None of the children were on $\geq$75% RDA for iron, reflected in the data. This in turn showed a significant difference compared to experimental groups. Percent consumption of other nutrients improved remarkably likewise experimental groups, whereas iron intake was the major concern.

Figure 4.18: Food frequency pattern of the population



	Iron Rich	Vit.C Rich	Iodine Source	Protein Rich	Nonveg	GLVS
Daily	10.63	7.29	14.33	11.64	1.30	0.28
5-6 times a week	4.91	5.48	8.67	5.48	1.42	1.79
2-4 times per week	6.92	11.43	16.12	12.78	4.36	5.94
Once a week	11.01	11.65	11.12	6.91	8.14	7.83
Fortnight	7.20	10.70	14.23	11.36	16.86	7.55
Once a month	4.75	2.79	2.17	2.70	6.60	9.53
Occassionally	8.46	7.11	6.22	6.81	6.01	4.25
Seasonally	6.48	17.16	4.81	0.38	0.59	10.57
Never	39.65	26.38	22.34	41.93	54.72	52.26

However, there were negligible percent of the children lacking 50% RDA intake for energy and protein. This reflected a positive impact of NHE provided on the dietary intake of the children was beneficial.

4.6.5.2 (A) Baseline Food Frequency

Food frequency pattern of the children is been revealed in **(Figure 4.18).** It demonstrates an overall scenario of the consumption of various food groups by the rural population, based on their major nutrient content.

Iron rich foods were consumed by 10.63% of the subjects on daily basis, and 40% of the children never consumed iron rich food items. For rest of the frequencies, the distribution varied from 5-11%. This group included Wheat, Baja, Rice flakes-puff, Semolina, Lentil, Bengal gram (roasted), Jaggery, Dates and Green leafy vegetables etc.

Wheat, Bajra, Jaggery, Rice flakes and Rice puff were consumed on daily basis by 72.17%, 11.79%, 8.96%, 8.02% and 16.04% respectively. However, consumption of GLVS was notably lower amongst the children due to seasonal availability, cost and universal unacceptability as a single vegetable by majority of the children. However, 36.79% of the children were consuming Jaggery on daily basis.

Vitamin C rich foods were consumed by 7.29% children on daily basis, 17.16% of the families were consuming them seasonally and 26.38% never consumed these foods. This group included Cabbage, Amla, Capsicum, Radish leaves, Citrus fruits, Lemon, Tomato, Onion stalks, Cauliflower, Guava etc. Tomato and Lemon are most frequently consumed by 23.11% and 18.87% of the subjects respectively on daily basis. Based on their affordable economy, cabbage is most frequently consumed food item of this group, with 40% - 2-4 times a week and 30% - once a week consumption. Cauliflower was also consumed by 28.30% of the subjects 2-4 times a week whereas, 81% never consumed capsicum. Rest of the items were consumed seasonally or based on the availability at an economical cost.

Iodine rich foods were consumed by 14.02% on daily basis, 16.12% consumed them 2-4 times a week, 11.12% once weekly, 3.52% subjects consumed these foods seasonally and one fourth of the subjects (23.7%) never consumed these foods. Other than iodized salt, foods like Milk, Milk products, Egg, Fish and Carrot were included

in this group. Milk was consumed by 48.11% of the subjects on daily basis; it was generally consumed as tea. However, most frequently consumed milk products were curd and buttermilk. These products were consumed by 8.62% of the subjects 5-6 times a week. However, 54.5% of the subjects consumed them frequently within week. Egg was consumed by 4.72% of the subjects on daily basis, Fish was consumed by 43.86% of the subjects at different frequencies and carrot is generally consumed based on the availability. At baseline 18.87% of the families were consuming carrot more frequently.

Protein rich foods were consumed daily and fortnightly by similar percent (12%) of the subjects. However, majority of the subjects never consumed (41.93%) these foods. The highest frequency observed was 2-4 times (12.78%) for the families. More than half (54.79%) of the subjects were vegetarian, since non-vegetarian foods are not being consumed by the subjects. This group included Milk, Milk products, Pulses and legumes. Most frequently consumed pulse was redgram dal, which was consumed by half (60.38%) of the subjects on daily basis. However, 12.26% and 20.28% of the subjects respectively consumed Bengal gram dal and green gram dal 2-4 times a week.

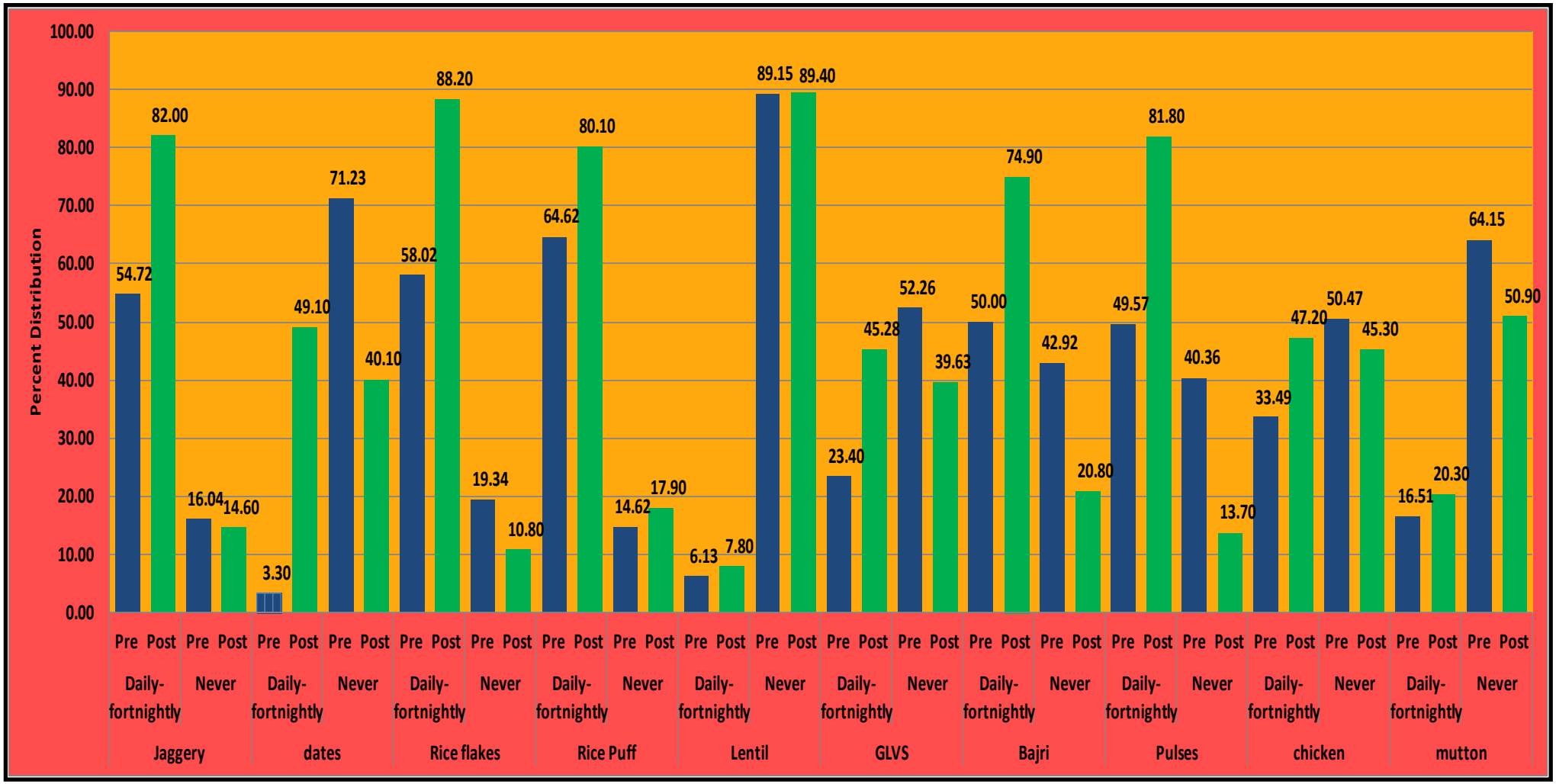
Non vegetarian foods were considered as a different group, since half of the subjects were vegetarians. This group included Egg, Chicken, Mutton and Fish. Of the total non-vegetarian subjects, the highest frequency (average 20%) of consumption was observed to be fortnightly by the families.

Green leafy vegetables (GLVS) were considered as iron rich and vitamin C rich foods. It was observed that, 10.57% of the subjects were consuming them based on the seasonal availability of GLVS. However, 52.26% of the subjects never consumed GLVS.

4.6.5.2 (B) Impact on Frequency distribution of recommended foods

Based on the available information on food frequency, some of the food items were selected considering their cost, availability throughout the year and acceptability by the subjects. These items included Orange, GLVS, Bajra, Lentil, Pulses (sprouted/roasted), Rice flakes-puff, Jiggery, Dates, Non veg., Milk and milk products, Carrot, Tomato, Lemon etc. were recommended to improve upon their iron status.

Table 4.19: Impact on frequency consumption of Iron Rich Foods



Post interventional impact was assessed with these foods only, since majority of the food frequencies were not expected to change within a span of 9 months. Data on pre and post intervention consumption frequencies of the above mentioned food items are depicted in **figures (4.19 to 4.22)** below based on their food groups.

The frequencies were divided into two categories, where cumulative frequency of Daily to Fortnightly was covered under one category and the second was for never consumed food item. The percentages have been distributed in these two categories and presented.

(1) Iron rich foods

Figure 4.19 reveals a difference in the intake of **iron rich foods** of the children after counseling the mother/caretakers. It was observed that, majority of the food items have been consumed more frequently by the children compared to the baseline data. Initially 3.30% of the children were consuming dates more frequently, whereas post intervention one half of the children consumed dates. Similarly for Jaggery, Pulses and Rice flakes there is approximately 30% increase in the consumption. However, there is a 25% and 23% increase in the consumption of Bajra and GLVS respectively. This suggests a positive shift in the consumption pattern. The increase in the consumption reciprocated with the other category.

(2) Vitamin C rich foods

Figure 4.20: Impact on frequency consumption of Vitamin C rich foods

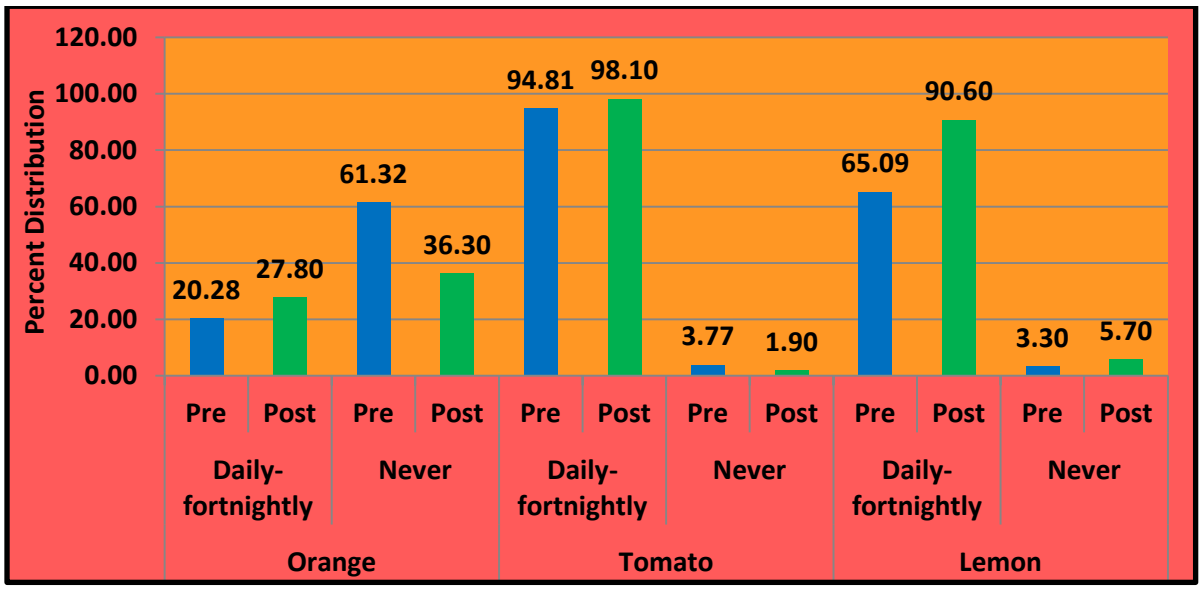


Figure 4.20 reveals a consumption pattern of Vitamin C rich foods before and after NHE. Consumption frequency of lemon has been increased by 25% (65.09 to 90.60%). However, the increase in consumption of Tomato and Oranges could be improved by 4% and 7% respectively.

(3) Iodine Rich foods

Figure 4.21: Impact on frequency consumption of Iodine rich foods

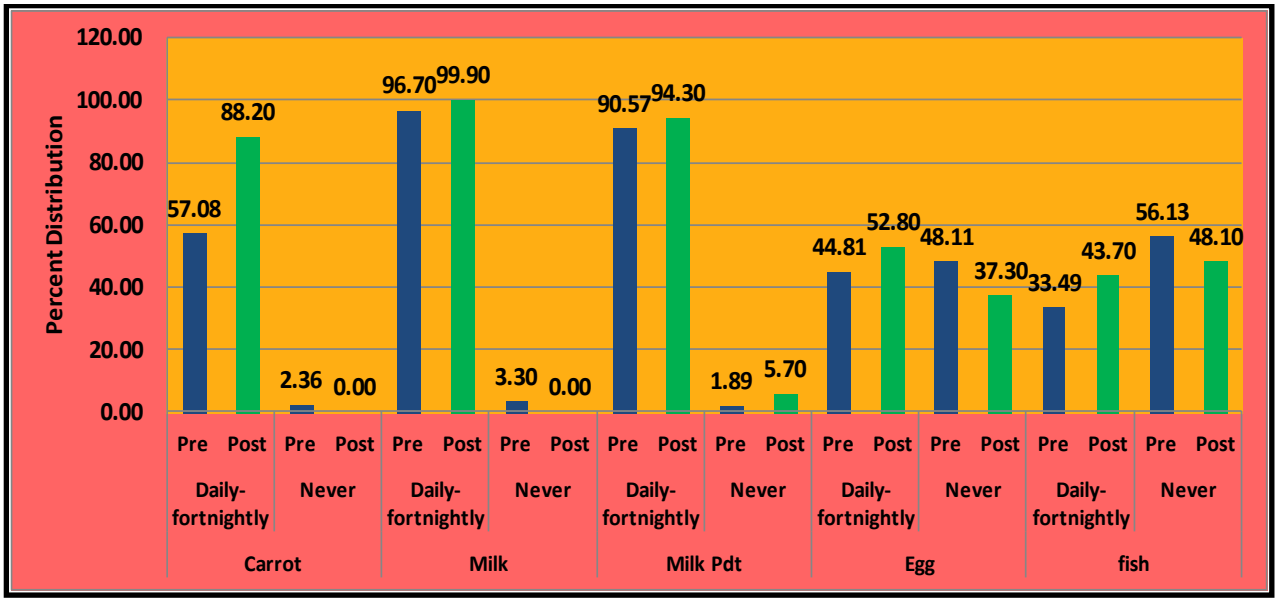
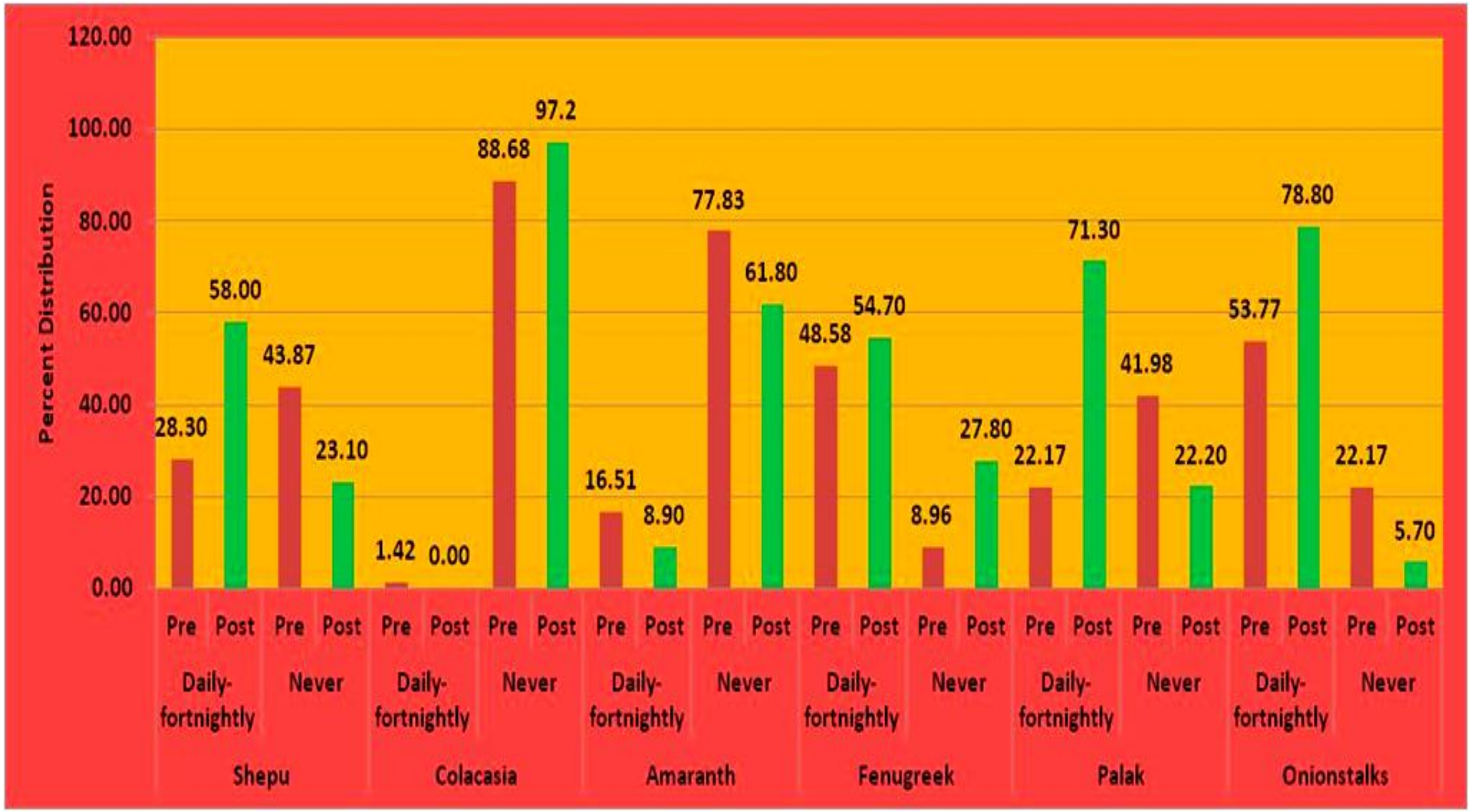


Figure 4.21 shows a consumption pattern of iodine rich foods amongst study population. There is a highest increase in the consumption frequency of Carrot (by 31%) observed, since post data collection was carried out during the seasonal availability of Carrot. However, 99.9% of the children were provided milk by their parents in one or the other form (Tea/milk/kadha-black tea) etc. Egg (8%) and Fish (10%) consumption also increased notably.

(4) Green Leafy Vegetables

Considering GLVS as iron rich and mineral rich foods in a vegetarian diet, frequency of available varieties was observed **(Figure 4.22)**. Majority of the leafy vegetables are available almost throughout the year and some are seasonally available. On observing the consumption frequency of the GLVS, Sheep (30%) and Spinach (50%) were more preferred by the families as observed from the increased percentages. However, consumption of Onion stalks also improved by 25% towards the end.

Table 4.22: Impact on frequency consumption of Green leafy vegetables



4.6.6 IMPACT ON KNOWLEDGE, ATTITUDE AND PRACTICES OF THE POPULATION

Information on knowledge, attitude and practices (KAP) was collected towards the end of the study, to assess the impact of nutrition, health education (NHE) and behavior change communication (BCC).

Table 4.95: Comparison of knowledge, attitude and practices regarding iodine nutrition before and after imparting Nutrition Health Education

Sr. No		Pre	Post	Chi square
1.	**Aware of iodine[(K)]**			
	Yes	30 (14.2)	177 (83.5)	**102.14*** **
	No	182 (85.8)	35 (16.5)	
2.	**Source of iodine[(K)]**			
	Yes	30 (14.2)	172 (81.1)	**92.20*** **
	No	182 (85.8)	40 (18.9)	
3.	**Source items[(K)]**			
	Iodized salt	24 (9.4)	171 (80.7)	**205.18*** **
	Milk and milk products	0 (0)	25 (11.8)	**334.55*** **
	Green leafy vegetables	0 (0)	0 (0)	**NS**
	Carrot	0 (0)	5 (2.4)	**5.06***
	Egg	0 (0)	0 (0)	**NS**
	Seafood	0 (0)	8 (3.8)	**8.25****
4.	**Source of information[(K)]**			
	Newspaper	0 (0)	0 (0)	**NS**
	Media	13 (6.13)	33 (15.6)	**9.75****
	Anganwadi worker	11 (5.1)	11 (5.1)	**NS**
	Researcher	0 (0)	166 (78.3)	**272.81*** **
5.	**Current Salt[(A) (P)]**			
	Refined	131 (61.8)	193 (91.0)	**50.30*** **
	Crushed	79 (37.3)	19 (9.0)	**133.38*** **
	Loose	2 (0.9)	0 (0)	**NS**
6.	**Cost of salt[(A)]**			
	1-3 Rs/kg	19 (9.0)	0 (0)	**19.89*** **
	>3-6 Rs/kg	133 (62.7)	113 (53.3)	**3.87***
	>6 Rs/kg	60 (28.3)	99 (46.7)	**15.31*** **
7.	**Recognize iodized salt[(K)]**			
	Label	5 (2.4)	2 (0.9)	**NS**
	Smiling sun logo	2 (0.9)	166 (78.3)	**265.16*** **
	Don't know	207 (96.7)	63 (29.7)	**211.45*** **
8.	**Salt consumption/month[(P)]**			
	1 kg	63 (29.7)	86 (40.6)	**5.47****
	2 kg	149 (70.3)	126 (59.4)	
	>2kg	0 (0)	0 (0)	

9.	**Point of adding salt in the food preparation(P)**			
	Beginning	182 (85.8)	44 (20.8)	**180.45*** **
	Midway	28 (13.2)	62 (29.2)	**16.31*** **
	Towards end	2 (0.9)	106 (50)	**134.38*** **
10.	**Storage of salt(P)**			
	In open jar	2 (0.9)	0 (0)	**NS**
	In closed jar	201 (94.8)	206 (97.2)	**NS**
	In packet	9 (4.2)	6 (2.8)	**NS**
11.	**Storage place**			
	Near cooking range	31 (14.6)	13 (6.1)	**8.22****
	Away from cooking range	181 (85.4)	199 (93.9)	
12.	**Consequences of IDD(K)**			
	Goitre	9 (4.2)	34 (16.1)	**16.18*** **
	Mental retardation	0 (0)	70 (33)	**83.84*** **
	Deafmutism	0 (0)	2 (0.9)	**NS**
	Squints	0 (0)	0 (0)	**NS**
	Physical defects	0 (0)	69 (32.5)	**57.41*** **

Values in the parenthesis depicts percentage, (K) Knowledge, (A) Attitude and (P) Practices.
**p<0.05, **p<0.01, ***p<0.001*

Data on KAP was combined for both the groups, since both the groups were treated equally for imparting the education **(Table 4.95).** It was observed that, majority of the mother's of the children did not have knowledge information on iodine (85.8%) and sources of iodine (85.8%) at baseline. However, towards the end the improvement on information could be improved in affirmation up to 83.5% and 81.1% respectively ($p<0.001$). Information on individual sources of iodine (90.6%) was non prevalent amongst the population, which could also be improved very significantly ($p<0.001$) amongst the respondents towards the end. This revealed a success of the base motto to create awareness and have efficiency to make right food choices to avail optimal iodine nutrition.

Attitude of the mothers on buying any cheapest salt packet has reverted towards to quality salt ($p<0.001$), identifying the iodized salt with its quality mark -smiling sun logo has also improved significantly ($p<0.001$) and the literate mothers have also started recognizing the iodized salt by its label. Knowledge on the various consequences has also improved significantly ($p<0.001$) on goiter, mental retardation and physical defects due to IDD in children and the fetuses of pregnant women has instilled amongst the respondents.

Data on practices on storage and usage of iodized salt also showed motivating results. This showed that, there were 93.9% of the homemakers ($p<0.01$) who have started storing iodized salt/DFS away from the cooking range, to prevent heat exposure for more stability of iodine into the salt. There were 85% of the mothers adding salt in the beginning of the cooking/ while seasoning, which has also improved to adding salt towards the end ($p<0.001$). However, data on storage of salt into a closed jar was already at >90%, which has improved to its optimal level.

Table 4.96: Comparison of knowledge, attitude and practices on iron nutrition amongst children before and after imparting NHE

Sr. No		Pre	Post	Chi square
1.	**Heard of iron (K)**			
	Yes	31 (14.6)	132 (62.3)	**101.67*** **
	No	181 (85.4)	80 (37.7)	
2.	**Source of iron(K)**			
	Yes	15 (7.1)	108 (50.9)	**99.05*** **
	No	197 (92.9)	104 (49.1)	
3.	**Source items (K) (P)**			
	Green leafy vegetables	9 (4.2)	64 (30.2)	**50.06*** **
	Jiggery	0 (0)	29 (13.7)	**31.13*** **
	Nonveg.	0 (0)	38 (17.9)	**41.47*** **
	Beet	0 (0)	45 (21.2)	**50.34*** **
	Rice flakes	0 (0)	33 (15.6)	**35.79*** **
	Pulses	0 (0)	7 (3.3)	**7.12** **
4.	**Source of information (K)**			
	Newspaper	0 (0)	0 (0)	**NS**
	Media	1 (0.5)	0 (0)	**NS**
	Anganwadi worker	20 (9.4)	0 (0)	**20.99*** **
	Researcher	0 (0)	133 (62.7)	**193.79*** **
	Others	10 (4.5)	0 (0)	**10.24*** **
5.	**Consequences of IDA (K)**			
	Weakness	3 (1.4)	71 (33.5)	**75.70*** **
	Lower work capacity	3 (1.4)	23 (10.8)	**16.39*** **
	Anemia	0 (0)	16 (7.5)	**16.63*** **
	Lower immunity	2(0.9)	0 (0)	**NS**
	Poor cognition	0 (0)	0 (0)	**NS**
	Don't know	204 (96.22)	131 (61.8)	**75.78*** **

Values in the parenthesis depicts percentage, (K) Knowledge and (P) Practices.
***p<0.01, ***p<0.001*

It was observed **(Table 4.96)** that, majority of the mothers of the children did not have knowledge information on iron (85.4%) and sources of iron (92.9%) at baseline. However, towards the end the improvement on information could be improved up to 62.3% and 50.9% respectively ($p<0.001$). Information on individual sources of iron (95.8%) was non prevalent amongst the population, which could also be improved very significantly ($p<0.001$) amongst the respondents towards the end. This revealed a success of the base motto to create awareness and have efficiency to make right food choices to avail optimal iron nutrition. Knowledge on the various consequences has also improved significantly ($p<0.001$) upon weakness, lowered work capacity and anemia due to ID in children and the fetuses of pregnant women has instilled amongst the respondents.

Table 4.97: Hygiene and sanitation practices in the children

		Pre	Post	Chi square
1.	**Hand washing before meal**			
	Yes	188 (88.67)	206 (97.2)	
	No	24 (11.32)	6 (2.8)	
2.	**If yes, how**			
	Soap	95 (44.51)	101 (47.6)	NS
	Water	93 (43.87)	105 (49.5)	
	Mud	1 (0.47)	0 (0)	

Mothers of the children or caretakers were also educated regarding the health benefits of hygiene and sanitation (hand washing and health). It was observed that, there was an improvement in the sanitation habit of washing hand with soap (47.6%) compared to the baseline (44.51%) **(Table 4.97)**.There were almost half of the children, who started washing their hands cautiously with soap and there were half of the children used water (49.5%) at least to wash their hands. Hence, in total majority of the school children were washing their hands before meals as per the availed information from the mothers.

Table 4.98: Acceptability of DFS by the families of experimental groups

Sr. No		N (%)
1.	**Salt consumption per month**	
	1 kg	45 (38.8)
	2 kg	71 (61.2)
2.	**Salt consumed daily by the children**	
	Yes	116 (100)

	No	0 (0)
3.	**Salt used for all foods during cooking and table**	
	Yes	116 (100)
	No	0 (0)
4.	**Color change in foods after adding salt in preparations**	
	Yes	111 (95.7)
	No	5 (4.3)
5.	**Which food preparations gave color change**	
	Rice	50 (43.1)
	Dal	67 (57.8)
	Starchy Vegetables	51 (44.0)
	Green leafy vegetables	53 (45.7)
6.	**Do you accept the salt provided**	
	Yes	116 (100)

Information on acceptability and usage of DFS from the mothers of the experimental groups revealed that, the acceptability of DFS was very high, since it appeared, tasted and textured like normal salt only **(Table 4.98)**. Initially there were 95.7% of the mothers who observed color change in the food preparations after adding salt to the food items. Majority of them observed color change in the food preparations like, Rice (43.1%), Dal (57.8%), Starchy vegetables (44%) and Green leafy vegetables (45.7%). This could have been due to longer cooking time and addition of salt directly to the oil at the time of seasoning. However, after providing NHE and BCC on cautious practices while using DFS, the problem of color change could be resolved to a great extent.

Table 4.99: Dietary modifications for school children- impact of NHE on mothers

Sr.No		N(%)
1.	**Have you modified the diet of your child as per NHE**	
	Yes	158 (74.53)
	No	54 (25.47)
2.	**Did you observed any change in the child after NHE**	
	Yes	136 (64.15)
	No	76 (35.85)

3.	**What kind of changes did you observe**	
	Increased physical activity/ strength	125 (58.96)
	Increased concentration	41 (19.34)
	Increased appetite/diet	71 (33.5)

Finally the data on dietary changes and impact on the children winded up our urge of collecting information on the impact of NHE. The data **(Table 4.99)** revealed that, 74.53% of the mothers tried following the information provided as the conditions allowed and there were 64.15% mothers who observed some amount of change in their children after the efforts made by them. The major observations involved increased physical activity (58.96%), increased concentration (19.34%) and increased appetite/diet (33.5%).

DISCUSSION

4.6 IMPACT ASSESSMENT

4.6.1 IMPACT ASSESSMENT ON NUTRITIONAL STATUS

Summing up, growth in terms of Height, Weight was significantly higher in experimental groups compared to control groups. The improvement in height was observed to be significantly higher in E+DW group compared to rest of the groups and weight gain was significantly higher among E group compared to rest of the groups. However, there was a non significant variation in the improvement in BMI amongst all groups. This in turn suggests that, DFS along with or without deworming did not show any significant impact upon the growth indices of the school children. The improved indices were observed due to the natural growth process of the children.

In many research studies, intermittent iron supplementation or daily lower doses of iron has been suggested to obtain the expected gain in developing countries. However, comparing DFS with weekly iron supplement has not shown any significant impact.

In support of our statement a review of 3 meta analyses reported by (Ramkrishnan U et al 2004) revealed that, iron or Vitamin A alone has non significant impact on the growth of the children <18 years compared to the multiple nutrients supplementation. Thus, the interventions with multiple nutrients are the best strategies to have an impact on the growth indices.

A study on Indonesian school children by (Soementri et al 1997) also reported non significant increase in growth indices after 3 months of weekly or daily iron supplementation.

4.6.2 IMPACT ON MICRONUTRIENT STATUS

4.6.2.1 Impact on hemoglobin status

In children, anemia prevalence decreased significantly in the double-fortified salt group over the nine-month intervention. Similar observation was reported by (Ashibey-Berko E et al 2007) with remarkable reduction due to DFS usage on regular basis.

As reported by NIN 2001 study, there was a significant reduction after 2 years of supplementation in the school children of residential schools of Hyderabad. The hypothesis put forth was similar to our study. It was assumed that, the supplemented iron from DFS (8-10 mg/day) was perhaps not adequate to increase mean Hb levels completely during their phase of increased physiological requirements for iron due to the growth process. However, when they considered children with mild anemia (10-12 g/dl Hb), slightly greater increments of hemoglobin were noted in those receiving DFS at the end of 1^{st} and 2^{nd} year.

When the limitations of the NIN 2001 study were referred, they were mentioned as (1) the iodine content of DFS in the first and the third batch was below acceptable limits (<15 ppm). Analysis of the quality of salt used for DFS production revealed that the defined quality of salt was not used. The salt supplier did not maintain good quality control. (2) The bulk packaging of the fortified salt supplied for the school (50 kg packets) as against 1 kg packets of salt. (3) Batch 3 contained only 713 ppm of iron compared to 1000 ppm in other batches of salt.

Quality of salt, usage of correct stabilizer and appropriate form iron fortificant plays a vital role in achieving an expected impact on both iron and iodine status. Since ferrous sulphate monohydrate and ferrous fumarate has been proven to be the effective fortificants. With respect to that, a study conducted by (Wegmuller R et al 2006) reported no change in hemoglobin concentration and a significant impact on UIE, using micronized ground ferric pyrophosphate (FePP) amongst school children.

Another representative data obtained on 5-14 years old children of Hyderabad in different studies by NIN showed, there was a significant improvement in hemoglobin status and the prevalence of anemia was reduced after introduction of IFS in both rural and urban communities. The increment in hemoglobin in anemic (Hb<12 g/dl) boys and girls was 18 g/dl and 32 g/dl respectively at the end of one year, on a diet which contributed an iron content of 35-40 mg/d (20-25 mg endogenous and 15 mg from iron fortified common salt with an absorption of 3.4%). The prevalence of anemia in boys was reduced to 19.4% from 51.9% whereas in girls it reduced to 15.5% from 22.5%. The reduction in prevalence rates was significant at $p<0.01$ (Nadiger et al 1980).

While comparing our study results with the quoted study, the data varied at three levels. One at the dietary intake of iron (with or without DFS supplementation), which is comparatively lower (6-10 mg/d) than the reference study and secondly the prevalence of anemia is very high amongst both the genders (98-99%) in our study population. Thirdly the supplementation period was 9 months. Hence, the final result in terms of increase in hemoglobin levels was lower than the reference study.

However, the most important similarities between our result and the findings by (Nadiger et al 1980) was, the increase in Hb concentration which was observed to be higher amongst children with lowest hemoglobin concentration at baseline.

Another finding presented in the (Report of the working group on fortification of salt with iron 1982), the mean change in hemoglobin in experimental and control groups was 0.53 g/dl in boys and 0.50 g/dl in girls; 0.14 g/dl in boys and -0.54 g/dl in girls respectively. There was also a significant reduction in the prevalence of anemia reported from 52.9% to 27% in boys and 63.8% to 33.7% in girls at the end of one year. This supported our findings demonstrated in **Table 4.77** and **Table 4.79**.

Looking into the percent prevalence of anemia, it was observed that, DFS supplementation with deworming (E+DW) could bring significant reduction in prevalence of anemia ($p<0.001$) compared to E and control groups (C and C+DW). Similar pattern study was also carried out by (Sood et al, UNICEF Report 1989-92) in Delhi among the children aged 6-15 years belonged to the orphanages to assess the effect

of iron fortified salt on prevalence of anemia. The population was a wheat based population. The results revealed that, in experimental group, male children showed a highly significant decline in the prevalence of anemia from 29.5% to 4.5% ($p<0.001$) and non significantly in the girls from, 28.9% to 25%. However, there was significant increase in the children belonged to control group with rise of 7% in boys and 23% in girls at the end ($p<0.001$). The population in a controlled condition showed this much high increase in the prevalence of anemia, than our free living population with insufficient stores of iron. Thus, it validates the efforts towards deworming and NHE-BCC made to improve the iron status other than DFS supplementation.

Iron content of DFS (10 mg/day = 70 mg/7 days) is comparable with the study on impact assessment of IFA /iron tablets supplementation once weekly or fortified foods supplementation to the school age children. The improvement in iron status amongst our study subjects could have been due to small but daily supply of iron through the daily diet of the children compared to the tablets or supplements which are not the part of routine food items. A study supporting our findings (Tee et al 1999) revealed that, the increase in anemics was twice than the borderline-anemics regardless of iron doses. The Malaysian adolescent girls in the study were supplemented with different doses of iron-folic acid supplements. (Either 60 mg or 120 mg Iron + 3.5 mg Folic acid once a week) for 22 weeks. With this regards, it can be stated for our study findings that, DFS also could bring better impact in the children, since majority of them were anemic. The cohort school study by NIN 2000 clearly demonstrated an improvement in hemoglobin concentration in mild-moderate anemic than in those with normal levels. There appears to be no additional benefit of DFS supplementation in those with normal status (Brahmam et al 2000). The supporting pillars like studies by NIN (Nair KM et al 1998) and (Zimmerman et al 2004) have also revealed a reduction in hemoglobin concentration and urinary iodine levels in the children belonged to control groups also motivates our findings on hemoglobin decline in control group. However, the urinary iodine concentration increased in control group due to optimal consumption of iodized salt by the population.

Another study conducted by (Thi Lee et al 2007) among Vietnamese school children reported the effect of fortified noodles supplementation 5 days a week for 6 months. Noodles were fortified with iron at 10.5 mg/ serving. The compared group was provided with 60 mg elemental iron per week. At the end of the study, a significant improvement in hemoglobin concentration was observed. Fortified noodles with deworming and without deworming groups showed average 0.26 g/dl compared to iron supplemented group (0.76 g/dl). But still the improvement was significant and remarkable, since it could improve their iron status with food based approach. On comparing our study, with these results, it can be stated that, DFS as a universal food ingredient to all homemade food items would be proved beneficial for the anemic population of the community.

4.6.2.2 IMPACT ON IODINE STATUS

DFS being an adequate source of iodine due to optimal fortification level (40 ppm) showed a similar effect to the optimally iodized salt amongst school children. There has been a significant ($p<0.001$) positive shift in the proportion of normal children based on urinary iodine excretion in all the study groups. The experimental population, where the availability of iodized salt was lower than the control group and thus chosen for DFS supplementation groups, showed an increase in urinary iodine excretion sufficiency to more than 80% in both the groups (E and E+DW). However, where the availability of iodized salt was almost 100% and due to improper dietary, cooking and storage practices of iodized salt were not able to achieve 100%, have also shown remarkable improvement in the excretion of urinary iodine levels with sufficiency at >90% in the children.

The median urinary iodine excretion also increased significantly ($p<0.001$) in all the groups intact and also based on the gender variations. When the improvement was compared based on the age groups (<12 years and $\geq$ 12 years), a remarkable improvement with visible variations was observed in the younger group compared to the latter group. This in turn proves school age children to be the indicator group for assessing the iodine nutrition status of the population.

In view of our findings, a study conducted by (Anderson M 2008) in south India amongst school children revealed a significant ($p<0.001$) increase in median urinary iodine excretion at the end of 10 months of supplementation compared to the baseline values. These values were 182, 143 and 133 µg/L in IS, DFS1 and DFS2 groups respectively at baseline and they increased to 355, 166 and 252 µg/L towards the end. In this study they have supplemented iodized salt (IS) to the control group, however in our study the NHE was provided on the consumption, since the availability of iodized salt was sufficient. The prevalence of iodine deficiency also decreased significantly in all the three groups ($p<0.01$) compared to the baseline.

As a secondary assessment of iodine status amongst the school children, their thyroid hormones were analyzed to assess the effect of iodine nutrition on the system. It was observed that, at baseline and at the end, there was a non significant difference between all the study groups, since all the subjects were falling into the normal ranges for thyroid hormones. The hormones levels were essential to be assessed, since it is proven by a study conducted by (Huda S. et al 1999) that, the school children in iodine deficient areas of Bangladesh, who had low T_4 levels, had poorer cognitive function and school achievement levels than children with higher T_4 levels. Thus, the effect of thyroid hormones and abnormal levels should be assessed amongst the children. However, our study population showed a normal range of thyroid hormones to judge their mental growth.

Another study conducted by (Huda S., McGregor-Grantham S and Tomkins A 2001) in Bangladeshi school children revealed no impact of Lipoidal oil supplementation on thyroid hormones status different than the control group. The effect was only observed significant ($p<0.001$) on urinary iodine excretion. This in turn reflects our study results partially, since it was observed that, the median TSH levels decreased significantly ($p<0.01$) in experimental groups (E and E+DW), whereas non significantly in control groups (C+DW and C). However, there was a significant ($p<0.01$) reduction in serum Tg levels in all the groups compared to baseline values, except E+DW group. This could be due to increased circulatory iron in the system and active role of iron in the action of TPO enzyme and incorporation of iodine in the Tg.

4.6.3 IMPACT ON IQ AND COGNITION TESTS

A number of studies have indicated that, in school children, initially lowered test scores of the IQ and cognition tests scores of cognition can be improved when their hemoglobin status is improved with the alleviation of anemia (Pollitt E et al 1985, Kashyap P et al 1987, Soemantri AG et al 1989, Bruner AB et al 1996). Most of the studies on effect of iron supplementation (iron alone or iron-folic acid) compared to control groups, generally showed a significant improvement on cognitive function or educational achievement amongst children (Sheshadri S and Gopaldas T 1989, Sheshadri S et al 1982). As discussed by (Malavika V and Rajgopalan S 2007), the adverse effects on cognition and educational test performance due to iron deficiency anemia in preschool and school children appear more transitory in nature than the effects on development on infants and imply that treatment of IDA through iron supplementation programmes may be beneficial and have immediate effects.

Anemia causes poor attentiveness, poor memory and academic performance in school age children. Anemic infants are often irritable, restless and show behavioral abnormalities like lack of attention, fatigue and insecurity. Poor attention span, memory and concentration as well as concept acquisition leading to poor school performance have been attributed to anemia during this phase of critical learning.

In a study conducted by (Pollitt E et al 1985) in Thai school children improved their cognitive scores at follow-up irrespective of their iron status. It was reasoned out by (Malavika V and Rajgopalan S 2007) that, there is always a familiarity element when a retest is given and this familiarity leads to improvement in scores in the control group also. To offset this improvement in scores due to prior practice, the end line scores were subtracted from baseline score and the increment in scores is taken to consider whether there is an improvement of experimental group over the control.

In our study, when DFS was supplemented for the period of 9 months, it has resulted in the increase in the scores of all the three tests (DMT, VMT and CT) in all the study groups. Increment in mean scores of each group was observed to be highest amongst experimental groups. In this respect the increase was significantly high ($p<0.001$) in

E+DW group for DMT test compared to rest of the groups. This in turn suggested the role of increased efficiency of iodine in the presence of iron due to its role in the action of thyroperoxidise enzyme and in the absence of worm infestation due to their irradiation by deworming. However, in the absence of iron supplementation C and C+DW groups could not show better improvement compared to experimental groups. Hence, an increase in the IQ scores of each group could be a result of the familiarity, but still varied on their iron and iodine status at the end.

The gender wise variations between groups in mean score (Anova test) was proved to be significant for boys ($p<0.001$) compared to the girls. The mean difference also varied between groups based on the reduction ($p<0.05$) in hemoglobin concentration and non significant for increased status. Hence, depicted less impact of variations in hemoglobin status. While comparing the mean difference in the scores compared to the variations in median urinary iodine status, the scores varied significantly between groups in iodine sufficient category, indicating the reliance of DMT scores (IQ) on iodine intake.

The mean IQ scores improvement has motivated our efforts by categorizing experimental groups into “Average” performance category compared to “Below average” scores for the entire population. However, control groups could not make that standard up (based on the IQ classification by Phatak P 2002).

Yehuda and Youdim (1989) reported that those who receive iron for controlling iron deficiency anemia commonly report improved memory, attention, mood and higher level of energy before any improvement in hemoglobin indices occurs. Our findings of the study also report that there is a significant improvement in short-term memory, attention and concentration of the children belonged to experimental and control groups, since there has been improved nutritional status. The improvement was observed to be significant amongst E group ($p<0.001$) compared to rest of the groups.

A review of literature shows that the intake of macro and micronutrients may have individual and interactive effects on brain and cognitive development. Cross- sectional studies indicate that there are associations between stunting, IQ and school performance

in children after controlling for key confounding factors such as socioeconomic variables (Granthan-Mcgregor 1993, Agarwal KN et al 1995).

In support of our study findings on improved VMT scores, a study conducted by (Sen A and Kanani S 2007) in preadolescent and adolescent girls of urban Vadodara also revealed a significant improvement in all the supplemented and control groups. The highest improvement was observed amongst the girls supplemented with IFA on daily basis compared to rest of the groups.

In the letter cancellation test/ Clerical test which is a measure of attention and concentration too, our study population demonstrated that the increment in mean scores was observed to be significant in all the groups. Gender wise variations revealed a significant difference between boys with higher difference compared to girls in all the study study groups. Between groups comparison revealed a difference to be non significant between experimental and control groups. However, the improvement in CT scores was observed to be higher amongst E+DW group compared to the rest groups, though non significant. This in turn suggests some role of iron release from DFS in absence of worm infection amongst children.

A study conducted by (Sen A and Kanani S 2007) showed a supportive evidence to our result indicating, the difference to be significant between experimental and control groups. However, the difference was remarkable than our findings. This can be reasoned out that, the supplementation groups were on IFA tablets, thus there was a significant difference between iron intake of experimental and control groups. Secondly there was no other strategy used like deworming along with the supplementation or in control to observe the effect same as our study. Thus it might not give appropriate differences on such basis. Hence, these two reasons justifies a non significant difference in our study population for CT.

Another study conducted by (Malavika V and Rajgopalan S 2007) has also revealed a significant improvement in CT amongst experimental group ($p<0.05$) compared to control group. The reason could be the difference in strategy, since they have supplemented the population with DFS fortified with multiple micronutrients (Vitamin A,

B complex and Niacin) other than iron and iodine. Hence, the observed results could be due to the cumulative effect of all the nutrients. Thus, the multiple micronutrients does not compare with DFS solely. Hence, looking into the reviewed findings DFS gave motivating results.

4.6.3 INTERRELATION BETWEEN PARAMETERS

When the mean change in all test scores were cross tabulated with 2 categories of mean change in hemoglobin (0.01->1 and 0.00-<-1 g/dl). The difference was observed to be significant for DMT and VMT between all four groups. However, when the cross tabulation was carried out with 2 categories for median change in UIE (>100 µg/L and <100 µg/L), a non significant difference between groups was observed. This can be justified as UIE being only a prior day output of iodine intake. However, hemoglobin reflects at least 3 months constant intake. Hence, the impact was observed to be more specific with change in hemoglobin concentration and not in UIE.

4.6.3 IMPACT OF SUPPLEMENTATION, NHE AND BCC ON DIETARY INTAKE

Data on impact of NHE on dietary intake revealed that, there was a significant improvement daily intake of macronutrients. A drastic improvement in the consumption of iron has improved amongst experimental groups due to addition of DFS in to the dietary practice, which might be the reason for increased hemoglobin concentration of the children belonged to the experimental groups. However, this positive shift was not observed among the control groups, thus it could be the reason for the reduced hemoglobin concentration at the time of their growth phase.

Percent distribution of the children meeting their RDA >50% has improved significantly in all the study groups, considering energy, protein and fat. However, dietary intake of iron has improved at a higher level of significance ($p<0.001$) compared to control groups due to DFS as an additional factor to their daily diet. These findings encouraged us by indicating majority of the children in both experimental group (E+DW and E) also achieved more than 75% RDA for iron intake, which in turn could have been the reason for increased hemoglobin concentration in the experimental group, where as reduction in

control groups was observed, since less than 20% of the children of control groups (C+DW and C) could achieve >75% RDA for iron after intervention.

There was also a significant improvement observed in the knowledge amongst the mothers of the children upon iodine and iron nutrition, their sources and cautious usage of these food sources in the daily diet of the children.

Attitude of the mothers on buying any cheapest salt packet has reverted towards making a wise choice by checking the smiling sun logo as a mark of quality iodized salt and the literate mothers have also stared recognizing the iodized salt by its label. The trend of choice on a packet of salt has improved to a significant level after knowing the significant consequences of IDD on children and the fetuses of pregnant women. Knowledge on the various consequences has also improved significantly. Impact on storage and cooking practices has also showed a significant improvement in the families.

Data on hygiene and sanitation section revealed that, majority of the children started washing their hands before taking meals. This data included shares of soap and water equally.

However, data on acceptability of DFS also revealed a very high rate of acceptance amongst the families, after understanding the health impact of DFS on their children. The constraint of color change in the preparations and thus affected acceptability by the children was observed by few mothers after cooking, but provided information on cooking practices and behavior could solve their problem of acceptance.

However, data of the impact of NHE on the children's health helped to end up the study phase, with positive results in terms of increased physical activity, increased concentration and increased appetite as observed by the mothers, who modified the diet of their children according to the provided information. These findings of ours were supported by the departmental study conducted by (Kanani and Agarwal 1997), clearly showed an impact of additional information and nutrition education may help to improve the dietary intakes of adolescent girls of urban Vadodara. This also indicated that, nutrition communication alone could significantly improve levels of awareness, intake of specific foods and nutritional status of the girls.

Thus, this phase has revealed the importance of daily supplements through a traditional food ingredient (Salt- double fortified) along with awareness campaigning, NHE, BCC and deworming plays a vital role to improve micronutrient status of the children, which is a prime goal of all the public-private sectors working in the area of nutrition. Further, this would help in attaining MDG 4 and MDG 5.

Phase III

Upgrading salt iodization at local level and feasibility for double fortified salt production

In this phase, salt producers from Anand, Kheda, Nadiyad, Vadodara and Bharuch were selected for up gradation of salt iodization process. Total (N=40) producers were covered. However, during at the time of baseline data collection, two producers shifted the plant out of our coverage periphery. Thus, baseline population was (N=38). Further, towards the end (N=34) producers could complete the study.

This phase was divided into three sections:

4.7 Baseline salt iodization levels

4.8 Interventions and monitoring of iodization levels

4.9 Initiating concept of DFS production at local level

4.7 BASELINE SALT IODIZATION LEVELS

A baseline mapping of small scale salt producers was carried out under a partnership program with MI, Gujarat and UNICEF, Gujarat. Preliminary data on the prevalence of iodine insufficiency in Anand district revealed 48.48% deficiency existed among the subjects enrolled from 20 PHC's. The subjects belonged to different age groups ranging from young children to pregnant and lactating women in Anand. Higher prevalence rate of still birth and lower UIE in pregnant women in Anand district triggered our concept of updating salt iodization at local level, since majority of the rural population was consuming locally produced salt.

Baseline data on salt production units **(Table 4.100)** revealed that, majority of the salt production units belonged to Anand and Nadiyad districts (63.20%). However, rests 26.80% of the units were from Vadodara, Bharuch and Kheda districts. Majority of the producers were from Anand and Nadiyad, since these two districts were the targeted regions according to UNICEF and MI, Gujarat data (2007-2008), as a poor salt production region. Thus, vigorous efforts were made by focusing these areas.

The production capacity of the salt production units reflected a mean value upto 10 tonnes/ month. The percent distribution revealed that, 13.2% of the producers were producing <5 tonnes of salt per month. However, 21.1% and 18.4% of the production units were producing 10-15 tonnes and >15 tonnes of salt per month. Majority (47.4%) of the production units were producing 5-10 tonnes of salt per month, indicating lower production capacity of the small scale salt crushers. These N=38 producers were producing almost 400 tonnes of salt per month, supplying their product to the rural and urban set up of the districts. Hence, the quality production by these producers was the major target to be achieved.

Table 4.100:Baseline characteristics of the salt production plants

Characteristics	N (%)	95% CI
District		
Anand/ Nadiyad	24 (63.20)	47.55-78.85
Vadodara/Bharuch	6 (15.8)	3.97-27.63
Kheda	8 (21.10)	7.86-34.34
Salt production capacity/ month		
<5 tonnes	5 (13.2)	2.22-24.18
5-10 tonnes	18 (47.4)	31.2-63.6
10.01-15 tonnes	8 (21.1)	7.86-34.34
>15 tonnes	7 (18.4)	5.82-30.98
Retail cost/kg		
1-4.99 Rs./kg	26 (68.4)	53.32-83.48
≥5 Rs./kg	12 (31.6)	16.52-46.68

Data on retail cost of one kg salt packets revealed, that 68.4% of the salt was sold at <5 Rs. per kg. However, there were one third of the salt units, which had retail cost of their salt above 5 Rs. per kg salt.

Further, data on mean salt iodine content of all the three districts is been depicted in **Table 4.101**. Data on mean iodine content revealed that, all the three districts were below the recommended levels (30 ppm) of iodine content.

Table 4.101: Iodine content of the salt samples at baseline (Using IT method)

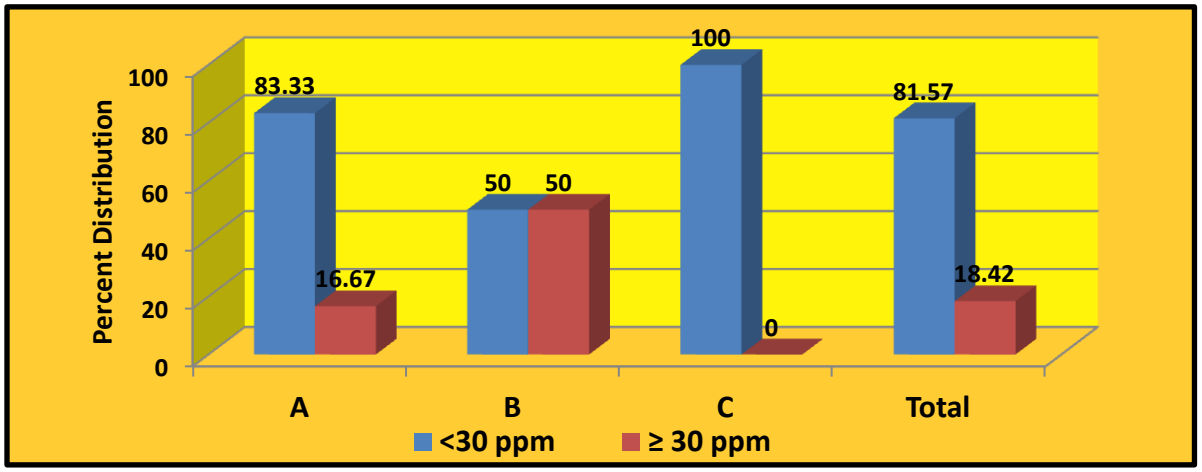
Place	Group*	N	Mean (ppm)	SD (ppm)	Median (ppm)	95% CI
Anand/Nadiyad	**A**	24	20.79	10.69	17.25	16.27-25.30
Vadodara/Bharuch	**B**	6	28.06	13.28	28.70	14.12-42.00
Kheda	**C**	8	20.00	4.47	18.80	16.25-23.74
Total		38	24.00	17.26	18.80	18.38-25.16

The districts have been coded as groups A,B and C for ease in expression in the text.

Mean values at baseline revealed that, there was no significant difference between all the three groups (F value-1.024, p>0.05). However, mean iodine content of BGroup was observed to be non significantly higher with 28.06 ± 13.28 ppm compared to rest 20.79 ± 10.69 ppm and 20.00 ± 4.47 ppm for the groups A and C respectively.

Further analysis on distribution of salt iodine content into classification based on the recommended level for salt iodine at production site **(Figure 4.23)** was carried out. It was observed that, majority of the salt samples from A and C group were falling into the first category (<30 ppm). However, this distribution was observed to be 50% in each category for Vadodara/Bharuch group. Due to the higher percent of the salt samples into <30 ppm, the distribution of the total samples also was skewed towards negative side with 81.57% heading towards the <30 ppm category.

Figure 4.23: Percent distribution of the districts based on salt iodine contents



A-Anand/Nadiyad, B-Vadodara/Bharuch and C- Kheda

However, it was also motivating to know that, there were 16.67% and 50% of the subjects in Anand/Nadiyad and Vadodara/Bharuch respectively. The difference was observed to be weakly significant between all the three groups (chi -5.84, $p<0.054$). This was suggestive that, either lack of technical skills or the lack of determination to produce iodized salt being reflective at this stage. Hence, we focused our interventional efforts focusing both the issues.

4.8 INTERVENTIONS AND MONITORING OF IODIZATION LEVELS

In this section, the enrolled producers were subjected to different strategies to improve their iodization levels. The strategies involved,

- Intermittent plant visits
- Regular monitoring by salt sample analysis
- Technical support at field level
- Review workshops
- Roles played by MI,Gujarat at different point of time - Subsidized supply of potassium iodate, partial payment for electricity bill, partial payment for packaging material.

The salt producers were communicated and potassium iodate was distributed by MI, Gujarat.

4.8.1 OVERVIEW OF THE REVIEW WORKSHOPS CONDUCTED

Majorly there were two review workshops involving government sectors, salt department and non government sectors were conducted at different point of time. Other than that, at plant level 3 more meeting were conducted to involve the producers into the iodization process and discuss the technical problems while iodization.

During this phase multiple approaches used were as follows:

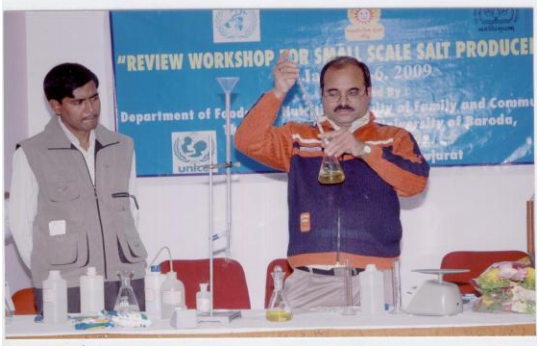
1. Personal visits made to the salt plants to check salt iodine level using mobile kits
2. Technical upgradation for iodization

3. Workshops organized to address general issues, technical aspects, STK distribution etc.
4. Distribution of KIO3 at subsidized cost.
5. Laisoning with all stakeholders

Figure 4.24: Review workshops with the producers

Addressing the producers by resource persons from the department;salt office-GOI;health and ICDS sectors

Training on technical aspects and morale boosting



Quality assurance training on salt titration

Oath ceremony to pledge adequate Iodization.

Figure 4.24 reveals the evidences for workshops conducted with the salt producers for generating awareness and improve their desire to produce quality iodized salt. The workshops involved speakers from GOG, salt department, Anganwadi, Health department etc**.** Training on quality assurance, technical aspects and ethical approaches of iodized salt production were provided.

Figure 4.25: Local level meetings

Registration of the producers for our study

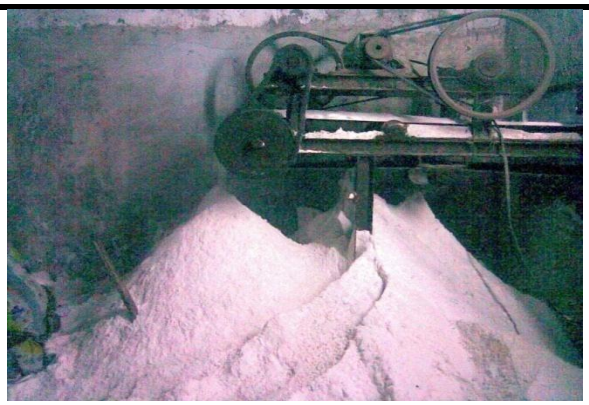
Subsidized supply of $KIO_3$and follow up

Figure 4.25 depicts active participation of the producers and desire to improve on their product quality, learning different approaches and discussing practical issues faced at the time of production. On the return, they were receiving a subsidized supply of Potassium iodate (KIO_3) (fortificant of iodized salt) by MI,Gujarat.

Figure 4.26: Plant visits for technical support and upgrade iodization at different levels

Measuring and transferring raw salt to the crushers

Indigenous method for iodization was made possible depending upon the plant facilities



Final product and packaging

Figure 4.26 reveals that, the salt iodization process was monitored and modified at each step, whenever there was a need. The producers were counseled and made understand from sequential steps from flow of KIO_3, packaging and quality assurance.

4.8.2 MONITORING OF THE SALT IODIZATION LEVELS

During the monitoring period 4 salt producers dropped out due to their unwillingness to participate and closing of the plants. Data on mean salt iodine content during and post interventional stage is been revealed in the **(Table 4.102)**.

Table 4.102 revealed that, there was a significant improvement ($p<0.001$) in mean final salt iodine content of A group compared to baseline (Manova test). However, rest two of the groups did not show significant improvement for intermittent or final data, when compared to the baseline values. This could have been due to higher baseline mean iodine content in Group B compared to Group A data.

Table 4.102 : Iodine content of salt at production unit during all the three intervals

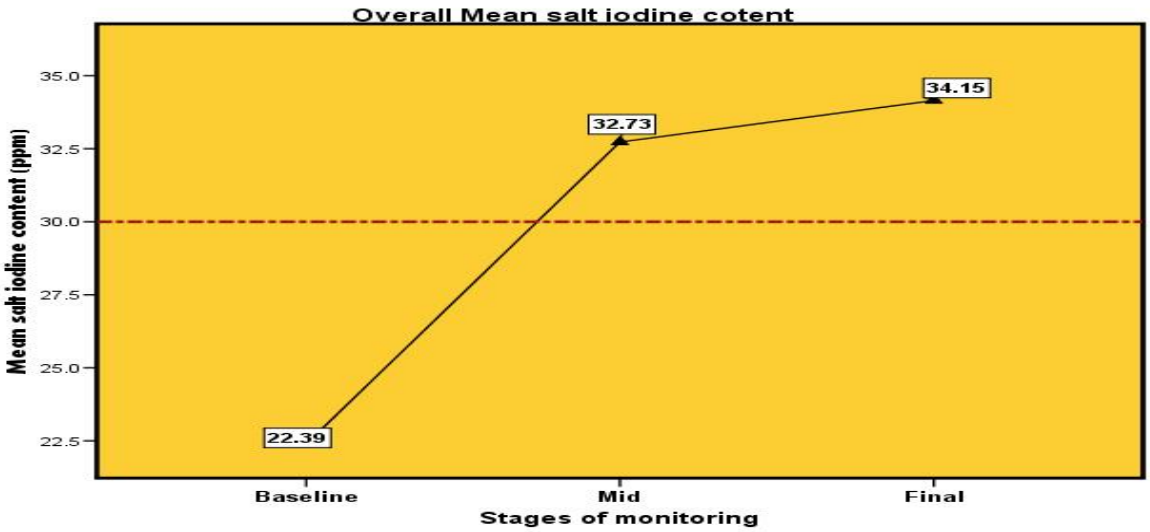
	N	Mean	SD	Median	95% CI
Anand/Nadiyad(A)	22				
Initial		21.10	11.10	17.25	16.17-26.02
Mid		30.51	16.75	23.80	23.08-37.94
Final		33.59**	14.08	31.35	27.34-39.83
Vadodara/Bharuch (B)	6				
Initial		28.06	13.28	28.70	14.12-42.00
Mid		40.43	9.59	42.05	30.36-50.49
Final		33.08	4.04	33.15	28.83-37.32
Kheda (C)	6				
Initial		21.43	4.24	19.90	16.97-25.88
Mid		33.16	27.85	22.95	3.93-62.40
Final		37.26	16.01	32.80	20.45-54.07
Total	**34**				
Initial		22.38	10.73	18.85	18.64-26.13
Mid		32.73*	18.00	27.20	26.44-39.01
Final		34.15***	13.02	32.30	29.60-38.69

p<0.05, p<0.01 and ***p<0.001 v/s Initial value*

While comparing total improvement including all the salt plants, revealed a consistent improvement during intermittent ($p<0.05$) and final visits ($p<0.001$) compared to baseline

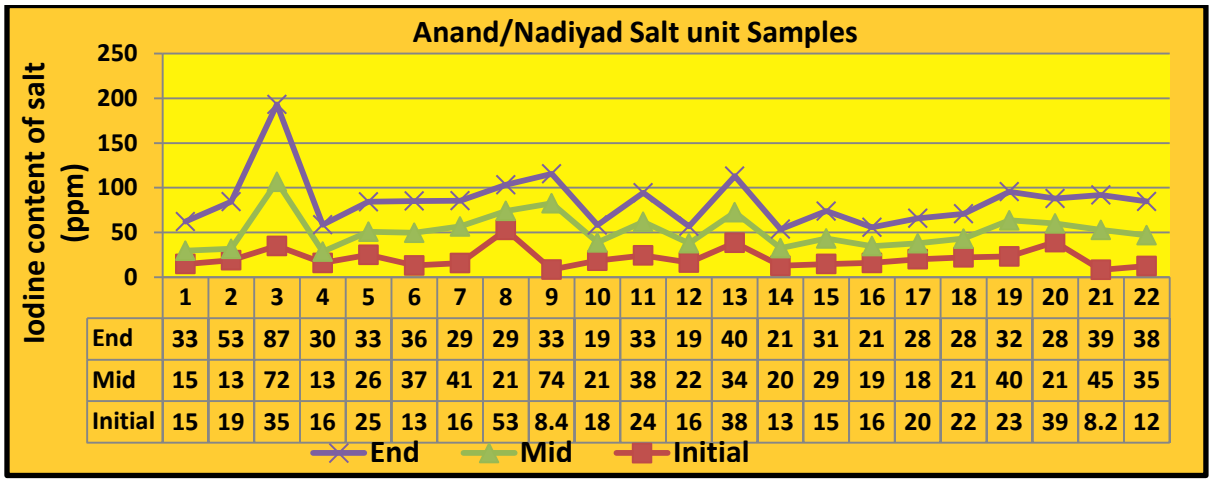
iodine content (Manova Test). This suggests steady and gradual success of our efforts towards upgrading the iodization process. This was also reflected in the **figures 4.27, 4.28, 4.29**and**4.30**, indicating all the mean iodine content above recommended level (30 ppm) for all groups and majority of the units.

Figure 4.27: Overview of salt iodization for all the production units



Data presentation of all the three groups for individual production units has been revealed in the **figures 4.28, 4.29** and **4.30**.

Figure 4.28: Data on salt iodine content of Ananad/ Nadiyad salt units at three intervals

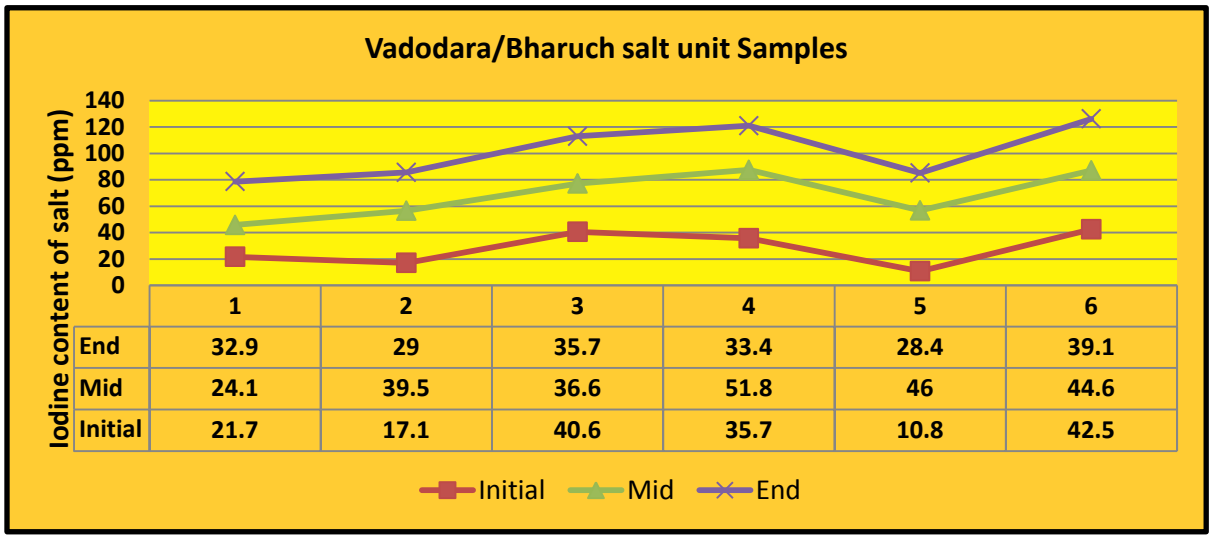


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
End	33	53	87	30	33	36	29	29	33	19	33	19	40	21	31	21	28	28	32	28	39	38
Mid	15	13	72	13	26	37	41	21	74	21	38	22	34	20	29	19	18	21	40	21	45	35
Initial	15	19	35	16	25	13	16	53	8.4	18	24	16	38	13	15	16	20	22	23	39	8.2	12

Figure 4.28 reveals that, there was a gradual improvement in the mean iodine content of the salt units. The improvement was steady but non significant (chi -0.683, p>0.05) for intermittent and end result compared to the baseline. However, the data on percent distribution for categories based on recommended salt iodization levels also supported the findings by revealing 81.81%, 59.09% and 40.90% samples below 30 ppm levels at baseline, intermittent and end result. This suggested 40% decrease (81% to 41%) in the lower category and thus improvement in higher category (>30 ppm). Thus, it was suggestive of a need for regular monitoring and improved public health concern amongst the producers.

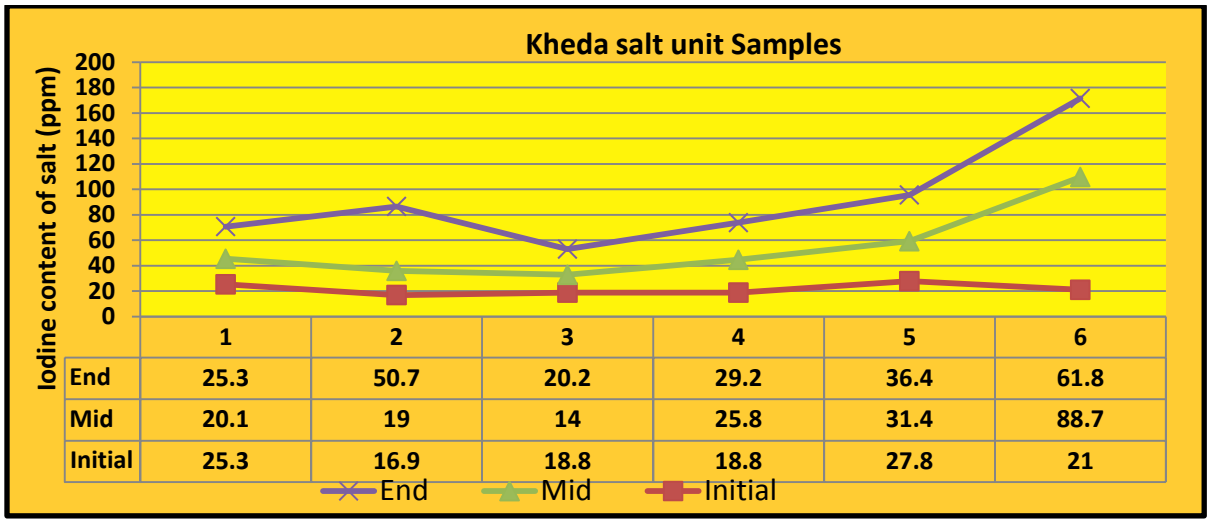
Figure 4.29 reveals that, there was a gradual improvement in the mean iodine content of the salt units. The improvement was steady but non significant (chi -0.273, p>0.05) for intermittent and end (chi -0.083,p>0.05) result compared to the baseline. However, the data on percent distribution for categories based on recommended salt iodization levels also supported the findings by revealing 50%, 16.66% and 33.33% samples below 30 ppm levels at baseline, intermittent and end result. This suggested 17% decrease (50% to 33%) in this category and thus improvement in higher category (>30 ppm). The higher level of fluctuations, yet non significant levels were due to small sample size (N=6). However, this suggests a need for regular monitoring and stability in iodization levels among the producers.

Figure 4.29: Data on salt iodine content of Vadodara/Bharuch salt units at three intervals



	1	2	3	4	5	6
End	32.9	29	35.7	33.4	28.4	39.1
Mid	24.1	39.5	36.6	51.8	46	44.6
Initial	21.7	17.1	40.6	35.7	10.8	42.5

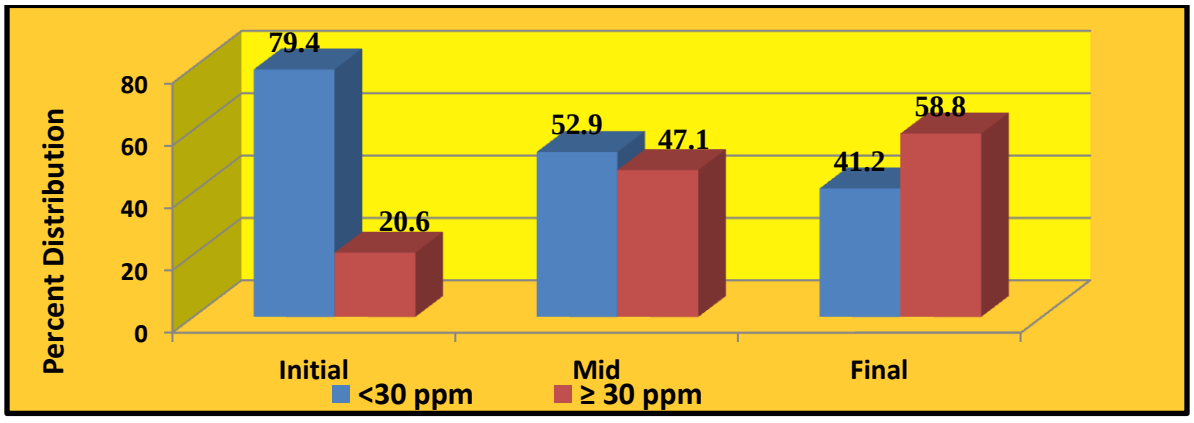
Figure 4.30: Data on salt iodine content of Kheda salt units at three intervals



	1	2	3	4	5	6
End	25.3	50.7	20.2	29.2	36.4	61.8
Mid	20.1	19	14	25.8	31.4	88.7
Initial	25.3	16.9	18.8	18.8	27.8	21

Figure 4.30 reveals that, there was a gradual improvement in the mean iodine content of the salt units. The improvement was steady but non significant for intermittent and end result compared to the baseline. However, the data on percent distribution for categories based on recommended salt iodization levels also supported the findings by revealing 100%, 66.66% and 50% samples below 30 ppm levels at baseline, intermittent and end result. This suggested 50% decrease (100% to 50%) in this category and thus improvement in higher category (>30 ppm). The higher level of fluctuations, though non significant due to small sample size (N=6). However, further this data suggests a need for regular monitoring, stable iodization process and improved public health concern amongst the producers.

Figure 4.31: Overview of percent distribution of salt iodine contents



Overall percent distributions of the salt units meeting the recommended levels of salt iodization are revealed in **Figure 4.31**. It was observed that, initially there were 79.4% of the salt producers who were producing <30 ppm of iodine content. Towards the end, it decreased to 41.2%, which suggested almost 40% improvement in the iodization levels, indicated in **Figure 4.31.**

4.9 INITIATING CONCEPT OF DFS PRODUCTION AT LOCAL LEVEL

Double fortified salt, is the salt fortified with Iodine as well as Iron as essential micronutrients. The concept, production and its establishment at a local level is a long way approach. However, generating awareness and interest of producing DFS amongst the salt producers was an objective of this section.

The Ministry of Health and Family Welfare, Govt. of India, constituted a technical committee with many of the eminent Govt. agencies working towards the public health on "Formulation of guidelines for use of double-fortified salt as a measure to reduce prevalence of anemia". The committee has approved NIN-DFS formula among the different formulation available for double fortification. The strengths depicted were stability and scientific publications on community acceptability, bioavailability, impact on iron-iodine status and last but not the least was the Cost.

After the release of the letter, based on the recommendations of the ICMR Expert Committee, NIN advertised in all leading newspapers and scientific fraternity calling for applications from salt manufacturers for the transfer of NIN-DFS technology during 2007. Based on the announcement, we approached NIN with our motto and they agreed for the transfer. Thus, majority of the purpose was fulfilled. But to initialize and convince the producers to avail the training were the main challenges.

Working towards the same, we identified N=3 salt producers within and around from Bharuch, Gujarat. Of these producers n=2 were large scale and rest n=1(group of 5 producers) was a medium scale salt producer. Thus, understanding the technical requirements, plant upgradation and determination to producer DFS were initiated as the base for the discussion for convincing the producers. The major points discussed were,

- NIN would sign a memorandum of understanding (MoU) with salt manufacturers for the transfer of NIN-DFS technology.
- NIN would not charge for the technology transfer, at a condition that, the manufacturers would supply at least 20% of the produce to the public distribution system (PDS) at prices fixed by the Government for the benefit of people living below the poverty line. [This was also one of our indirect motto by running a supplementation phases, thus it was more emphasized.]
- Cost of DFS would be 1 Rs./kg more than the cost of iodized salt **(Table 4.103)**.

The evidences were explained to the producers and the result of our vigorous efforts earned a success for us in the form of readiness of all the targeted salt producers for undergoing training.

Table 4.103: Approximate cost (in rupees) per kilogram of (NIN-DFS) and iodized salt (IS)

Constituent	Cost per Kilogram (Rs)	
	NIN-DFS	IS
1. Salt	2.00	2.00
2. Chemicals	1.00	0.10
3. Processing	0.40	0.30
4. Packaging material	1.00	1.00
5. Amortization	0.20	0.20
6. Overheads	0.25	0.25
Production cost (1to7= A)	4.85	3.85
7. Profit: (a) Factory	0.40	0.40
(b) Dealer	0.25	0.25
(c) Wholesale	0.20	0.20
(d) Retail	0.15	0.15
Total profit: 7 (a) to 7(d)= B	1.00	1.00
8. Transport : C	1.00	1.00
9. Total cost: A+B+C	6.85	5.85

(Source: S.Ranganathan and B.Sesikeran 2008)

Currently the producers are in a process of availing funding support for advanced infrastructure for DFS production, since refined salt and dried mixing are the two essentialities of the stable DFS formulation. Hence, we expect that, the production units can meet the requirement in the nearing future and then can initiate DFS production; by

taking few more steps ahead from the starting line which we have drawn and made them walk towards the track to successful production of DFS in their units.

DISCUSSION

According to UNICEF global database of the state of the World's Children 2007 indicated that the proportion of households in the developing world consuming adequately iodized salt officially remains at about 70% (UNICEF 2006). The challenges for the status could be lack of program maturation, which some of the countries face but is not visible. Further, data limitation is also one of the confounding factors. This was the time when India was also struggling for the fight against IDD by using salt iodization as a tool (ICCIDD 2008), since the ban on uniodized salt production and consumption has undergone a lot of controversies and finally been reimplemented in May 2006. The national iodized salt consumption rate was 51% reported by NFHS III (2005-2006) survey. In the consequent year (2007), the proportion of iodized salt production had then improved very significantly, since government and Non government sectors joined their hands together to defeat the challenge of inadequate or nil salt iodization at small scale and unregistered salt units.

Data on salt iodization by (UNICEF 2007) revealed that, there were 169 units in and around Anand district. On the contrary the consumption of iodized salt was 52%, which has decreased from the previous year (71%) (State IDD cell, Health and FW 2007).However, health department revealed a higher prevalence of still birth and iodine insufficiency in urinary excretion of the samples availed from 20 PHCs. Hence, when both the data are was compared, the relative risk was reflected to be high. The numbers of salt production units were more but QA and QC procedures were not met by the producers or any other agencies. Thus, the work on upgrading salt iodization was initiated as the partnership approach with MI, Gujarat and UNICEF, Gujarat.

Baseline data on iodization revealed very lower rate of quality production (salt iodine content $\geq$ 30 ppm). However, when we initiated the work for upgradation, the levels started increasing. This was also supported by the data reported by UNICEF 2008, which reflected household iodized salt consumption to be 56% (4% increase) within a span of 3

months. This further reflected the positive impact of our efforts and motivated us to bring the levels ≥70%, which is the indication of iodine sufficiency in this region.

We provided interventions as technical support, review workshops to impart knowledge, increase public health concern and morale boosting of the producers.This eventually showed a positive impact at post interventional stage where the quality iodization levels improved significantly for overall salt plant and it was observed to be more significant for Anand/Nadiyad district.

Thus, it can be revealed, that our efforts brought a desire among the producers to produce quality product thereby saving our coming generation from the impaired mental and physical development. After completing the final data, monitoring and upgradation was handed over to MI, Gujarat, since there was no technical officer for IDD from UNICEF. It is currently being sustained. This indicates a greater level of success of the work carried out, since the level of salt iodization has improved tremendously among our study subjects.

It is also reported that, except iodized salt, the success and coverage of supplements or fortified foods have been limited in developing countries (ACC/SCN 2004). Their limited success might have been due to inadequate supply of supplements, cultural beliefs or the need to adhere to a daily regimen (Galloway et al 2002). Thus, salt has been proven to be the best vehicle for producing and supplying DFS at local level was conceptualized.

The selected producers were also provided with narrations and calculations on the benefits (socially and financially) of DFS production at their units. This was initiated after a thorough strategy work out based on requirement and feasibility of DFS production at local level.

The cost calculation and benefit: cost ratio was also explained to the producers with the success of DFS production. The benefit: cost ratio reported by (Horton S et al 2011) on DFS in India would be 2:1 and 5:1 with 0.25$/person/year. This ratio is comparatively lower than the wheat fortification (9:1) with 0.17 $/person/year (Horton and Ross 2003-2006; Fiedler et al 2008). However, DFS would be widely distributed and accepted in the

regions also where wheat is not used as the only staple food. This ultimately may meet or give higher ratio than the wheat fortification.

Further, in the study after convincing the producers for plant up gradation, undergoing training and technology transfer, now we are into process on deciding towards the period of the training and rest of the formalities for the MoU. We hope that, the program works out in a recent future and we also have full-fledged functioning salt units producing DFS at local level so as to provide the benefits of these essential micronutrients to the most deprived population in their daily diet at a very nominal cost.

CHAPTER 5
SUMMARY AND CONCLUSION

PHASE I
EFFICACY OF DOUBLE FORTIFIED SALT SUPPLEMENTATION AMONGST PREGNANT WOMEN

SUMMARY

Introduction

Globally iron and iodine deficiencies affects one third of the total population. However, the pregnant women and their fetuses are most affected by these deficiencies. Iodine is an essential micronutrient for the synthesis of thyroid hormones, which regulates the metabolic pattern of most cells and plays a vital role in the process of early growth and development of most organs, especially the brain. Iodine deficiency at critical stages of foetal life and early childhood remains single most important and preventable cause of mental retardation globally (WHO/ICCIDD/UNICEF 2007). However, iron plays a vital physiological role in growth, development, metabolic reactions, cofactor for most of the enzymatic activities and cognitive development of the fetus. Untreated chronic/severe maternal IDA may prove to be detrimental to the developing fetus. The prevalence of anemia amongst Indian pregnant women is more than 60% (NFHS-III. 2005-2006).

Methodology

The present research phase was undertaken with the broad objective "To study the impact of double fortified salt supplementation amongst pregnant women"

Sample selection and site of the phase I

A semi-government hospital - having antenatal clinic with higher number of inflow of pregnancy registrations and an accountable delivery rate (250-300/month) throughout the year in the centre of the Vadodara city was selected as a study site. Pregnant women, who were attending antenatal services at the study site were selected and enrolled from the study setting.

This phase was designed as a hospital based longitudinal study including experimental-control samples, where impact of two types of intervention (given throughout gestation) was compared.

1. Double fortified salt supplementation to the experimental group and counseling on consuming iodized salt was provided to the control group
2. Nutrition health education was provided to both the groups.

Prior permission for the study was obtained from the hospital authorities. Written consent from the pregnant women, at the time of registration was availed.

Experimental design of the phase I

This phase was divided into 3 major sections:

Section I: Baseline survey

Data from (N=256) all enrolled pregnant women was collected on their socio-economic status, anthropometry parameters, hemoglobin concentration, urinary iodine concentration and thyroid analytes.

Specific objectives:

- Screening of pregnant women from urban Vadodara for iodine and iron deficiencies
- To assess their nutritional status through anthropometry standards
- To assess their socio-economic status

Total enrolled population (N=256) at the first visit were subsampled N=150 owing to drop out rate due to various reasons such as migration to maternal place or other place, abortions, miscarriages, unwillingness to participate, family disagreement and non-reachability.

Further, baseline data was screened to maintain the homogeneity of the enrolled population and have a representative scenario of the maternal health belonged to LIG in Vadodara city.

The sample size and methods are summarized in **Table 5.1.** Before and after intervention, data of the study groups were collected on nutritional status, hemoglobin concentration, urinary iodine concentration, thyroid analytes, cord blood samples, KAP, dietary intake etc. were assessed and results were recorded.

Pregnant women during first trimester (<15 weeks) of gestation, singleton pregnancy and without visible signs of hypothyroidism were included in the study.

Section II: Interventional strategies

This section comprised of the interventional strategies to be implemented upon our study subjects- pregnant women. These strategies included,

(1) Double fortified salt supplementation amongst experimental group and advocacy for optimally iodized salt consumption amongst control group.
(2) Nutrition Health Education to the pregnant women belonged to both the groups.

Table 5.1: Study indicators and tools for data collection

Sr.No	Indicators	Tool	Sample Size
1	Socio-economic status	Structured questionnaire	256
2	Anthropometry indices	Standard methods	256 (Baseline) 121 (Final)
3	Hemoglobin Estimation	Cynmet-hemoglobin method	256 (Baseline) 121 (Final)
4	Urinary iodine excretion	Sandell-kolthoff reaction(Modified microplate technique)	256 (Baseline) 121 (Final)
5	DFS content estimation	BIS standards	3
6	Thyroid hormones-TSH, FT_4,TT_4,Tg	RIA technique	256(Baseline) 121 (Final)
7	Cord blood Analysis-TSH, FT_4,TT_4,Tg	RIA technique	64
8	Dietary intake- 24 hr. dietary recall and FFQ	Structured questionnaire	121 (Baseline and Final)
9	Knowledge, attitude and practices	Semi-structured questionnaire	121 (Baseline and Final)

The specific objectives of this section were,

- To Supplement DFS to the experimental group and counsel control group to consume optimally iodized salt
- Monitoring salt iodine content of the household samples of pregnant women belonged to control group
- Provide nutrition health education regarding iodine and iron nutrition, best practices for storage and usage of DFS/ iodized salt to the pregnant women belonged to both the groups
- To collect blood samples for Hb estimation, urine samples for urinary iodine concentration and serum samples to analyze thyroid hormones at the end of each trimester
- To monitor the compliance of DFS consumption and provide counseling whenever problem in the usage aroused.

Out of (N=150) sub grouped subjects, experimental and control group were formed by incorporating n=75 pregnant women to each group randomly. With the progression of gestation and our study phase, 121 pregnant women made the final number of the subjects. Of which N=67 from experimental and N=54 from control group completed the study.

Section III: Impact assessment

This section was formed for assessment of DFS supplementation and non supplementation amongst pregnant women of both the groups using various indicators for iodine, iron and nutritional status. Impact on pregnancy outcome and neonatal parameters were also included in this section to assess the extent of efficacy of DFS giving health benefits.

There was also an assessment on the impact of NHE on knowledge, attitude and practices of the subjects, their dietary habits (quantity and quality) and frequency as well.

The specific objectives of this section were:

- To study the impact of DFS supplementation and non supplementation on various indicators :

- ✓ on nutritional status
- ✓ on the prevalence of anemia
- ✓ on prevalence of iodine deficiency disorders and iodine status
- ✓ on pregnancy outcome
- ✓ on neonatal anthropometry parameters
- ✓ on neonatal thyroid analytes

- To compare the relative impact of NHE on
  - ✓ 24 hr. dietary recall
  - ✓ FFQ
  - ✓ KAP

Post intervention data was collected with regards to change in hemoglobin concentration, urinary iodine excretion, thyroid analytes, nutritional status, dietary intake, KAP, neonatal anthropometry and neonatal thyroid analytes.

Data Analysis

Mean, median and standard deviations were calculated for maternal height, weight, age, BMI, hemoglobin concentration, urinary iodine concentration, thyroid analytes, dietary intake and neonatal height, weight, head circumference, gestational age, Z scores (WHO growth standards)and thyroid analytes. 95% CI and percentiles (95^{th} and 5^{th}) were revealed wherever required. Correlation coefficients used for assessing correlation between different indicators. Paired‘t’ test, chi square and independent ‘t’ test were used to assess significant difference within and between groups. Minimum level of significance was kept at $p<0.05$. Post hoc analyses were carried out whenever further analytes on data reevaluation was required.

MAJOR FINDINGS OF PHASE I

5.1 BASELINE SURVEY

At baseline, a survey on assessment of general characteristics, nutritional status, iodine and iron status of the pregnant women enrolled was carried out.

- Forty four percent of the subjects were primiparous and rests of them were multiparous. All the subjects were enrolled during first trimester. Majority were enrolled towards the end of first trimester (68.53%)
- Thirty five percent of the subjects were underweight at the time of registration and half of the subjects were in the normal category of BMI.
- There were 90% subjects with iron deficiency anemia at the time of registration and majority were moderately (62.1%) anemic. Mean hemoglobin concentration was 9.26 g/dl depicting the population as moderately anemic.
- The median urinary iodine excretion (297.14 µg/L) showed that population was iodine sufficient at the time of enrollment in all the 3 months of first trimester. However, there were 16.79% of the subjects, who had insufficient UIE (<150 µg/L).
- Mean TSH, FT_4 and TT_4 were observed to be in normal range for the population. A reciprocal pattern ($r= -0.178$, $p<0.01$) between TSH and FT_4 levels was observed as normal pregnancy induced physiological fluctuations.
- Inter relation between anthropometry parameters and hemoglobin concentration revealed significant correlation (height= $p<0.05$ and weight-BMI= $p<0.01$). However, UIE did not give any correlation with any of the parameters.
- Month of gestation during first trimester correlated negatively ($r= -0.248$, $p<0.01$) with FT_4 levels.
- More than 70% of the subjects were consuming optimally iodized salt (iodine content $\geq$ 15 ppm).
- Sub grouped population (N=150) also revealed a more or less similar set of results with regards to above indicators and represented the enrolled population.

5.2 INTERVENTIONAL STRATEGIES AND MONITORING

5.2.1 Distribution of the population into groups

- The sub grouped population was divided equally into experimental (n=75) and control (n=75) groups randomly.
- The experimental group was subjected to DFS supplementation and control group was advocated to consume adequately iodized salt.

5.2.2 DFS as a supplement

- Double fortified salt was used as a supplement for the pregnant women, who were grouped into experimental subjects.
- Before supplementation, iodine and iron content of DFS were assessed using titrimetric method (BIS standards). Mean iodine content was 40 ppm (recommended 30 ppm) and iron content was 1050 ppm (recommended 1000 ppm) (Recommended by BIS 1999, GOI/WHO 2004).
- On initiation and during supplementation process, experimental group was provided ample advocacy regarding the usage and storage of DFS towards increasing acceptability and stability of the contents.
- DFS was very widely accepted by the subjects (95.5%) and their family members (82.1%). Initially 70.1% of the subjects observed colour change in the food preparations after cooking, but the percentages could be reduced by providing counseling on the issue.

5.2.3 Nutrition Health Education as a tool

- NHE was provided to both the groups regarding iodine and iron nutrition, storage and usage in cooking practices for DFS/iodized salt to avail optimum iodine from the salt.
- Control group was counseled for iodized salt and iodine rich foods consumption to have a comparable iodine intake to the experimental group.

5.3 IMPACT ASSESSMENT ON THE PARAMETERS

5.3.1 Impact on nutritional status

- There was an improvement in nutritional status of the subjects compared to the stage of registration. This improvement was reported in terms of BMI (kg/m^2). There was a significant ($p<0.001$) gradual reduction in the percentage of underweight subjects with the progression of gestation. However, there was no significant difference observed in BMI distribution of both the groups.
- With the observed change in percent distribution in BMI categories, there was also a significant difference observed in mean BMI within groups but no difference was

observed between both the groups. There was a similar effect of NHE observed in both the groups.

5.3.2 Impact on iron status

- All the subjects were classified according to WHO recommendation on classification for anemia, 2001 and Shobeiri et al 2006 with trimester specific cutoffs during pregnancy. Based on their percent distribution during first trimester results, 90% of the subjects were anemic in experimental and 85.2% in control group.
- At the end of gestation, 1.5% increase in proportion of the normal subjects (nonanemic- from mild to normal) of experimental group and 11.1% reduction in normal subjects of control group was reported. However, both the groups were on IFA supplements, DFS showed stability in the hemoglobin levels of the non anemic subjects but still there was a remarkable difference in both the groups for normal category.
- At the end 50% and 25% of the subjects in remained mildly anemic in experimental and control group respectively. However, the positive shift in the experimental group and negative shift in the control group was observed in the classification of anemic subjects.
- During second trimester there was non significant reduction ($p>0.05$) in Hb concentration of experimental group and significant reduction ($p<0.05$) in Hb concentration of control group.
- During third trimester, the Hb concentration improved significantly in experimental group compared to both the trimesters ($p<0.01$). However, non significant improvement in control group was observed.
- There was a significant improvement in mean hemoglobin concentration amongst experimental group compared to control group ($p<0.05$).
- This in turn suggests the beneficial effect of DFS as a dietary source of iron compared to experimental group.

5.3.3 Impact on iodine status

All the subjects were classified according to the WHO, ICCIDD,UNICEF recommended classification for iodine deficiency disorders, 2007 during pregnancy. Based on their percent distribution during first trimester results 14% of the subjects were iodine deficient and the percentage remained same till the end. There were 16.4% of the subjects in experimental group and 11.1% subjects in control group as iodine insufficient based on the urinary iodine levels during first trimester.

- Median urinary iodine levels were non significantly different on comparing both the groups. Significant increase in median urinary iodine of experimental group during second trimester was observed ($p<0.05$). On comparing with first trimester urinary iodine levels decreased significantly towards the end ($p<0.05$).
- However, urinary iodine levels decreased gradually in control group. The difference was non significant between first and second trimester and it was significant between first and third trimester ($p<0.05$).
- It can be stated that there was no significant difference between both the groups using urinary iodine as an indicator, which could be achieved, since majority of the subjects were on optimal iodine intake. This in turn reveals a successful functioning of salt iodization programme and impact of NHE to meet the optimal iodine nutrition in non supplemented group compared to DFS group.

5.3.4 Impact on thyroid analytes

- Mean serum TSH and FT_4 did not vary significantly throughout gestation. However, within group variation for FT_4 was observed among the subjects belonged to control group ($p<0.05$).
- Mean TT_4 level improved significantly in both the groups during third trimester ($p<0.001$), which is a physiologically driven condition and DFS did not play a significant role to bring an influence on hormone levels.
- Based on the urinary iodine deficiency and sufficiency classification, thyroid analytes were assessed for both the groups. It was observed that, the subjects with iodine deficiency based on first trimester UIE levels showed higher levels of TSH and lower

levels of FT_4 compared to iodine sufficient subjects. This difference was observed throughout gestation.

- Baseline assessment of thyroid hormones recommended by "The Endocrine Society" 2007 cutoffs for TSH; recommendations provided by commercial kits for FT_4 and TT_4 , the subjects were classified with SCH (17.91%-experimental and 27.77%-control group), OH (11.29%- experimental and 9.26%-control group), Hypothyroxinemia (40%- experimental and control) and Euthyroidism (36.36% - experimental and control).

5.3.5 Impact assessment with pregnancy outcome

- There was no significant difference on neonatal parameters between both the groups was observed. It was observed that, none of the maternal parameters correlated with neonatal parameters.
- There were 20.48% of the neonates, who were born with low birth weight (<2.5 kg) irrespective of groups for both the groups and 13.25% of the neonates born premature (<37 weeks of gestation).
- Maternal hemoglobin at 3^{rd} trimester correlated significantly with neonatal birth weight ($p<0.05$).This in turn suggests the importance of normal hemoglobin concentration during pregnancy.
- Neonatal TSH showed a significant correlation with LBW. Odd's ratio and 95% CI limits were (3.55 : 0.97,13.44). Relative risk of LBW was higher in neonates with cord blood TSH >10 µIU/ml or vice versa.
- Using WHO growth standards, nutritional status of the neonates was assessed. It revealed that, 27.71% of the subjects were moderately and 19.28% were severely stunted. Moderate underweight was observed in 10.854% and 7.22% were falling in severely wasted class.

5.3.6 Impact assessment with dietary intake

- The mean energy, protein, CHO and iron consumptions were comparatively lower in both the groups with deficit percent as 49.42%, 62.31%, 10.45% and 84.42%

respectively for experimental group. However, these deficit percentages were 52%, 64.34%, 14.07% and 85.97% respectively for control group.

- After imparting NHE as an interventional strategy, data on mean intake of energy, protein, CHO and iron was recollected during third trimester and a significant difference was observed for each nutrient in both the groups.
- The differences for energy, protein and CHO were approximately 10-11%, 10-12%, 20% respectively for both the groups. However, iron intake improved significantly in both the groups ($p<0.001$-experimental and $p<0.01$-control). Iron intake improved significantly ($p<0.01$) in experimental group compared to control group due to DFS consumption also.
- Overall food frequency did not show any significant change in both the groups. However, change in the consumption frequency of the recommended iron and Vitamin C rich foods was observed amongst both the groups as a success of NHE.

5.3.7 Impact on KAP

- There was a significant improvement on KAP of the subjects, which was observed in both the groups. An improvement in knowledge about iodine, iodized salt, storage and usage practices for DFS/iodized salt was observed after NHE.
- There was almost 40% increase in awareness regarding sources of iodine amongst experimental and control groups. Increase in knowledge regarding individual sources in both the groups ranged from 10-40%.
- In both the groups there were 70% of the subjects, who started recognizing iodized salt from it's "Smiling sun logo" and 30% of the subjects with its label. Thus, 100% of the subjects became aware of the identification of the iodized salt from the packet.
- Almost 55-66% of the subjects started following best practice to maintain iodine in the preparation and majority of the subjects were using best practices of the storage of DFS/iodized salt.
- Majority of the subjects attained knowledge on consequences of IDD on physical and mental development of the growing fetuses and children.
- More than 85% of subjects could respond on various sources of iron and almost 80% were aware about specific consequences of iron deficiency anemia on maternal and fetal health.

- Compliance to IFA and frequency of consumption could be improved by 30% amongst control group.
- Increased impact on food intake (quantity) also was observed with 9% in experimental group and 35% in control group.
- There was a significant reduction in food taboos and majority of the food items which were restricted due to blind believes, hence the consumption of these foods was initiated.

  Thus, it could be stated that, providing NHE or parallel counseling, helps in improving knowledge levels of the populations to attain betterment in nutrition and health status.

CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE I

To conclude the present phase, validation and usefulness of double fortified salt for pregnant women and emphasizes the need for including DFS into life cycle approach. It is more effective when used with other supportive strategies such as improvised nutrition intakes based on nutrition health education/counselling.

The results of the study, viewed collectively, lead to the rejection of the hypothesis stated at the onset of the study (Described in methodology), which were as follows:

1. There is no significant impact of DFS supplementation (compared to control) on iron and iodine status.
2. There is no significant impact of NHE on nutrient intake and KAP of the subjects

Contrary to the hypothesis, the experimental group was highly benefited from DFS supplementation:

- Experimental group had an improvement in iron status and iodine status.
- The study population was iodine sufficient but neonatal parameters showed moderate iodine deficiency, so efforts should be targeted in maintaining iodine sufficiency.

- The hemoglobin status of the population calls for an immediate action to be improved since most of the subjects are anemic and hence, the prevalence of LBW is very high (20%).
- Among pregnant women from lower strata of the community, whether supplemented with IFA tablets or not, should be supplemented with DFS throughout gestation and even before and after gestation to have a sustained liberation of iron from the salt in the gut.
- Prevalence of thyroid dysfunction as SCH, OH and hypothyroxinemia is comparatively higher than the reviewed literature. There is a need for careful monitoring of the subjects and if necessary they should be put on hormone therapy, till they achieve normal levels.
- All pregnant women need to be screened for iodine deficiency and thyroid dysfunction as soon as the pregnancy is confirmed.
- Pregnant women and their concerned family members (whenever required) should be made aware about the importance of iodine and iron nutrition and their consequences resulted by their deficiencies during pregnancy or prepregnancy stage.
- Multiple approaches should be used to combat micronutrient deficiencies as an initiative and support strategies from all stake holders and the government for a permanent solution.

PHASE II
IMPACT ASSESSMENT OF DOUBLE FORTIFIED SALT SUPPLEMENTATION AMONGST SCHOOL CHILDREN

SUMMARY

Introduction

Evidence based studies have reported that, **physical growth** and **cognitive development** in children are faster during early years of life, and that by the age of four years, 50% of the adult intellectual capacity has been attained and before thirteen years, 92% of adult intellectual capacity is attained (Vernon 1976). However, it is affected significantly due to micronutrient deficiencies, especially iron and iodine.

Thus, to combat these deficiencies NIN has ventured with a stable formula as Double fortified salt (DFS) to meet the major part of the RDA of iron and iodine requirements of the children during their growth spurt and pubertal stages. This phase is an effort to achieve the same amongst children belonged to endemic region for iron and iodine deficiencies.

Methodology

The present research phase was undertaken with the broad objective "To study the impact of double fortified salt supplementation amongst school children"

Sample selection and site of phase II

Waghodia, a rural block of Vadodara district was considered as a study site. According to State Nutrition Cell 2008-2009, Waghodia has 12.6% goiter prevalence, depicting it as an endemic region. Out of 172 primary government schools in the block, 4 schools were randomly selected on the same belt. Based on the percent availability of iodized salt, the schools were distributed into experimental (n=2) and control groups (n=2). All the school children from the standard 1^{st} to 6^{th} (5-15 years) made the final sample size N=1184, where N=947 could complete the study.

This phase was designed as an interventional longitudinal experimental-control study, where impact of DFS supplementation was compared and assessed for the initial and post interventional difference.

1. DFS supplementation to the experimental group was carried out. Counseling was provided to consume optimally iodized salt to the control group.
2. NHE and BCC to the children and their mother/caretakers was provided on iodine and iron nutrition, consequences of the deficiencies, storage and cooking practices using iodized salt and DFS.

Informed consent for the study was obtained from education officials, school principals and parents of the children. However, children were also explained the purpose of the study.

Experimental design of the Phase II

This phase was divided into 3 major sections.

Section I: Baseline survey

Data from (N=1184) children belonged to four purposively selected schools was collected on their anthropometry indices, hemoglobin concentration, urinary iodine excretion, thyroid analytes and IQ/cognitive scores.

Specific Objectives:

- To map the availability of iodized salt in the study area
- To map the prevalence of iodine deficiency and iron deficiency amongst school children from the selected schools.
- To assess baseline nutritional status through anthropometry indices.
- To assess baseline IQ and cognition scores of the study population.

Total N=1184 children were enrolled from the four schools of Waghodia block at baseline. Later the final sample size reached to N=947, since the rate of absenteeism was very high (30%) amongst rural school children. The exclusion criteria included the unavailability of the same child for 3 consecutive visits during supplementation period,

non reachability of the child or unwillingness of the children/parents to be a part of the study. The sample size and methods are summarized in **Table 5.2.**

Table 5.2: Study indicators and tools for data collection

S.No.	Indicators	Tools	Samples Size
1.	Socio-Economic Status	Structured Questionnaire	212 (Subsample)
2.	Anthropometry	Standard methods and tools	1184 (Baseline) 947(Final)
3.	Hemoglobin Estimation	Cynmet-hemoglobin method	972 (Baseline) 947 (Final)
4.	Urinary iodine excretion	Sandell-kolthoff reaction (Modified microplate technique)	1034(Baseline) 947 (Final)
5.	DFS content estimation	BIS standards	3
6.	Thyroid hormones-TSH, FT_4,TT_4,Tg	RIA technique	212 (Baseline) 189 (Final)
7.	Dietary intake- 24 hr. dietary recall and FFQ	Structured questionnaire	212(Baseline) 212 (Final)
8.	Knowledge, attitude and practices	Semi-structured questionnaire	212 (Baseline) 212 (Final)
9.	Household salt samples	Spot testing kit	302
10.	IQ and Cognition Tests- Draw-a-man, Visual Memory test, Clerical Test	Standardized methods	823-864 (Baseline) 700 (Final)

Before and after intervention, data of the study groups were collected on nutritional status, hemoglobin concentration, urinary iodine concentration, thyroid analytes, IQ and cognition scores, dietary intake and parental SES-KAP etc were assessed and the results were recorded.

Section II: Interventional strategies

This section comprised of the interventional strategies to be implemented on the study population-school children. These strategies included,

(1) Double fortified salt supplementation amongst experimental group and advocacy for optimally iodized salt consumption amongst control group.

(2) Deworming among half of the children belonged to both groups, which lead to final number of study groups to be four:
    - Experimental and dewormed group (E+DW)
    - Experimental group (E)
    - Control and dewormed group (C+DW)
    - Control group (C)
(3) Nutrition health education was provided to the mothers of the children belonged to all the groups, regarding iodine and iron nutrition.

Specific objectives:

- To classify study population into experimental and control based on the availability of iodized salt in the village (household and retail shop samples).
- To administer deworming tablets amongst both the groups, subdivision into 4 groups.
- To collect information on SES and KAP on the subsample.

Out of N=4 schools selected for the study phase, 2 schools to experimental and control group each, who were divided based on the availability of iodized salt. In control group, there were almost all population were consuming iodized salt (>15 ppm). However, the experimental group showed below 70% consumption of iodized salt. This observed difference in both the groups could have been the result of the distance from the city area. Since schools included in experimental group were 5-10 kms more into the deep of rural area than the control group school from the border of urban Vadodara.

Section III: Impact assessment

This section was comprised of impact assessment of DFS supplementation and non supplementation amongst school children, along with and without deworming. The indicators included iodine and iron status, nutritional status and IQ/cognition tests.

There was also an assessment on the impact of NHE and BCC on the knowledge, attitude and practices of the mothers/caretakers of the children, dietary intake (quantity and quality) of the children and frequency as well.

The specific objectives of this section were:

- To study the impact of DFS supplementation and non supplementation on various indicators:
  - ✓ On nutritional status
  - ✓ On the prevalence of anemia
  - ✓ On the prevalence of iodine deficiency
  - ✓ On IQ/cognitive parameters
- To compare the relative impact of NHE on,
  - ✓ 24 hours dietary recall
  - ✓ FFQ
  - ✓ KAP (parental)

Post intervention data with regards to change in hemoglobin concentration, urinary iodine excretion, thyroid analytes, nutritional status, dietary intake, IQ/cognitive parameters, KAP of the mothers etc. was collected.

Data analysis

Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square (χ^2) when appropriate for categorical data. Normal distribution of the data was assessed by the Kolmogorov-Smirnov test. Where indicated, the data was normalized using log transformation to facilitate the use of normal-theory analytic methods. Nonparametric (Mann-Whitney U test, Kruskal-Wallis test and Wilcoxan rank test) or parametric (Student's t-test, paired t test and ANOVA) statistical tests, depending on the normality of the data, were used to detect within-group and between group differences. Further post-hoc Bonferroni analysis was carried out. To determine associations between analytes Pearson's correlation or Spearman's rank correlation were calculated. A two-tailed p values <0.05 was considered statistically significant.

MAJOR FINDINGS OF PHASE II

5.4 BASELINE SURVEY

At baseline, a survey on assessment of anthropometry indices, iodine and iron status, IQ and cognitive scores of the school children was carried out.

- Baseline data on anthropometry revealed mean height and weight of the children were 122.80±12.75 cm and 21.21±6.20 kg. Data on malnutrition based on CDC standards revealed 44.60% stunting, 70.78% underweight and 54.16% thinness in the study population. There was a significant difference for WAZ score observed between both the genders.
- Mean hemoglobin concentration of the population was observed to be 9.17±1.23 g/dl, indicating population into moderately anemic category. However, gender wise significant difference was observed with hemoglobin concentration at 9.27 g/dl and 9.04 g/dl amongst boys and girls respectively.
- According to WHO classification, overall prevalence of anemia was 98%, with majority of the children to be moderately anemic (70%) and 24% with mild anemia.
- Median UIE of the population was 146.33 µg/L, indicating the population to be iodine sufficient. There was no significant difference observed between both the genders.
- However, there were 30% of the children who were iodine insufficient, including 18.2% mild and 8.5% moderately deficient. There were 3% of the children with severe deficiency also. The classification followed was recommended by WHO/ICCIDD/UNICEF, 2007.
- Based on thyroid analytes assessment, normal range for TSH, FT_4 and TT_4 were observed amongst the study population. Incidence of subclinical hypothyroidism was observed amongst 2.4% children.
- Analysis of IQ and cognition test results revealed that, the population was below average IQ scores (Draw- a man test) using standard classification recommended by Phatak P (2002) and Wechsler's scale. However, mean score for Visual memory test (VMT) was 0.40 and 0.48 amongst boys and girls respectively, where as it was 0.72 and 0.76 for Clerical test (CT) for both the gender respectively.

- VMT scores varied significantly based on the presence of iron deficiency anemia where it was observed that, the anemic population (moderate-severe) scored lower than the normal-mild anemic). When the results were compared based on the gender wise category, higher scores were observed among girls compared to boys. Similar pattern was also observed for the presence of iodine deficiency.
- Nutritional status classification (deficient and normal) of the children cross tabulated mean hemoglobin concentration showed a significant difference for HAZ and WAZ. However, the difference was non significant for iodine deficiency. This indicates hemoglobin concentration varies with the nutritional status, where as UIE does not depends upon the Z scores.

5.5 INTERVENTIONAL STRATEGIES

5.5.1 Distribution of the population into study groups

- The study population was divided into two groups, as experimental and control for double fortified supplementation as a tool. These groups were further subdivided as dewormed and non dewormed groups, resulting in two subgroups into each study group. This was done to assess the impact of DFS with deworming compared to deworming alone. Hence, final number of groups was four: E+DW, E, C+DW and C groups.

5.5.2 Supplementation

- Before supplementation, iodine and iron content of DFS were assessed using titrimetric method (BIS standards). Mean iodine content was 40 ppm and iron content was 1050 ppm.
- On initiation of DFS supplementation amongst experimental groups (E+DW and E), control groups (C+DW and C) were recommended to consume optimally iodized salt. The supplementation continued for 9 months.
- SES of the study groups was collected, data revealed that, majority of the children were Hindus, with majority belonged to joint families. Parental literacy rate revealed that, majority of the mothers of the children were illiterate or educated till primary

school. However, fathers of the children were educated till primary or secondary school.

- Majority of the mothers were housewives, one fifth of the percent fathers of the children were doing Jobs and half of the total were on labor contracts, classifying majority of the families into lower income groups.
- Based on the data on KAP and dietary intake, NHE to all the groups was provided on iodine and iron nutrition, their importance in their children's health, cooking and dietary modification for optimal gain of the nutrients, consequences of these micronutrient deficiencies, consumption of iodine and iron rich foods etc were provided throughout the study period.

5.6 IMPACT ASSESSMENT ON THE INDICATORS

5.6.1 Impact on nutritional status

- Impact on nutritional status was observed among all the study groups. Since there was almost 3.4 kg weight gain within 1 year in the children. However, height increased to 6.3 cm including all the study groups. These increases were significant in all the study groups ($p<0.001$).

- However, there was significant difference between groups for difference in height, which varied significantly between E+DW and rest of the groups, indicating significant impact of DFS on height gain of the children compared to their counterparts in rest of the groups. This resulted due to highest increased height in E+DW compared to rest of the groups. However, the increase in weight varied significantly between C+DW and rest of the groups, since C+DW showed lowest weight gain. The increased weight and height could have been due to the growth spurt of the children and slightly favored by iron source from DFS. Overall prevalence of malnutrition (lower HAZ, WAZ and BMZ) decreased significantly with growth spurt of the children.

5.6.2 Impact on micronutrient status

5.6.2.1 Impact on iron status

- Mean hemoglobin concentration of experimental group (+0.42 g/dl) increased significantly ($p<0.001$) compared to decrease (-0.54 g/dl) in control group. At baseline

there was a significant difference between both the groups ($p<0.001$), however the difference has remained non significant towards the end.

- When mean hemoglobin improvement was compared for all 4 study groups, a significant ($p<0.001$) improvement among E+DW group was observed with 0.60 ±1.09 g/dl was observed and also among E group with 0.21 ± 1.04 g/dl at level of significance with ($p<0.01$).
- On the contrary, there was a significant reduction in hemoglobin concentration of both control groups (C+DW and C) with mean -0.5 g/dl.
- Prevalence of mildly anemic subjects increased in experimental groups with the positive shift of moderately anemic children in the category. However, control groups showed negative shift with increased percentage of moderately anemic children.
- Total prevalence of anemia decreased by 6.3% in E+DW group, where as it increased by 1.5%, 22.5% and 21.0% in E, C+DW and C groups respectively.

5.6.2.2 Impact on iodine status

- Median UIE of the both the groups was above 100 µg/L, which is a cutoff for classifying the population into iodine sufficient category at baseline. This was reflected by UIE at 132.13 µg/L and 158.18 µg/L for experimental and control group respectively.
- Experimental group were supplemented with DFS, which replaced the iodized salt in the area, where as consumption of optimally iodized salt was emphasized for the children belonged to control group. Further, post intervention data on UIE revealed median levels to be 177.02 µg/L and 238.22 µg/L for experimental and control groups respectively. The improvement was observed to be significant at higher level ($p<0.001$) for both the groups.
- Proportion of iodine sufficiency increased for all groups with significant reduction ($p<0.001$) in iodine insufficiency for all four groups. Percent reduction of iodine insufficiency was observed to be 24.3%, 20.3%, 20.6% and 13.8% for E+DW, E, C+DW and C groups respectively.
- This ended with improved percentages of normal children in E+DW, E, C+DW and C group with 84.9%, 88.2%, 93.5% and 91.8% respectively. This suggests the efficacy

of DFS in improving UIE similar to optimally iodized salt and reflects the active supply of iodine into the system.

5.6.2.3 Impact on thyroid analytes

- At baseline and towards end median TSH levels were observed to be within normal range for all study groups. They also differed non significantly throughout the study period between all groups. Within group comparison for median TSH levels revealed that, towards the end E+DW ($p<0.001$) and E ($p<0.01$) groups showed significant reduction in TSH values compared to their baseline values. However, control groups did not show any significant reduction at significant level.
- Median FT_4 values varied significantly between groups at baseline and at the end. There was also a significant reduction in FT_4 value in each group compared to their baseline values.
- This in turn suggested non significant impact of DFS on thyroid analytes compared to control groups.

5.6.3 Impact on IQ and cognition

- Data on IQ and cognitive scores revealed that, DMT scores improved ($p<0.001$) significantly amongst E+DW group compared to rest of the groups, indicating better impact of iodine and iron through DFS on IQ of children than iodine only.
- Results on VMT also revealed improved scores for VMT in experimental (E and E+DW) groups with higher level of significance ($p<0.001$). However, the improvement was significant in C+DW group ($p<0.001$), where as non significant in C group.
- CT scores improved significantly in all the groups, indicating impact of NHE and improved diet on the concentration of the children, other than the familiarity factor. However, the improvement was observed to be significantly high ($p<0.01$) amongst E+DW group compared to rest of the group, indicating the role played by iron supplies by DFS along with deworming.
- Overall experimental group dominated the improved scores for all three tests.

5.6.4 Interrelation between parameters

- Interrelation between parameters revealed that, there was a significant difference between all four groups for VMT scores ($p<0.01$) when compared for mean change in hemoglobin 0.01->1 g/dl and also for DMT scores ($p<0.05$). However CT did not show any significant difference between the groups. This in turn suggests that, DMT and VMT scores are influenced by improved iron nutrition. However, CT is influenced by overall nutrition.
- When all groups were compared based on variation in UIE by raise or decrease in median UIE by 100µg/L, there was no difference observed for all the tests which were dependent on UIE.

5.6.5 Impact of supplementation, NHE and BCC on the KAP and dietary intake

- Based on the data on mean dietary intake of school children before and after NHE revealed a significant improvement amongst all age groups of the children compared to their RDA for energy, protein and fat.
- Improvement in mean dietary intake of iron was resulted due to NHE in control group, where as NHE+DFS intake brought highly significant improvement ($p<0.01$) in the intake of children belonged to experimental groups.
- When the dietary intake of children was compared for before and after intervention with median percent RDA, it was observed that, there was a significant improvement in macronutrients like energy, protein and fat. However, the increase with regards to iron was highly significant ($p<0.001$) for experimental group, where as non significant for control groups.
- Percent distribution of RDA intake into 4 categories- <25%, 26-50%, 50-75% and >75%, revealed a significant improvement in the percentages of two later categories compared to baseline, indicating positive impact of NHE along with supplementation.
- After collecting data on FFQ at baseline, which was observed to be poor with respect to iron, iodine, Vitamin C rich and protein rich foods; post intervention data was collected only on the recommended foods.

- This reflected a significant improvement in frequency of the recommended foods compared to baseline. This in turn indicated by the increased frequency for the consumption of iron rich, Vitamin C rich, protein and iodine rich foods.
- There has also been a remarkable impact on the knowledge, attitude and practices of the mothers of the children upon targeted topics like iodine and iron nutrition, their importance, the storage and cooking practices for iodized salt/DFS for optimum gain of the nutrients, modified dietary practices and most cost effective modes to improve the nutritional status of their children.
- Last but not the least, the acceptability of DFS was extremely high, though initially there were certain issues related to colour change, which could be sorted out and the usage of DFS could be assured for the children and their families.

CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE II

To conclude the present phase, validation and usefulness of double fortified salt for school aged children and adolescents which emphasize the need for consuming DFS by one and all. It has been observed to be more effective when used with other supportive strategies such as improvised micronutrient intake (iron and iodine) using DFS, improved nutrition intakes based on nutrition health education/counseling and deworming, since the children are more prone to worm infestation during their growth years.

The results of the study, viewed collectively, lead to the rejection of the hypothesis stated at base,

3. There is no significant impact of DFS supplementation (compared to control) on iron and iodine status.
4. There is no significant role of deworming in improving the efficacy of DFS (compared to controls).
5. There is no significant impact of DFS consumption on IQ/cognition test scores of the children (compared to controls).
6. There is no significant impact of NHE on nutrient intake and KAP of the subjects

Contrary to the hypothesis, the experimental group was benefited from DFS supplementation:

- Experimental group had an improvement in iron status and iodine status.
- The study population was iodine insufficient, so efforts should be targeted in achieving iodine sufficiency.
- The hemoglobin status of the population calls for an immediate action to be improved since most of the children are anemic >90%.
- Among children from rural settings, there are limited dietary sources for iron due to limited purchase power of the families and ignorance as major causes. Hence, DFS should be included in the daily diet of the families to meet partial RDA for iron and complete RDA for iodine at an economic cost and to have a sustained liberation of iron from the salt in the gut.
- Prevalence of urinary iodine insufficiency is higher (>20%) and prevalence of goitre is 12.87%. Hence, there is a need for careful monitoring of the subjects and consumption of optimally iodine fortified salt (DFS).
- Parents/ concerned family members (whenever required) should be made aware about the importance of iodine and iron nutrition and their future consequences resulted by their deficiencies during growth spurt/adolescence.
- As suggested in the phase I, it is advisable to use multiple approaches to combat micronutrient deficiencies as an initiative and support strategies from all stake holders and the government for a permanent solution.

PHASE III
UPGRADING SALT IODIZATION AT LOCAL LEVEL AND FEASIBILITY FOR DOUBLE FORTIFIED SALT PRODUCTION AT LOCAL LEVEL

SUMMARY

Introduction

Globally salt iodization has been proven to be one of the most renowned, workable strategy to combat iodine deficiency disorders with all its controversies. However, many of the countries have achieved the goal of reaching >90% iodization and some are still striving to achieve the same. India is one of these countries, though we had a tremendous economic growth from last decade, we have reached 71% in the year 2010 towards processing of Iodizing all edible salt respite the strict regulatory mandates from the government. Considering India's political upheavals', this improvement is a 20% increase from 51% reported in the year 2006.

Micronutrient malnutrition, especially iodine and iron are detrimental to the health of pregnant women and children. Therefore, it is essential to produce fortified food products which can be consumed by one and all at daily basis. Salt has been chosen to be the vehicle meeting the requirement most efficiently compared to rest of the vehicles among dietary diversified country like India. Thus, it becomes a responsibility of the salt producers to make these micronutrient reach at one go to the population striving these micronutrients.

This phase is an effort to take a step ahead towards this direction by motivating salt producers to produce DFS at local level and thereby increasing the availability and cost of DFS for a common man.

Methodology

The present research phase was undertaken with the broad objective "To upgrade the iodization among small scale salt producers and workout feasibility for DFS production".

Sample selection and site of the phase III

Higher prevalence of still birth and lower UIE excretion in pregnant women attending 20 PHC's from Anand district contributed to the conceptualization of this phase. On initiating a partnership work with MI, Gujarat and UNICEF, Gujarat on upgradation of salt iodization program, N=38 producers from Anand, Nadiyad, Vadodara, Bharuch and Kheda made our study samples.

Experimental design of the Phase II

This phase was divided into 3 major sections.

Section I: Baseline salt iodization

At baseline mapping and salt sample collection was carried out from N=38 salt production units from Anand, Nadiyad, Vadodara,Bharuch and Kheda.

Specific objectives:

- To map the small scale salt units and assess the salt iodization levels in Anand, Nadiyad, Vadodara, Bharuch and Kheda districts of Gujarat.

Section II: Interventions and monitoring of iodization levels

This section included the N=34, owing to drop outs of the salt producers due to various reasons. The producers were provided with technical support and public health concern ethically by using different strategies like one to one communication at plants and conducting training-review workshops as and when required.

Specific objectives:

- To build up the capacity of small scale salt producers of these districts for salt Iodization.
- To provide technical support for optimal iodization.

Post intervention data was collected on upgraded salt iodization levels in the produced salt in the production units.

Section III: Initiating concept of DFS production at local level

This section included N=3 of the selected medium and large scale salt producers. They were communicated on the role of DFS, need for technicalities, benefit: cost ratio at producer and consumer level.

- Selecting salt producers for initiation of double fortified salt at medium scale and large scale.
- To initiate the conversation on feasibility for DFS production at local level.

Feasibility trials are on progress to manufacture DFS at local levels. Three salt producers are on the move to undergo training from NIN. Initial processing and cost effectiveness of the programme have been discussed with them. Further, their convenience is awaited

Data analysis Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square (χ^2) when appropriate for categorical data. The groups were compared using 'F' test.

MAJOR FINDINGS OF PHASE III

- At baseline, 63.20% of the salt producers were from Anand/Nadiyad and rest were from Vadodara/Bharuch and Kheda.
- Majority of the producers were producing ≥5 tonnes salt/month, except 13.2% below 5 tonnes/month. Thus, the concern was more to be focused since majority of the population was consuming inadequately iodized salt.
- Baseline data revealed that, none of the districts had mean iodine content of the salt on the recommended level (≥30 ppm).
- There were >80% of the units in each district which were having below 30 ppm of iodine content in the salt. This suggested lack of either technical know-how or less determination to produce quality product. Hence, the interventional strategies addressed both the issues.
- Interventional strategies included, training of the salt iodization procedure at production units, technical workshop, review workshop and subsidized supply of potassium iodate.

- Towards the end of the study, the salt iodization level improved significantly ($p<0.05$) in Anand/Nadiyad districts and non significantly, yet improved for rest of the two districts.
- In total there were 58.8% of the producers producing iodized salt at optimal level (≥30 ppm), which is a tremendous success in itself since the improvement within a span of 9 months has been 38% (baseline 20.6% to 58.8%- end).
- Conceptualization of DFS has also achieved success, having N=3 of the medium and large scale salt producers being eager to become a part of our efforts towards establishing DFS (NIN formula) production at local level.
- To overcome the need as a participatory approach National Institute of Nutrition was approached for facilitating the premix supply and technology transfer. Discussions for training the producers and technology transfer have been initiated. Further procedures are being addressed. The work is in progressive state.

CONCLUSION AND UNIQUE CONTRIBUTION AS RECOMMENDATIONS OF RESEARCH PHASE III

It can be stated that advocacy effort provided towards technical salt iodization, and availing concern from the salt producers for double fortified salt production at local level could be achieved successfully.

The results of the study phase, viewed collectively, lead to the rejection of the hypothesis stated at base indicated the following:

1. There will be no significant impact of advocacy measures on achieving optimal salt iodization by the small and medium scale salt units.
2. It is not feasible to achieve production of DFS at local level.

Contrary to the hypothesis, the results of the study phase revealed that,

- Majority of the producers have achieved recommended levels of salt iodization at production level (30 ppm) (60%).
- Majority of the producers have started putting their conscious efforts and are willing to produce quality product.
- Three of the producers are eager to produce DFS at local level, undergo technical training and salt unit upgradation as an initiative towards DFS production.

RECOMMENDATIONS

- ✓ Double fortified salt should be implemented as nationwide strategy to combat two essential micronutrient deficiencies among all age groups.
- ✓ Screening for thyroid dysfunction and iodine deficiency should be implemented as target coverage under government programmes for safe pregnancy.
- ✓ Nutrition health education and campaigning should be carried out at massive scale to create awareness amongst general public regarding micronutrient deficiencies, their consequences for all age groups at various life stages and strategies to combat these deficiencies.
- ✓ Deworming should be implemented as compulsory procedure for school curriculums under the government schemes for all school aged children and adolescents.
- ✓ Salt producers should be advocated and motivated to achieve optimal salt iodization with apt technical proficiency.
- ✓ Technical training, financial and plant upgradation support-machineries (subsidized cost) should be provided by the government to the salt producers who are willing to initiate DFS production at their units.

RECOMMENDATIONS FOR FUTURE RESEARCH

- ✓ DFS has been proven efficacious enough to improve iron and iodine status of the study population- pregnant women and school aged children. However, due to various limitations, the study was conducted for a stipulated time period, population and region. Thus, long term supplementation and its impact need to be studied on larger sample size and on regional groups. To sum up there is a need to carry out multicentric studies by the government agencies including nutrition institutions to assess the blanket impact of DFS consumption.
- ✓ School children and adolescents are very sensitive, rather indicative group for assessing micronutrient deficiencies. Thus, longitudinal studies including adolescent

girls supplemented with DFS, their pregnancy and birth outcome could be one of the key research to observe the contribution of iron present in DFS when consumed for a long term.

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