# MATERNAL THYROID DYSFUNCTION AND IODINE DEFICIENCY, ITS IMPLICATIONS ON INFANT DEVELOPMENT AND IMPACT OF DOUBLE FORTIFIED SALT SUPPLEMENTATION

**Ph.D. THESIS**

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# MATERNAL THYROID DYSFUNCTION AND IODINE DEFICIENCY, ITS IMPLICATIONS ON INFANT DEVELOPMENT AND IMPACT OF DOUBLE FORTIFIED SALT SUPPLEMENTATION

**A Dissertation submitted in partial fulfilment of the requirement for the award of**

## Doctor of Philosophy

**(Foods and Nutrition)**


**By**

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**Vadodara, Gujarat, India**


**SEPTEMBER - 2012**

# *CERTIFICATE*

*This is to certify that* ***RITU RANA,*** *candidate for the award of Ph.D. in the department of Foods and Nutrition of this Faculty has fulfilled the requirements for Degree of Doctor of Philosophy in Foods and Nutrition. The thesis entitled* ***"Maternal thyroid dysfunction and iodine deficiency, its implications on infant development and impact of double fortified salt supplementation"*** *was carried out under my direct guidance and supervision. This thesis incorporates the results of independent investigations carried out by the candidate. The content of this thesis, in full or parts have not been submitted to any other Institute or University for the award of any other degree or diploma.*

*The attendance requirements as per The Maharaja Sayajirao University of Baroda have been fulfilled by the candidate.*

***Dr. Sirimavo Nair*** ***Prof. Uma Iyer***
***Guide*** ***Head***

***SEPTEMBER, 2012***

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Ritu Rana

(Mrs. Ritu Hardik Kapadia)

September, 2012

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PICTURES

**Dedicated to my grandfather**

*Prof. Kantilal Kapadia*

## List of Abbreviations

| | |
|---|---|
| AACE | American Association of Clinical Endocrinologists |
| ACC | Administrative Committee on Coordination |
| ACTH | Adrenocorticotrophin |
| AITD | Auto immune Thyroid Disorders |
| BDSTI | Baroda Development Screening Tests for Infants |
| BMI | Body Mass Index |
| BMIC | Breast Milk Iodine Content |
| BSID | Bayley Scales of Infant Development |
| CB | Cord Blood |
| CES | Coverage Evaluation Survey |
| CH | Congenital Hypothyroidism |
| CH | Clinical hypothyroidism |
| CNS | Central Nervous System |
| CoA | Co-activators |
| CoR | Co-repressor |
| CP | Choroid Plexus |
| CSF | Cerebrospinal Fluid |
| D1 | Deiodinase 1 |
| D2 | Deiodinase 2 |
| D3 | Deiodinase 3 |
| DFS | DoubleFortified Salt |
| DLHS | District Level Health Survey |
| EDTA | Ethylene di-amine Tetra Acetic Acid |
| FAO | Food and Agriculture Organization |
| FT3 | Free tri-iodothyronine |
| FT4 | Free Thyroxine |
| HB | Hemoglobin |
| hCG | human Chorionic Gonadotropin |
| HPT | HypothalamicPituitaryThyroid |
| HT | Hypothyroxinemia |
| ICCIDD | International Council for Control of Iodine Deficiency Disorders |
| ICDS | Integrated Child Development Services |
| ID | Iodine Deficiency |
| IDA | Iron Deficiency Anemia |
| IDD | Iodine Deficiency Disorders |
| IFSD | Iron Fortified Salt Distribution |

| | |
|---|---|
| IOM | Institute of Medicine |
| IQ | Intelligence Quotient |
| ISCS | Iodized Salt Coverage Study |
| IUFD | Intra Uterine Fetal Death |
| KAP | Knowledge Attitude and Practices |
| LIG | Low Income Group |
| MCT8 | Mono-carboxylate transporter-8 |
| MI | Micronutrient Initiative |
| NBAS | Neonatal Behavioural Assessment Scale |
| NFHS | National Family Health Survey |
| NHE | Nutrition Health Education |
| NHNES | National Health and Nutrition Examination Survey |
| NIN | National Institute of Nutrition |
| NNNB | National Nutrition Monitoring Bureau |
| NRHM | National Rural Health Mission |
| OATP | Organic Anion Transporter Protein |
| OH | Overt Hypothyroidism |
| PP | Post-partum |
| PPD | Post-partum Depression |
| PPT | Post-partum Thyroiditis |
| PVN | Para Ventricular Nucleus |
| RDA | Recommended Dietary Allowances |
| RIA | Radio Immuno Assay |
| rT3 | Reverse tri-iodothyronine |
| RXR | Retinoid X Receptor |
| SAM | Severe Acute Malnutrition |
| SCH | Sub Clinical Hypothyroidism |
| SCN | United Nations Subcommittee on Nutrition |
| SHMP | Sodium Hexa Metaphosphate |
| T3 | Tri-iodothyronine |
| T4 | Total Thyroxine |
| TBG | Thyroxine-binding Globulin |
| Tg | Thyroglobulin |
| TG-Ab | Thyroglobulin Antibody |
| TPO | Thyroid Per-oxidase |
| TPO-Ab | Thyroid Per-oxidase Antibody |
| TRE | Thyroid Hormone Response Elements |
| TRH | Thyrotropin Releasing Hormone |

| | |
|---|---|
| TRs | Thyroid Hormone Receptors |
| TSH | Thyroid Stimulating Hormone |
| TTR | Transthyretin |
| UI | Urinary Iodine |
| UIC | Urinary Iodine Concentration |
| UIE | Urinary Iodine Excretion |
| UNICEF | United Nations Children's Fund |
| USI | Universal Salt Iodization |
| WHO | World Health Organization |

**ABSTRACT**

Aim: To screen pregnant women during early gestation for thyroid dysfunction; to observe thyroid hormone changes throughout pregnancy; to observe its impact on infant development and to study impact of DFS supplementation on iodine and iron status of mothers during lactation.

Methods: A hospital based follow up and interventional study was carried out in urban Vadodara. Thyroid hormone analysis was performed using RIA technique, urinary iodine and hemoglobin estimation was carried out using simple micro-plate method and cyanmethemoglobin method respectively.

Results: Screening of pregnant women for thyroid dysfunction during early gestation revealed that 28% women were at low risk (TSH >2.5mIU/l) and 5.5% women were at high risk (TSH >5.0mIU/l) of developing hypothyroidism. Mean TSH, FT4, TT4 and TG were falling under normal range (adult reference value). Thyroid dysfunction was found in 32.88%, 43.84% and 31.51% during first, second and third trimester respectively. There was an increase in mean TSH, FT4 and TG with advancing gestation; however FT4 decreased from first to second trimester and then increased from second to third trimester. Raised CBTSH was found in 12.60% neonates and low CBFT4 was found in 9.25% neonates. A significant difference of 0.2 and 0.4 was found between mean BDSTI scores of both groups (with thyroid dysfunction and with normal thyroid function) at 6 months and 12 months respectively. There was a significant increase of 0.22 g/dl in hemoglobin in experimental group, while in control group there was a significant decrease in hemoglobin of 0.17 g/dl. Median urinary iodine increased by 78 mcg/l ($p<0.05$) in experimental group and in control group it decreased by 16 mcg/l ($p=0.964$).

Conclusion: Screening of pregnant women during early gestation using a lower TSH cut-off can result in identifying pregnant women who may be at risk of developing hypothyroidism. Since thyroid dysfunction during early gestation effects infant development, it is of great importance to diagnose thyroid dysfunction during early gestation and start appropriate treatment. DFS can be used as an additional strategy to combat anemia among women along with IFA supplementation and dietary modification.

# INTRODUCTION

**Pregnancy and increased micronutrients requirement: a concern**

Pregnancy is associated with increased nutritional needs due to the physiologic changes and the metabolic demands of the embryo/fetus. Hence, the nutritional status of mother prior to conception establishes the quality of the environment in which the fetus will develop and is a key determinant in the life of the newborn. Proper maternal nutrition during pregnancy is thus imperative for the health of both the woman and the offspring. Daily requirements for micronutrients particularly iodine and iron during pregnancy are higher to meet the physiologic changes and increased nutritional needs of pregnancy. Both iodine and iron requirements are increased by 40% during pregnancy.

| Micronutrient | RDA non pregnant women | RDA pregnant women | Increase in requirement |
|---|---|---|---|
| Iodine | 150µg/d | 250 µg/d | 40% |
| Iron | 21 mg/d | 35 mg/d | 40% |

**Source: NIN, 2010; WHO/UNICEF/ICCIDD, 2007**

Iodine is an essential micronutrient for normal growth and development. The human body contains 15–20 mg of iodine, of which 70–80% is concentrated in the thyroid gland (FAO, 2005).

At present, the only physiological role known for iodine in the human body is for the synthesis of thyroid hormones by the thyroid gland. Therefore, the dietary requirement of iodine is determined by normal thyroxine (T4) production by the thyroid gland without stressing the thyroid iodide trapping mechanism or rising thyroid stimulating hormone (TSH) levels. Iodine from the diet is absorbed throughout the gastrointestinal tract. Dietary iodine is converted into iodide ion before it is absorbed. The iodide ion is 100% bio-available and absorbed

totally from food and water. This is however not true for iodine within thyroid hormones ingested for therapeutic purposes.

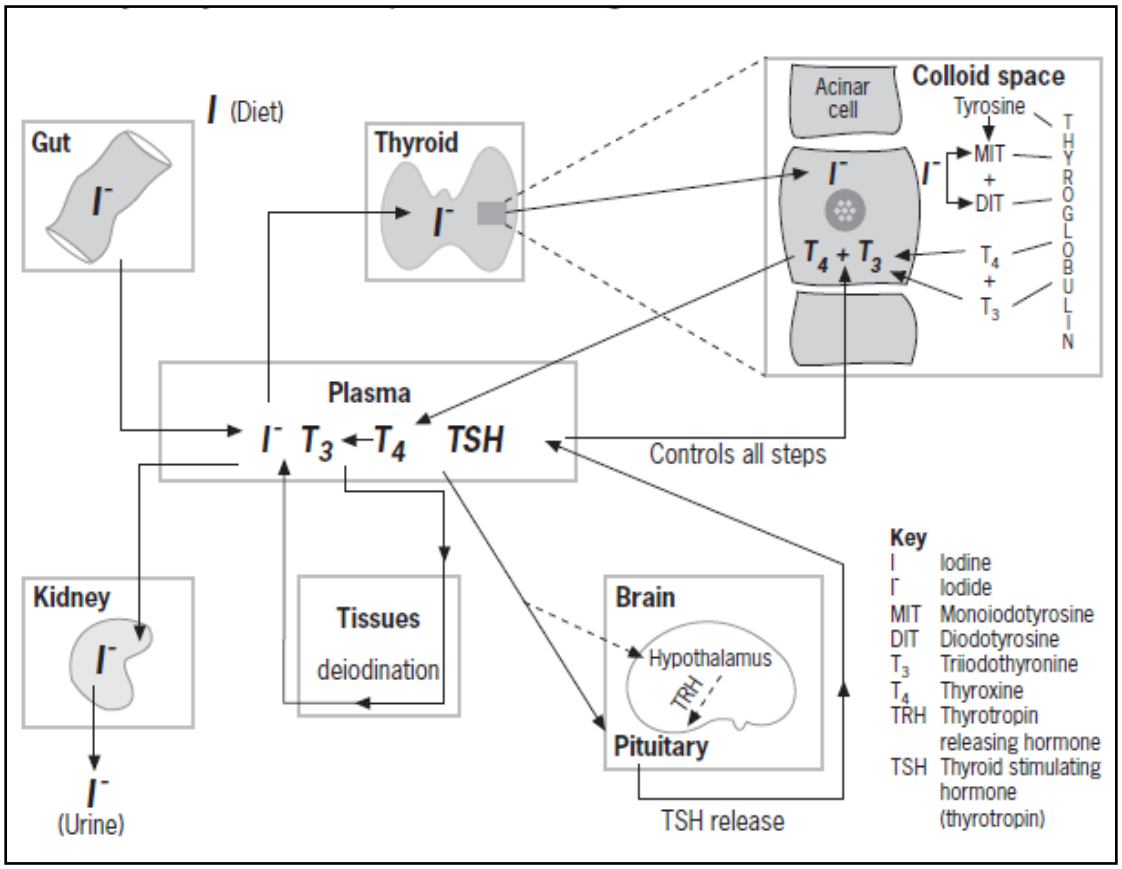
Iodine enters the circulation as plasma inorganic iodide, which is cleared from the circulation by the thyroid and kidney. The iodide is used by the thyroid gland for synthesis of thyroid hormones, and the kidney excretes excess iodine with urine. The excretion of iodine in the urine is a good measure of iodine intake. In a normal population with no evidence of clinical iodine deficiency either in the form of endemic goitre or endemic cretinism, urinary iodine excretion reflects the average daily iodine requirement. Therefore, for determining the iodine requirements and iodine intake, the important indices are serum T4 and TSH levels (exploring thyroid status) and urinary iodine excretion (exploring iodine intake). A simplified diagram of the metabolic circuit of iodine is given in Figure 1.1. All biological actions of iodide are attributed to the thyroid hormones. The major thyroid hormone secreted by the thyroid gland is T4. T4 in circulation is taken up by the cells and is de-iodinated by the enzyme 5'-monodeiodinase in the cytoplasm to convert it into triiodothyronine (T3), the active form of thyroid hormone. T3 traverses to the nucleus and binds to the nuclear receptor.

All the biological actions of T3 are mediated through the binding to the nuclear receptor, which controls the transcription of a particular gene to bring about the synthesis of a specific protein.

The physiological actions of thyroid hormones can be categorized as 1) growth and development and 2) control of metabolic processes in the body. Thyroid hormones play a major role in the growth and development of the brain and central nervous system in humans from the 12th week of gestation to 3 years of age. If iodine deficiency exists during this period and results in thyroid hormone deficiency, the consequence is derangement in the development of the brain and

central nervous system. These derangements are irreversible; the most serious form being that of cretinism.

**Figure 1.1: Summary of thyroid hormone production and regulation**




**Source: Stanbury, 1960**

The effect of iodine deficiency at different stages of life is given in Table 1.1. The other physiological role of thyroid hormones is to control several metabolic processes in the body. These include carbohydrate, fat, protein, vitamin, and mineral metabolism. For example, thyroid hormone increases energy production, increases lipolysis, and regulates neoglucogenesis, and glycolysis.

**Table 1.1: Effect of iodine deficiency by life stage**

| Life stage | Effects |
|---|---|
| All ages | Goitre<br>Hypothyroidism<br>Increased susceptibility to nuclear radiation |

| Life stage | Effects |
|---|---|
| Fetus | Abortions |
| | Stillbirths |
| | Congenital anomalies |
| | Increased perinatal mortality |
| | Increased infant mortality |
| | Neurological cretinism: mental deficiency, deaf mutism, spastic diplegia, and squint |
| | Myxedematous cretinism: mental deficiency, hypothyroidism and dwarfism |
| | Psychomotor defects |
| | Impairment in development of brain, lung, muscle, nerves, adipose tissue, heart and cardiovascular function |
| Neonate | Neonatal goitre |
| | Neonatal hypothyroidism |
| Child and adolescent | Goitre |
| | Juvenile hypothyroidism |
| | Impaired mental function |
| | Retarded physical development |
| Adult | Goitre with its complications |
| | Hypothyroidism |
| | Impaired mental function |
| | Iodine-induced hyperthyroidism |

**Source: Zimmermann et al, 2008**

## IODINE DEFICIENCY, THYROID DISORDERS AND PREGNANCY

The high global prevalence of iodine deficiency and autoimmune thyroid disorders, the mental and physical consequences of these disorders create a huge human and economic burden that can be prevented, in large part, by early detection and therapeutic measures.

Over the past several years it has been proved that maternal thyroid disorders influence the outcome of mother and fetus, during and also after pregnancy. The most frequent thyroid disorder in pregnancy is maternal hypothyroidism, which is associated with fetal loss, placental abruptions, pre-eclampsia, preterm delivery and reduced intellectual function in the offspring (Abalovich et al, 2002).

**Thyroid disorders during pregnancy**

Prevalence of thyroid disorders during pregnancy is, hypothyroidism-2% (Vanderpump et al, 1995), congenital hypothyroidism-1/4,000 (Vanderpump et al, 1995), overt hypothyroidism-0.2-0.5% (Allan et al, 2000; Casey et al, 2005), subclinical hypothyroidism-2.2-2.5% (Allan et al, 2000; Casey et al, 2005), hypothyroxinemia-1.3-2.1% (Casey et al, 2007; Cleary-Goldman et al, 2008) and TPO-Ab or TG-Ab positive test results-5% (Cleary-Goldman et al, 2008).

Worldwide more than 20 million people develop neurological disorders due to intra uterine iodine deprivation (Girling, 2008). Another problem related to thyroid disorders during pregnancy is postpartum thyroiditis.

**Physiology of thyroid in pregnancy**

Thyroid hormones consist of thyroxine (T4) and triiodothyronine (T3) of which active forms are the free portions (fT3, fT4) consisting of 1% of total hormones. The fT3 fraction is biologically more significant and derived from conversion of fT4 at liver, kidney and muscle. The fT3 hormone acts through specific nuclear receptors of fT3, situated in most of the tissues. TSH secreted from anterior pituitary act as negative feedback from fT3 levels. Dietary iodine is essential for this thyroid hormone synthesis.

***Fetus:*** In pregnancy, fetus receives iodine from maternal source in all the trimesters. Fetus receives thyroxine from mother up to 12 weeks through placental circulation but not TSH or fT3. Thyroxine is partially converted to fT3 and combines with receptors in fetal brain and is responsible for fetal brain development. From 12th week, placental changes resist T4 passage to fetus and fetal pituitary thyroid axis start functioning like adult (Girling, 2008).

***Pregnant women:*** Pregnancy has an appreciable effect on thyroid economy. There is an increase in thyroid binding globulins, increase

in total T4 and T3, thyroid stimulation by hCG, increase in renal iodine clearance and increase in serum thyroglobulin during normal pregnancy (Fantz et al, 1999). Hence as a result of these changes, iodine requirements increase during pregnancy.

**Maternal aspects of hypothyroidism**

Women with hypothyroidism have decreased fertility; even if they conceive, risk of abortion is increased, and risk of gestational hypertension, anemia, placental abruption and postpartum hemorrhage is increased (Abalovich et al, 2002). The risk of these complications is greater in women with overt, rather than subclinical hypothyroidism.

**Fetal and neonatal aspects of maternal hypothyroidism**

Untreated maternal hypothyroidism can lead to preterm birth, low birth weight, and respiratory distress in the neonate. Enough evidence has accumulated over the years about the role of thyroxine in normal development of the fetal brain. The presence of specific nuclear receptors and thyroid hormone found in fetal brain at 8 week of gestation, free T4 found in the coelomic and amniotic fluids and demonstration of the transfer of maternal thyroid hormones across the placenta, underline the role of thyroid hormones in fetal brain development. Complex interactions between the D2 and D3 iodothyronine deiodinases during gestation help to fine tune the supply of adequate amounts of T3 required for normal brain development.

A number of pioneering studies by Man et al (1971), Haddow et al (1999), Pop et al (1999) and newer studies by Rovet et al (2004) and Vermiglio et al (2004) have conclusively proved that children born to mothers with hypothyroidism had a significantly increased risk of impairment in IQ scores, neuropsychological developmental indices and learning abilities. Children born to untreated hypothyroid women

had an IQ score that was 7 points below the mean IQ of children born to healthy women and women given thyroxine supplements. This risk applies to children born not only of untreated women, but also women with suboptimal supplementation. A study by Rovet et al (2004) found that such children had mild defects in global intelligence, but visual-spatial ability, language, fine motor performance, and preschool ability were unaffected. This study emphasizes the need to follow-up women adequately after initiating treatment. Children born to mothers with iodine deficiency fared even worse, with a greater than 10-point average deficit in global IQ and quite a few also had attention deficit hyperactivity disorder (Vermiglio et al, 2004).

**Need for trimester specific reference intervals**

Because of physiological changes, values of thyroid hormones during pregnancy differ from non-pregnant values. Values in pregnancy also vary from trimester to trimester and from method to method. Since more and more researchers are aware of the importance of evaluating maternal thyroid function during pregnancy by gestation-specific reference intervals, manufacturer's reference range should not be used for pregnant women. If a non-pregnant reference interval is used, a number of pregnant women with thyroid dysfunction could be potentially misclassified.

**IODINE DEFICIENCY AND PREGNANCY**

Iodine plays a critical role in the neuropsychological development of the fetus throughout gestation and in the first two years of life. Iodine uptake by the thyroid is higher in pregnancy and iodine reserve in the thyroid can decrease to approximately 40% of preconception levels (Glinoer, 1997*). World Health Organization (WHO) has recently increased their recommended iodine intake during pregnancy from 150 to 250 µg/day.

Thyroid gland stores iodine from the diet and as such maternal iodine status is not entirely dependent on the current dietary intake during gestation. If preconception iodine nutrition is adequate there will be sufficient stores of thyroid hormone to support the mother and foetus, 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 hypothyroxinemic 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, 2009*).

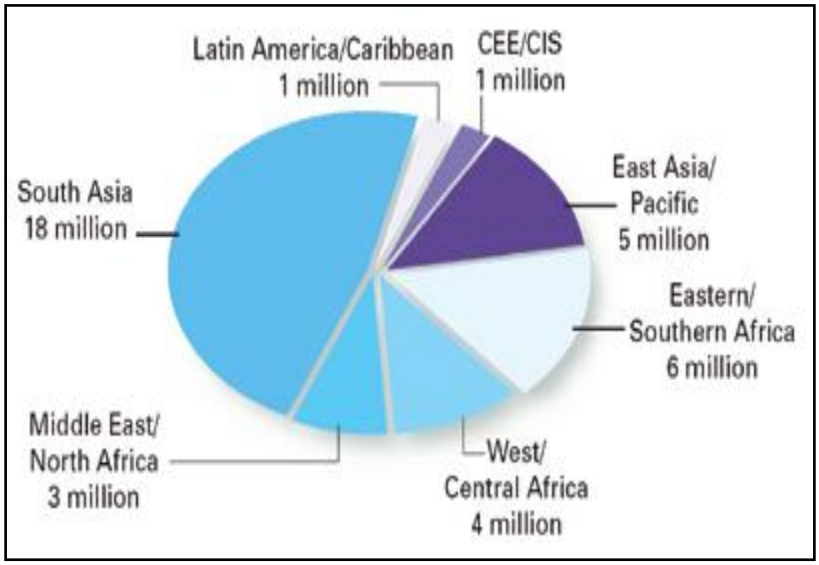
Over the past decade, there has been increasing focus on iodine deficiency during pregnancy as iodine is critical for optimal fetal development, yet 38 million newborns in developing countries every year remain unprotected from the lifelong consequences of brain damage associated with iodine deficiency (Figure 1.2).

**GLOBAL iodine nutrition**

Only a few countries, Switzerland, some of the Scandinavian countries, Australia, the United States and Canada were completely iodine sufficient before 1990. Since then, there has been a major global effort to introduce salt iodization to ensure sufficient intake in deficient areas. Over two-thirds of the world's population is now covered by iodized salt (UNICEF, 2012).

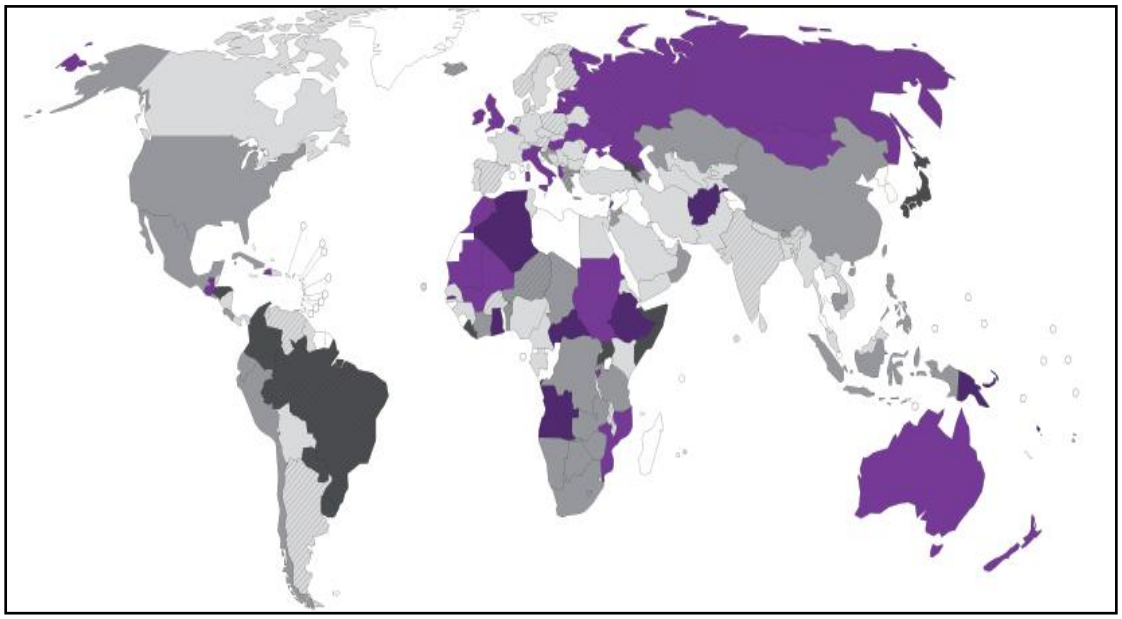
Global iodine nutrition has markedly improved over the past decade (but with strong regional differences) and the number of iodine deficient countries has decreased from 54 in 2003 to 32 in 2011 (Figure 1.4). Yet despite remarkable progress, 1.88 billion of the global population, including 241 million school children, still has insufficient dietary iodine intakes.

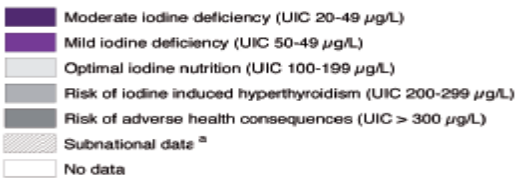
**Figure 1.2: Distribution of infants born in developing countries annually who are unprotected against IDD, by region 2000-2006**




**Source: UNICEF, 2012**

**Figure 1.3: National iodine status based on urinary iodine concentrations in school aged children**

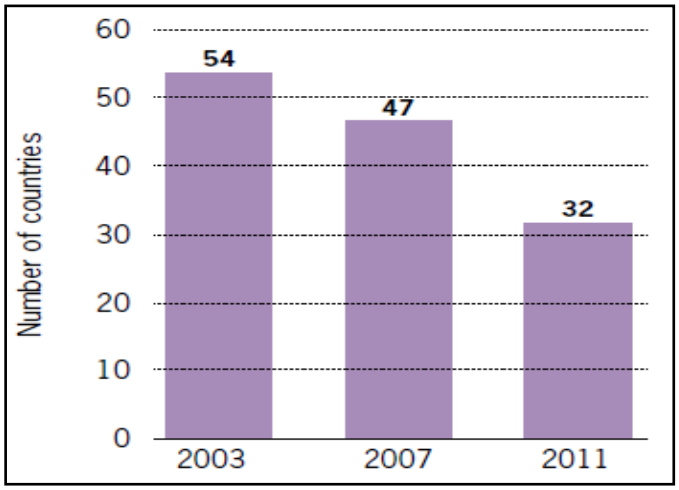





*The country estimates in the cross-hatched countries are based on sub-national data. The national coverage of iodized salt in these countries is incomplete, there are large variations in the iodine intake and some regions likely remain deficient.

**Source: IDD Newsletter, Feb 2010**

**Figure 1.4: Number of iodine deficient countries in 2003, 2007 and 2011**




**Source: IDD Newsletter, Feb 2012**

**Table 1.2: Countries (number) by iodine status over the period 2003-2011**

| Iodine intake | 2003 | 2007 | 2011 |
|---|---|---|---|
| Insufficient: severe iodine deficiency | 1 | 0 | 0 |
| Insufficient: severe iodine deficiency | 13 | 10 | 9 |
| Insufficient: severe iodine deficiency | 40 | 37 | 23 |
| Adequate | 43 | 49 | 69 |
| More than adequate | 24 | 27 | 36 |
| Excessive | 5 | 7 | 11 |
| Countries with data | 126 | 130 | 148 |
| **Total countries** | **192** | **193** | **193** |

**Source: IDD Newsletter, Feb 2012**

### Iodine nutrition of pregnant women

Only a limited number of countries have completed UIC surveys in pregnant women and women of reproductive age on the national or large sub-national level. Thus, there are insufficient data to directly estimate the regional or global prevalence of low iodine intake in these important target groups. This is a major limitation of the current

estimate because although the median UIC in children may be used to represent iodine status of most of the population, it should not be used as a proxy for iodine status in pregnant women (Wong et al, 2011).

**Iodine nutrition in INDIA**

There is insufficiency of national data on urinary iodine concentration of population (Figure 1.3). According to global iodine nutrition scorecard of 2010 and 2012, percentage of households consuming iodized salt in India has improved with an improvement in median UIE. However, proportion of population having UIE <100 has not improved (Table 1.3).

**Table 1.3: Comparison of country data on iodine nutrition**

| **Global scorecard values for INDIA** | | **2010*** | **2012**** |
|---|---|---|---|
| Annual no. of births in 2008* and 2009** (000) | | 26'913 | 26'787 |
| Household consuming iodized salt (%) | | 51.1 | 71 |
| Median UIE (μg/L) | | 133 | 154 |
| Proportion of population with UIE <100 (%) | | 31.3 | 34.4 |
| General Population | Iodine deficiency protected population (000) | 602'520 | 625'778 |
| | Iodine deficiency unprotected population (000) | 576'892 | 598'836 |
| Infants | Iodine deficiency unprotected infants (000) | 13'726 | 13'688 |

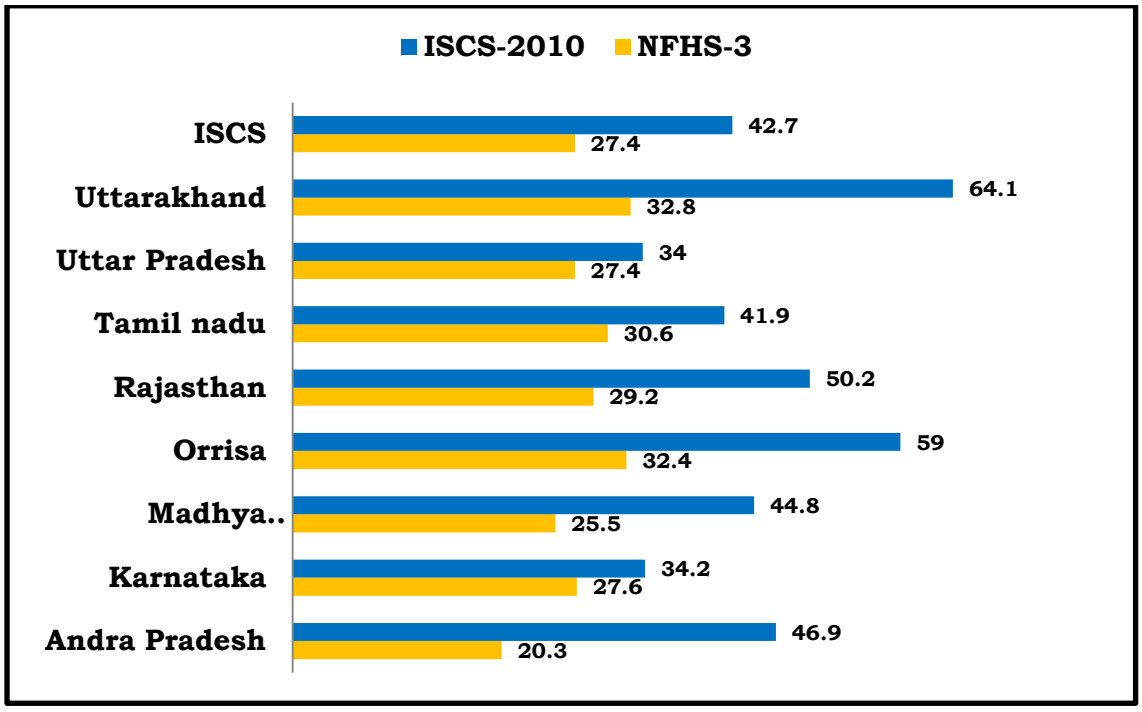
**Source: *IDD Newsletter, Feb 2010; ** IDD Newsletter, Feb 2012**

***Adequately iodized salt availability at household level in 8 states***

Recently in 2010, Micronutrient Initiative (MI) has conducted a study on iodine content of edible salt at household level in rural areas of eight states. A comparison between NFHS-3 and Iodized Salt Coverage Study (MI-ISCS, 2010) is shown in Figure 1.5. The use of adequately

iodized salt in rural households has increased across all states. The 8 state averages have gone up from 27% during NFHS-3 to 47.2% during the 2010 study. The highest increase was evidenced in Uttarakhand followed by Orissa, Rajasthan, Andhra Pradesh and Madhya Pradesh. Tamil Nadu and Uttar Pradesh have also reported modest increases. Use of iodized salt has gone up significantly in rural areas in states which were previously considered to be problem states. The amount of adequately iodized salt at the household level has increased and the amount of non-iodized salt has dropped dramatically (MI-ISCS, 2010).

**Figure 1.5: A comparison between NFHS-3 and Iodized Salt Coverage Study**




**Source: MI-ISCS, 2010**

**Recommendations of thyroid societies (worldwide):**

Recently in 2007 (Abalovich et al), all thyroid societies have worked towards development of guidelines for treatment of thyroid disorders. These societies are-Latin American Thyroid Society, the Asia and Oceania Thyroid Society, the American Thyroid Association, the European Thyroid Association and the American Association of Clinical Endocrinologists.

***Hypothyroidism and pregnancy: Maternal and fetal aspects***

- Both maternal and fetal hypothyroidism is known to have serious adverse effects on the fetus. Therefore maternal hypothyroidism should be avoided.
- If hypothyroidism has been diagnosed before pregnancy, we recommend adjustment of the preconception thyroxine dose to reach a TSH level not higher than 2.5 μU/mL prior to pregnancy.
- The T4 dose usually needs to be incremented by 4-6 wk gestation and may require a 30-50% increase in dosage.
- If overt hypothyroidism is diagnosed during pregnancy, thyroid function tests (TFTs) should be normalized as rapidly as possible. Thyroxine dosage should be titrated to rapidly reach and thereafter maintain serum TSH concentrations of less than 2.5 μU/mL in the first trimester (or 3 μU/mL in the second and third trimester) or to trimester-specific normal TSH ranges. Thyroid function tests should be re-measured within 30-40 days.
- Women with thyroid autoimmunity who are euthyroid in the early stages of pregnancy are at risk of developing hypothyroidism and should be monitored for elevation of TSH above the normal range.
- Subclinical hypothyroidism (serum TSH concentration above the upper limit of the reference range with a normal free T4) has been shown to be associated with an adverse outcome for both the mother and offspring. T4 treatment has been shown to improve obstetrical outcome but has not been proved to modify long-term neurological development in the offspring. However, given that the potential benefits outweigh the potential risks, the panel recommends T4 replacement in women with subclinical hypothyroidism.
- After delivery, most hypothyroid women need a decrease in the thyroxine dosage they received during pregnancy.

***Iodine nutrition during pregnancy***

- Women in the childbearing age should have an average iodine intake of 150 µg per day. During pregnancy and breast-feeding, women should increase their daily iodine intake to 250 µg on an average.
- Iodine intake during pregnancy and breastfeeding should not exceed twice the daily recommended nutritional intake for iodine, i.e. 500 µg iodine per day.
- To assess the adequacy of the iodine intake during pregnancy in a population, urinary iodine concentration (UIC) should be measured in a cohort of the population. UIC should ideally range between 150 and 250 µg/L.
- To reach the daily recommended nutrient intake for iodine, multiple means must be considered, tailored to the iodine intake level in a given population. Different situations must therefore be distinguished: a) countries with iodine sufficiency and/or with a well-established universal salt iodization (USI) program; b) countries without a USI program or an established USI program where the coverage is known to be only partial; and finally c) remote areas with no accessible USI program and difficult socioeconomic conditions.

***Postpartum thyroidtis***

- There are insufficient data to recommend screening of all women for postpartum thyroiditis (PPT).
- Women known to be thyroid peroxidase antibody positive should have a TSH performed at 3 and 6 months postpartum.
- The prevalence of PPT in women with type 1 diabetes is threefold greater than in the general population. Postpartum screening (TSH determination) is recommended for women with type 1 diabetes mellitus at 3 and 6 months postpartum.

- Women with a history of PPT have a markedly increased risk of developing permanent primary hypothyroidism in the 5 to 10 year period following the episode of PPT. An annual TSH level should be performed in these women.
- Asymptomatic women with PPT who have a TSH above the reference range but below 10 μU/mL and who are not planning a subsequent pregnancy do not necessarily require intervention, but should, if untreated, be re-monitored in 4–8 weeks. Symptomatic women and women with a TSH above normal and who are attempting pregnancy should be treated with levothyroxine.
- There is insufficient evidence to conclude whether an association exists between postpartum depression (PPD) and either PPT or thyroid antibody positivity (in women who did not develop PPT).
- However, as hypothyroidism is a potentially reversible cause of depression, women with postpartum depression should be screened for hypothyroidism and appropriately treated.

***Screening for thyroid dysfunction during pregnancy***

Although the benefits of universal screening for thyroid dysfunction (primarily hypothyroidism) may not be justified by the current evidence (presented above), we recommend case finding among the following groups of women at high risk for thyroid disease by measurement of TSH:

- Women with a history of hyperthyroid or hypothyroid disease, PPT, or thyroid lobectomy.
- Women with a family history of thyroid disease.
- Women with a goiter.
- Women with thyroid antibodies (when known).

- Women with symptoms or clinical signs suggestive of thyroid underfunction or overfunction, including anemia, elevated cholesterol, and hyponatremia.
- Women with type I diabetes.
- Women with other autoimmune disorders.
- Women with infertility who should have screening with TSH as part of their infertility work-up.
- Women with previous therapeutic head or neck irradiation.
- Women with a history of miscarriage or preterm delivery.

**IRON DEFICIENY ANEMIA DURING PREGNANCY**

Iron deficiency anemia is the most common nutritional deficiency in the World. Anemia is a condition of low levels of hemoglobin in the blood. It is a widespread public health problem associated with increased risk of morbidity and mortality. Young children, pregnant and postpartum women are the most severely affected by iron deficiency because their demand for iron is high.

**National and state prevalence of anemia during pregnancy**

According to DLHS survey percentage of pregnant women having anemia in India is 55.3% with 38.6% mildly anemic, 15% moderately anemic and 1.8% severely anemia (Kothari and Noureddine, 2010). According to NFHS 3 data, 57.9% pregnant women (15-49 years of age) are anemic in Gujarat state.

In developing countries, the majority of women are anemic in the second half of pregnancy. Pregnant women are often iron deficient and iron deficiency has adverse effects on thyroid function. During the second and third trimester, pregnant women are highly vulnerable to iron deficiency because their increased iron needs are rarely met by dietary sources. Iron deficiency has multiple adverse effects on thyroid metabolism. It decreases circulating thyroid hormone concentrations, likely through impairment of the heme-dependent thyroid peroxidase

(TPO) enzyme. Iron deficiency blunts the efficacy of iodine prophylaxis, and iron repletion improves the efficacy of iodized salt in goitrous children with iron deficiency (Zimmermann, 2007).

**MICRONUTRIENT DEFICIENCIES AND MDGs**

Recent evidence suggests that, micronutrient deficiencies (especially iodine and iron) may play a role in children's development. Micronutrient deficiencies are a critical concern among children throughout the world. Approximately 30% of the world's population lives in iodine-deficient areas and 25% of the world's children <3 years of age have iron-deficiency anemia, with higher rates in developing countries. The relationship between micronutrient deficiency and early cognitive development has captured recent attention because micronutrients are related to specific physiological processes. Therefore, programs designed to prevent or treat micronutrient deficiencies can be targeted toward specific recommendations. The fortification of salt with iodine has been hailed as one of the world's great public health advancements. Now breakthrough technology that allows salt to be double fortified with iron as well as iodine has created an exciting new opportunity to reach the world with supplemental iron easily and inexpensively, without having to change people's habits.

Nutrition actions are critical to achieve the Millennium Development Goals (MDGs). Micronutrient interventions are suggested as cost-effective and programmatically feasible to scale-up worldwide.

***Elimination of Iodine Deficiency will contribute to at least six of the Millennium Development goals:***

MDG 1, Eradicate extreme poverty and hunger: Eliminating iodine deficiency will increase learning ability and intellectual potential, leading to higher earnings. In addition, the burden of diseases and pathologies related to ID will be eliminated.

MDG 2, Achieve Universal Primary Education: Children will have improved cognitive development and learning capacity,

leading to improved school performance and reduced drop-out rates.

MDG 3, Promote gender equality and empower women: Eliminating ID in children reduces child care burden for women, frees up household resources and allows women more time for income generating work.

MDG 4, Reduce child mortality: Reduced ID contributes to decreased rates of miscarriages, stillbirths, and other pregnancy complications, as well as early neonatal deaths.

MDG 5, Improve maternal health: Eliminating ID in women will reduce rates of miscarriages, thyroid diseases and other clinical outcomes of ID, thus improving the health status of women of reproductive age.

MDG 8, Develop a global partnership for development: The programs for sustainable elimination of iodine deficiency ensure a strong partnership of public, private and civil society at the global, regional, and country level.

***DFS can play a major role:***

MDG 2, Achieve Universal Primary Education: DFS will improve children's cognitive development and educational outcomes through increased and sustained intake of iron and iodine.

MDG 5, Improve Maternal Health: DFS will improve the survival and health of women by increasing and sustaining their iron and iodine intake and, in turn reducing the consequences of iron deficiency anemia and of poor pregnancy outcomes.

From the above discussion it was worthwhile to work towards-

(1) Preventing fetal brain damage by screening pregnant women during early gestation for thyroid dysfunction, iodine deficiency and iron deficiency anemia.

(2) Improving maternal iodine and iron status with supplementation through double fortified salt.

Apart from screening, pregnant women should also be provided with knowledge regarding antenatal care, delivery care and postnatal care. Correction of iodine deficiency and iron deficiency alone may not result in delivering a healthy baby. In order to ensure healthy baby, it also becomes necessary to check that pregnant women are availing good antenatal care, delivery care and postnatal care. Welfare check-ups for babies once in three months till one year will further reduce morbidity/mortality in children.

In view of the previous section discussion, present study was planned with major objective-

**"Maternal thyroid dysfunction and iodine deficiency, its implications on infant development and impact of double fortified salt supplementation"**

Specific objectives were:

- To screen pregnant women during first trimester for thyroid dysfunction, iodine deficiency and iron deficiency anemia.
- To provide Nutrition Health Education (NHE) to pregnant women regarding importance of iodine and iron nutrition during early pregnancy.
- To provide knowledge to pregnant women regarding maternal health components (antenatal care, delivery care and postnatal care).
- To record thyroid hormone levels, urinary iodine concentration and hemoglobin status during each trimester.
- To assess the nutritional status of pregnant women (anthropometry, biochemical indicators and 24 hr dietary recall).
- To screen the neonates.
- To test infant development.
- To study postpartum maternal thyroid status.
- To study the impact of double fortified salt supplementation (DFS) on iodine and iron status during lactation.
- To study infant iodine status.

# REVIEW OF LITERATURE

# REVIEW OF LITERATIRE

2.1 Thyroid hormone production and metabolism

Effects of thyroid hormones

2.2 Thyroid diseases

Hypothyroidism

Hypothyroxinemia

Chronic autoimmune thyroiditis

Hyperthyroidism

2.3 Thyroid function during pregnancy

Factors affecting thyroid physiology during normal pregnancy

Thyroid stimulation by hCG

Increase in thyroid binding globulins

Increase in total T4 and T3

Increase in serum thyroglobulin

Increase in renal iodide clearance

2.4 Development of the thyroid gland and fetal thyroid hormone supply

Thyroid hormones and neurodevelopment

Timing of thyroid hormone action in the brain

Circulating thyroid hormone concentration in human pregnancy

Thyroid hormone concentrations in fetal brain

Utero–placental transfer of thyroid hormones

Delivery of thyroid hormones to target tissues

Thyroid hormone action

Thyroid hormone metabolism (iodo-thyronines)

Thyroglobulin

Thyroid hormone concentrations

Assessment during pregnancy

Assessing status during lactation

Assessing status during infancy

2.13 Ioidne deficiency, thyroid dysfunction and infant development

Infant development testing scales

2.14 Iron deficiency during pregnancy

Iron requirements during pregnancy

Iron balance in pregnancy

Importance of iron stores

Assessment of iron status in pregnancy

Strategies to combat iron deficiency in pregnancy

2.15 Double fortified salt (DFS)

Physico-chemical Feature of DFS

Bio-impact features of DFS

Promotion of DFS to battle malnutrition in India

Response of various public health experts on DFS

## 2.1 THYROID HORMONE PRODUCTION AND METABOLISM

The thyroid gland secretes two main hormones; thyroxine (T4) and tri-iodothyronine (T3) (Figure 2.2). Thyroxine is produced in greater quantity than T3 (at a rate 10:1), but T3 is the major biologically active thyroid hormone and is mostly derived from T4 in the peripheral tissues. The thyroid gland utilizes and conserves iodine to produce thyroid hormones. Iodine is obtained from the diet, converted to iodide, actively transported to the thyroid, and incorporated into thyroglobulin (TG) by way of the enzyme thyroid peroxidase (TPO). This leads to production of mono-iodotyrosine and di-iodotyrosine, which are then coupled to form T4, T3 and reverse T3 (rT3). Reverse T3 has no biological activity. The thyroid hormones are part of the TG stored in the colloid of thyroid follicles until excreted into the circulation.

Thyroid hormone production is regulated by the pituitary through the action of thyrotropin (thyroid-stimulating hormone, TSH). TSH comprises two subunits and it has one alpha-subunit in common with luteinizing hormone, follicle stimulating hormone and human chorionic gonadotropin (hCG), and one specific beta-subunit. TSH shows circadian and pulsatory secretion – its secretion peaks at around midnight and declines during the day. The function of the pituitary is controlled by the hypothalamus, which excretes thyrotropin-releasing hormone (TRH). It accelerates the production of TSH, whereas dopamine and somatostatin hinder it. The thyroid hormones have a negative feedback effect on the pituitary and hypothalamus (Figure 2.1), which is modified by the T4 concentration in the serum and the conversion of T4 to T3 locally in the brain. Therefore, if T4 concentration in the serum drops, the inhibitory stimulus is decreased due to a diminished local effect of T3 in the pituitary and TSH levels rise to stimulate the thyroid gland (Ganong, 2005; Hadley and Levine, 2007).

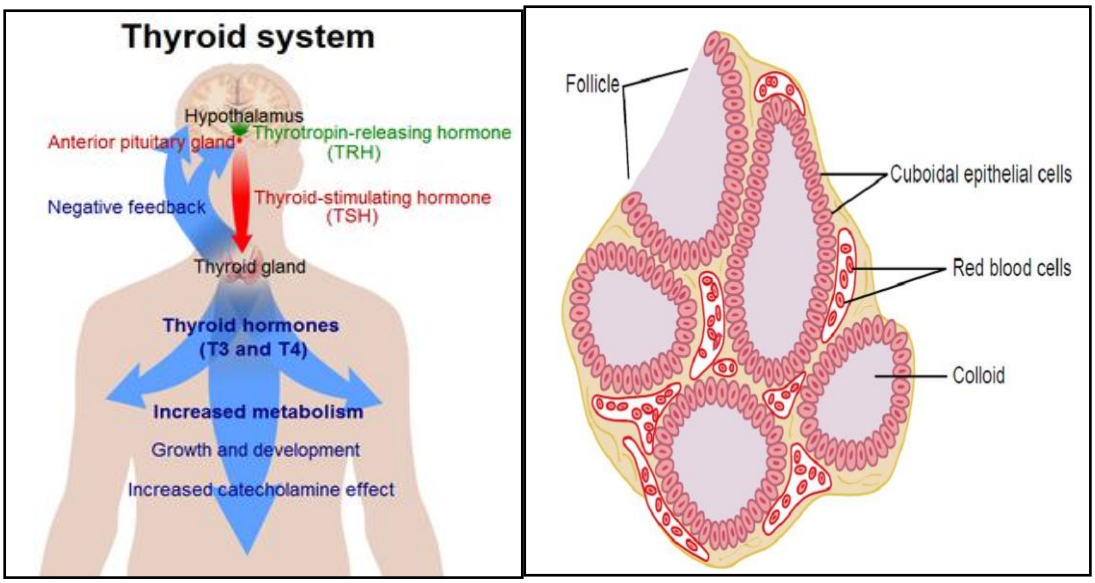



**Figure 2.1: Thyroid gland and follicle cell**

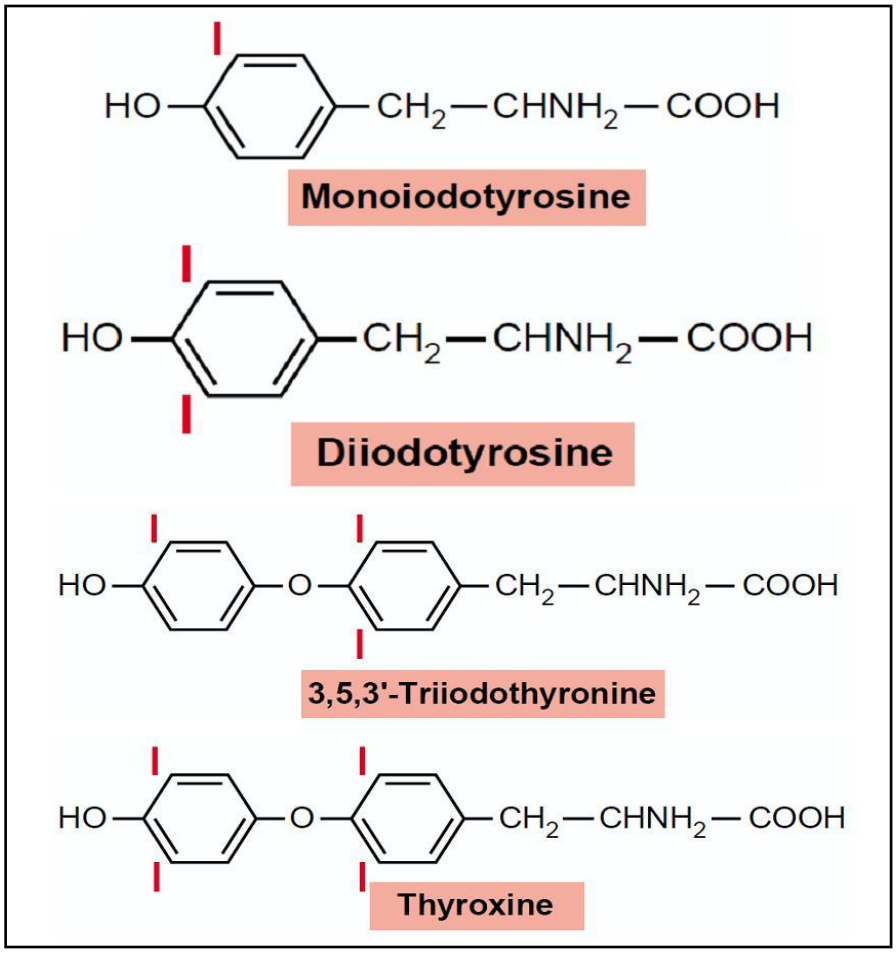
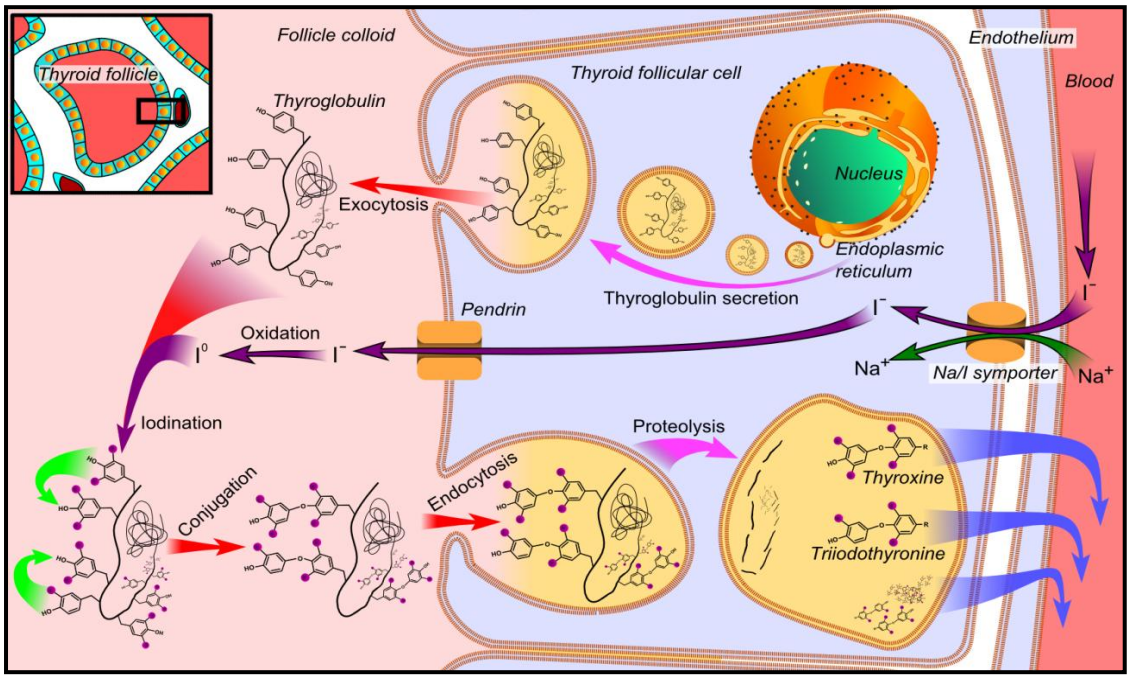



**Figure 2.2: Structure of thyroid hormones**

**Figure 2.3: Synthesis of the thyroid hormones in thyroid follicular cell**

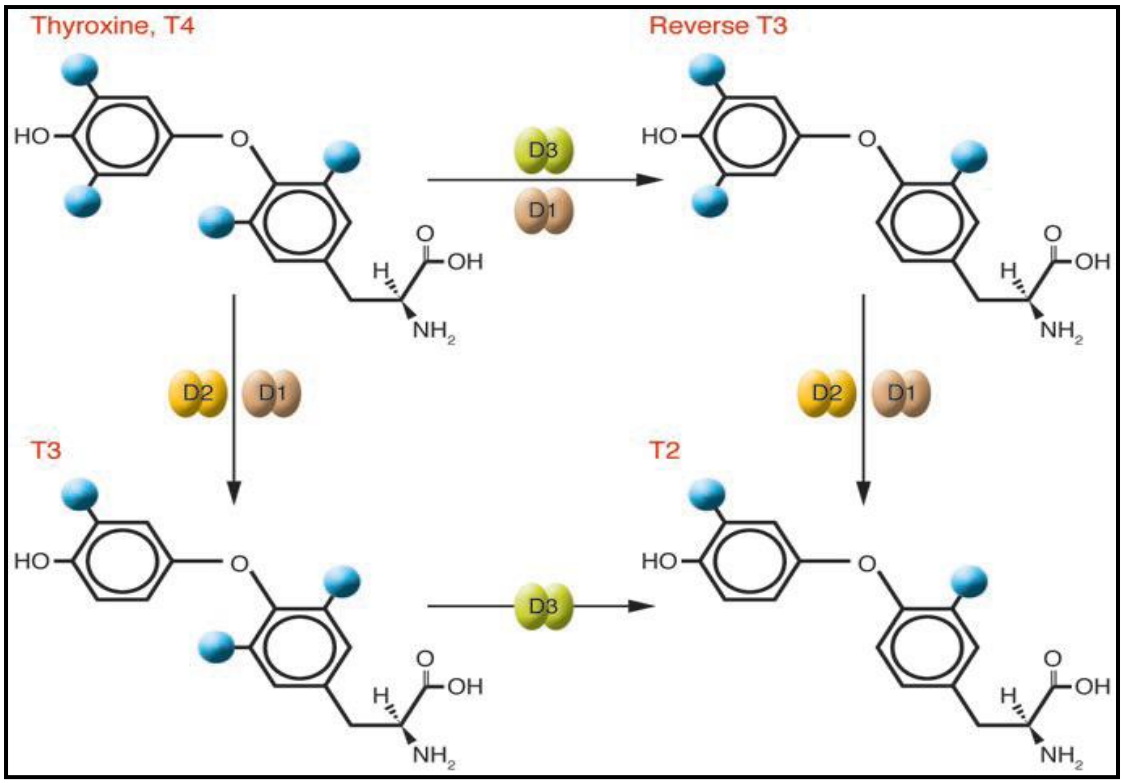



- Thyroglobulin is synthesized in the rough endoplasmic reticulum and follows the secretory pathway to enter the colloid in the lumen of the thyroid follicle by exocytosis.
- Meanwhile, a sodium-iodide (Na/I) symporter pumps iodide (I) actively into the cell, which previously has crossed the endothelium by largely unknown mechanisms.
- This iodide enters the follicular lumen from the cytoplasm by the transporter pendrin, in purportedly passive manner.
- In the colloid, iodide (I-) is oxidized to iodine (I) by an enzyme called thyroid peroxidase. Iodine (I) is very reactive and iodinates the thyroglobulin at tyrosyl residues in its protein chain (in total containing approximately 120 tyrosyl residues).
- In conjugation, adjacent tyrosyl residues are paired together. The entire complex re-enters the follicular cell by endocytosis.
- Proteolysis by various proteases liberates thyroxine and triiodothyronine molecules, which enter the blood by largely unknown mechanisms.

**Source: Peterson, 2011**

The thyroid hormones are protein-bound in the serum, and only 0.02% of T4 and 0.2% of T3 are free, biologically active hormones. 45-70% of thyroid hormones are bound to thyroxine-binding globulin (TBG), and the rest to transthyretin and albumin.

**Figure 2.4: The basic deiodinase reactions**



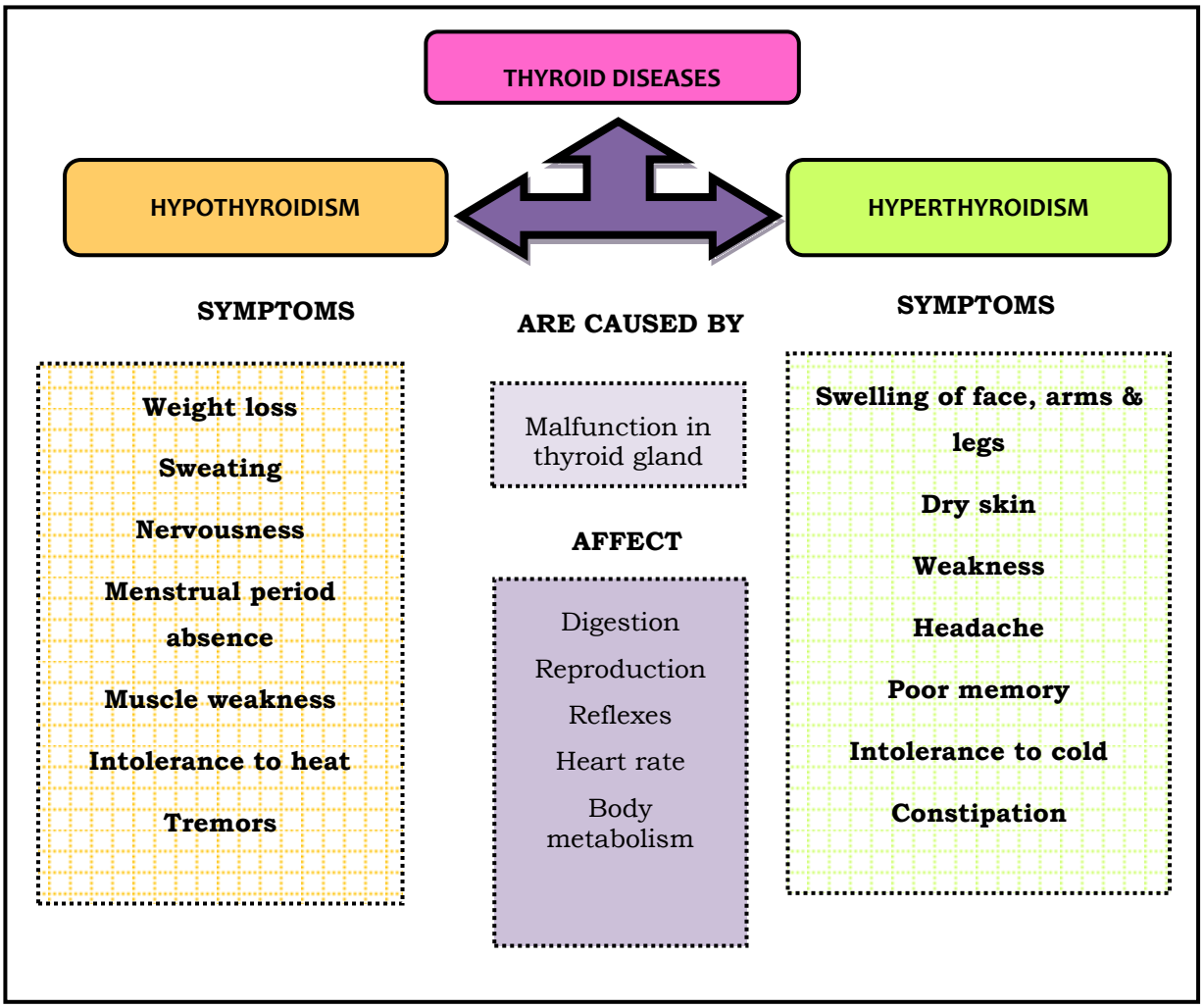

**Source: Bianco and Kim, 2006**

Free T4 (fT4) is metabolized in the tissues to the active form free T3 (fT3) by three deiodinase enzymes. The tissues have different rates of fT3 production and uptake according to the presence of the deiodinase enzymes. Type I deiodinase (D1) is located in the liver, kidneys, thyroid and pituitary and is primarily responsible for fT3 formation. Its activity is low in the fetus. Type II deiodinase (D2) is located in the central nervous system and pituitary and it produces a supply of fT3 to the brain. Type III deiodinase (D3) is located in the brain and in reproductive tissues and it inactivates both fT4 and fT3, maintaining an equilibrium in the fT3 concentration. Only D2 and D3 have been detected in human placental tissue, the former providing the placenta with a supply of fT3, and the latter maintaining its equilibrium (Ganong, 2005).

**Effects of thyroid hormones**

The thyroid hormones stimulate oxygen consumption and increase the metabolic rate. They have an effect on the heart and connective tissues and affect growth and development. Thyroid hormones have a marked effect on brain development, especially on the cerebral cortex and the basal ganglia. Lack of thyroid hormones during development due to iodine deficiency leads to cretinism- a condition which can be fully prevented with iodine prophylaxis. In addition, congenital hypothyroidism, if unnoticed and untreated, leads to intellectual deficiency, which fortunately can be prevented by screening programmes and thyroxine treatment started in infancy (Hadley and Levine, 2007).

**Figure 2.5: Thyroid diseases and their symptoms**



High concentrations of thyroid hormones can elevate the body temperature as they increase the metabolic rate. A rise in body temperature activates the cardiovascular system to dissipate heat. Also, peripheral vascular resistance decreases due to vasodilatation, and blood volume and cardiac output increases through the direct actions of thyroid hormones. Thyroid hormones also lead to protein catabolism from muscles and increase carbohydrate absorption. Therefore they have an effect on glucose metabolism. They also lower circulating cholesterol levels by increasing the hepatic removal of cholesterol (Ganong, 2005).

## 2.2 THYROID DISEASES

### Hypothyroidism

Hypothyroidism is a deficiency of T4. It is present in approximately 2% of women and 0.1–0.2% of men (Tunbridge et al, 1977; Vanderpump et al, 1995). The prevalence of congenital hypothyroidism is 1/4000. Hypothyroidism can present with different symptoms such as fatigue, dry and coarse skin, puffiness, weight gain, diminished perspiration and poor cold endurance, as well as menstrual abnormalities and infertility in women (Figure 2.5). Hypothyroidism is often caused by factors associated with the thyroid itself (primary hypothyroidism). Only 5% of hypothyroidism is the result of central hypothyroidism, i.e. due to lack of TSH or its effects. The most common causes of hypothyroidism are autoimmune thyroiditis, radioiodine therapy and thyroid surgery, but also iodine, medicines or rare genetic disorders may cause hypothyroidism.

#### *Overt hypothyroidism*

Overt hypothyroidism is diagnosed by laboratory testing and is presented as low concentrations of circulating thyroid hormones with raised concentrations of TSH. Overt hypothyroidism is often detected and treated before the onset of pregnancy, since it causes infertility and recurrent miscarriages (Krassas et al, 1999). However, 0.2–0.5%

of all pregnant women have overt hypothyroidism (Allan et al, 2000; Casey et al, 2005), representing either new undiagnosed cases or inadequate treatment of previously detected disease.

***Subclinical hypothyroidism***

Subclinical hypothyroidism is present when TSH concentrations are raised but thyroid hormone concentrations are still normal. The presence of thyroid antibodies reveals if hypothyroidism is caused by chronic autoimmune thyroiditis. Subclinical hypothyroidism has a prevalence of approximately 2.2–2.5% in pregnant women (Allan et al, 2000; Casey et al, 2005). Hypothyroidism is treated with thyroxine (Valimaki and Schalin-Jantti, 2009). Nearly all levothyroxine treated mothers need an approximately 30–50% increase in their dosage to maintain euthyroidism during pregnancy (Mandel et al, 1993; Alexander et al, 2004). The dosage should be raised to 2.0–2.4 μg/kg when trying to conceive or at least when pregnancy tests are positive. It is noteworthy that women without residual thyroid function (after radioiodine treatment or thyroidectomy) require a greater increase in their daily dose of levothyroxine than women with chronic autoimmune thyroiditis.

Hypothyroid mothers should be followed by using thyroid function tests (serum TSH and fT4) throughout pregnancy (4 weeks apart if test results are abnormal and 6–8 weeks apart if results are within reference intervals) and their thyroxine dosage altered when necessary. This can be based on the degree of TSH elevation: for women with serum TSH levels of 5–10 mIU/L the increment in thyroxine is 25–50 μg/day; for those with serum TSH levels of 10–20 mIU/L the increment is 50–75μg/day and for those with serum TSH levels of > 20 mIU/L the increment is 75–100 μg/day.

A newly diagnosed case of hypothyroidism during pregnancy should be vigorously treated. Thyroxine treatment should be initiated with a dose of 100–150 μg thyroxine/day or a dose calculated according to body weight. In severe hypothyroidism, therapy may be initiated with

a double dose of the estimated final daily dose for the first few days. The treatment of hypothyroidism during pregnancy should be adjusted so that TSH and fT4 levels remain within the established reference ranges for pregnant women (Abalovich et al, 2007).

**Hypothyroxinemia**

Hypothyroxinemia is defined as a low serum fT4 concentration with normal TSH concentrations. Its prevalence during pregnancy is approximately 1.3–2.1% (Casey et al, 2007; Cleary-Goldman et al, 2008). The clinical significance of hypothyroxinemia is still largely unknown. It is noteworthy that central hypothyroidism shows similar features. Hypothyroxinemia has been particularly associated with iodine deficiency and it has a high prevalence in iodine-deficient populations. Due to lack of raw materials (iodine), the thyroid produces more T3 instead of T4, thus leading to hypothyroxinemia (Glinoer, 1997).

**Chronic autoimmune thyroiditis**

Chronic autoimmune thyroiditis is the most common cause of primary hypothyroidism. A high affinity marker of the disease is thyroid peroxidase antibody (TPO-Ab), and high concentrations of TPO-Ab can be measured in most cases of autoimmune thyroiditis. A similar marker of autoimmune thyroiditis is thyroglobulin antibody (TG-Ab). Autoimmune thyroiditis is a silent disease process in which the thyroid may be enlarged or atrophied and the production of thyroid hormones may decrease resulting in hypothyroidism. The prevalence of positive thyroid auto antibodies increases with age, with the highest frequency observed in women aged 40–60 years (Vanderpump et al, 1995; Hollowell et al, 2002).

Positive TPO-Ab and/or TG-Ab test results are found in approximately 5% of euthyroid pregnant women. However, a thyroid autoantibody prevalence of up to 15% has been found in pregnant populations (Cleary-Goldman et al, 2008). At parturition, 56% of thyroid antibody-

positive mothers have been reported to have high TSH values. During gestation, a significant amount of TPO-Ab and/or TG-Ab positive women are at risk of developing hypothyroidism, since they have lower thyroid function reserve (Glinoer et al, 1994). Chronic autoimmune thyroiditis is the most important cause of hypothyroidism in pregnant women, and up to 90% of women with hypothyroidism during pregnancy test positive for thyroid antibodies (Klein et al, 1991).

**Hyperthyroidism**

Hyperthyroidism, overproduction of thyroid hormone, has a prevalence of approximately 1% in the population. Thyrotoxicosis refers to increased amounts of thyroid hormones in the circulation, the cause of which can be also other than thyroid hormone overproduction. The symptoms of hyperthyroidism include anxiousness, tachycardia or even atrial fibrillation, increased heat sensitivity, perspiration, skin changes, loss of weight, insulin resistance, fatigue and shivering. Overt hyperthyroidism complicates approximately 0.05–0.2% of pregnancies (Burrow, 1993). Hyperthyroidism is associated with ovulatory dysfunction, miscarriages and difficulties conceiving (Krassas et al, 1999; Anselmo et al, 2004) unless treated. Subclinical hyperthyroidism has a prevalence of approximately 1.7% in pregnant women (Casey et al, 2006).

**Figure 2.6: Normal, over-active and under-active thyroid gland**

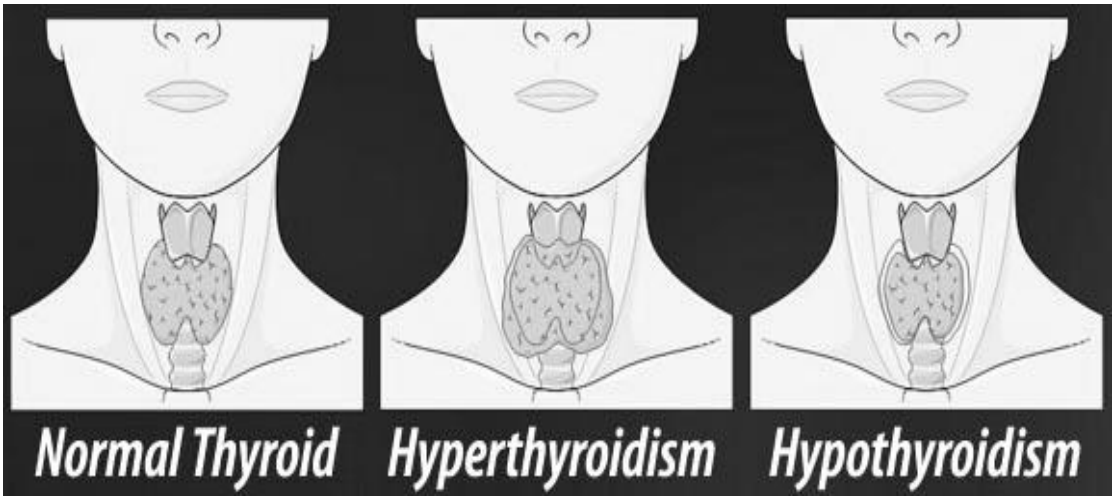


**Table 2.1: Etiology of hypothyroidism in pregnancy**

| |
|---|
| Hashimoto disease |
| Post-thyroid ablation/removal |
| Iodine deficiency |
| Primary atrophic hypothyroidism |
| TSH-dependent hypothyroidism |

**Source: Mestman et al, 1995**

**Table 2.2: Etiology of hyperthyroidism in pregnancy**

| |
|---|
| Graves's disease (85–90% of all cases) |
| Sub-acute thyroiditis |
| Toxic multi-nodular goiter |
| Toxic adenoma |
| TSH-dependent thyrotoxicosis |
| Exogenous T3 or T4 |
| Iodine-induced hyperthyroidism |
| Pregnancy-specific associations |
| Hyperemesis gravidarum |

**Source: Browne-Martin and Emerson, 1997**

**Table 2.3: Etiology of postpartum thyroid dysfunction**

| |
|---|
| Hyperthyroidism |
| *Primary* |
| Postpartum thyroiditis |
| Postpartum Graves's disease |
| *Secondary* |
| None* |
| Hypothyroidism |
| *Primary* |
| Postpartum thyroiditis |
| *Secondary* |
| Lymphocytic hypophysitis |
| Postpartum pituitary necrosis |

**Source: Mulder, 1998**

*TSH-producing pituitary tumors and thyroid hormone resistance are secondary causes of hyperthyroidism, but they do not have a predilection for the postpartum period.

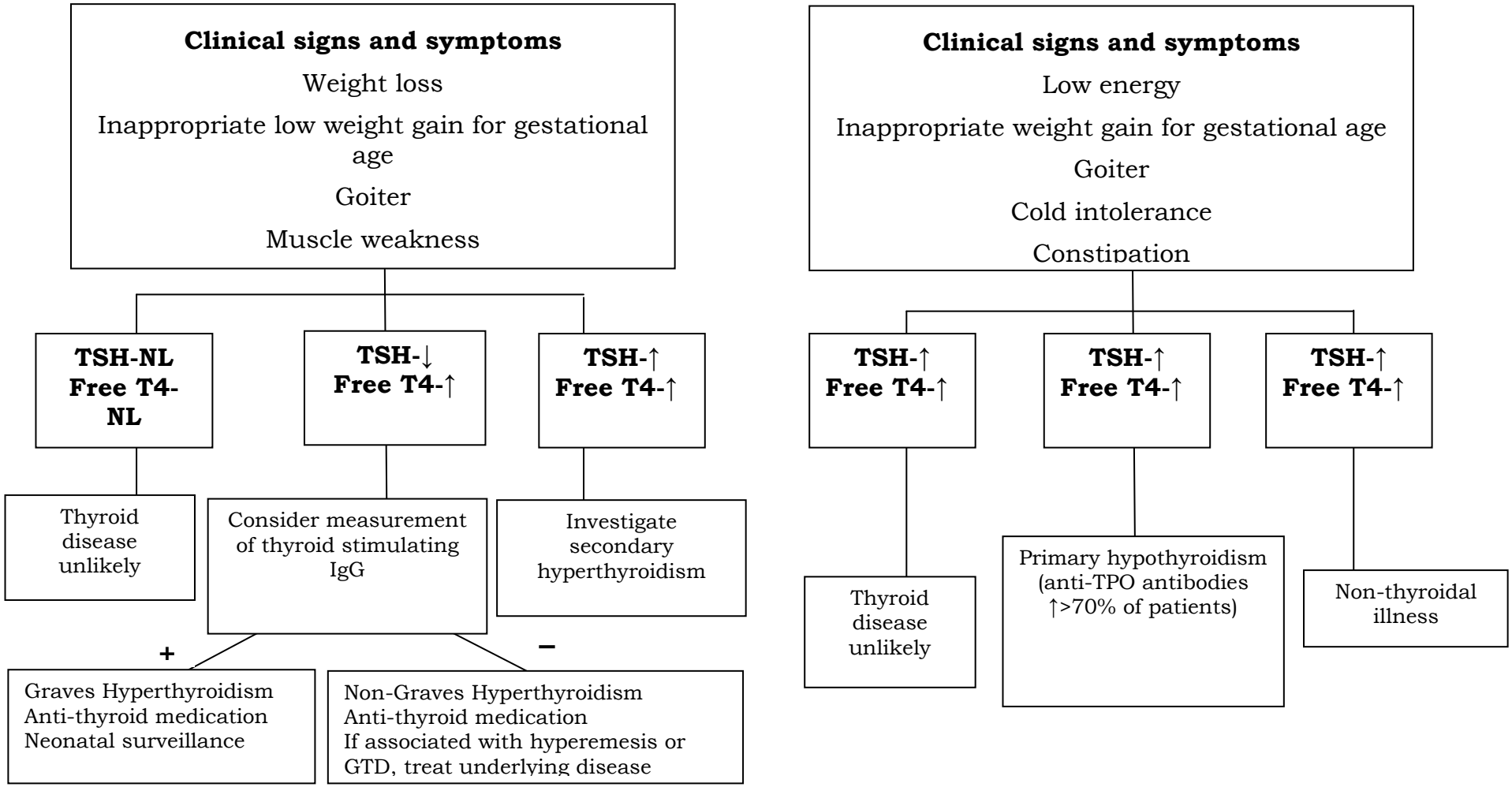



**Figure 2.7: Algorithm for the evaluation of hyperthyroidism [left] and hypothyroidism [right] during pregnancy**

**Source: Fantz et al, 1999**

## 2.3 THYROID FUNCTION DURING PREGNANCY

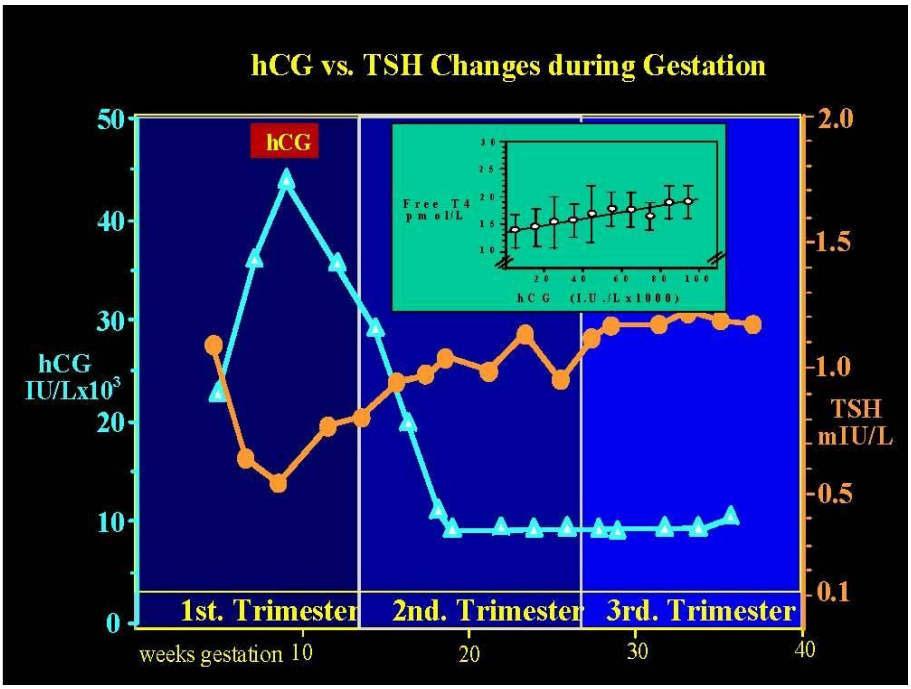
During normal pregnancy, the maternal thyroid produces up to 50% more thyroid hormones. This rise in thyroid hormones results from physiological changes in pregnancy.

**Factors affecting thyroid physiology during normal pregnancy**

### *1. Thyroid stimulation by hCG*

hCG has mild thyrotropic activity (Yoshimura, 1995; Goodwin, 1997). During the first trimester of pregnancy, when hCG is at its greatest concentration, serum TSH concentrations drop, creating the inverse image of hCG (Figure 2.8). In most pregnancies, this decrease in TSH remains within the health-related reference interval (Glinoer, 1990). Under pathological conditions in which hCG concentrations are markedly increased for extended periods, significant hCG-induced thyroid stimulation can occur, decreasing TSH and increasing free hormone concentrations.

**Figure 2.8: Changes in TSH and hCG during gestation**




**Source: Glioner, 1990**

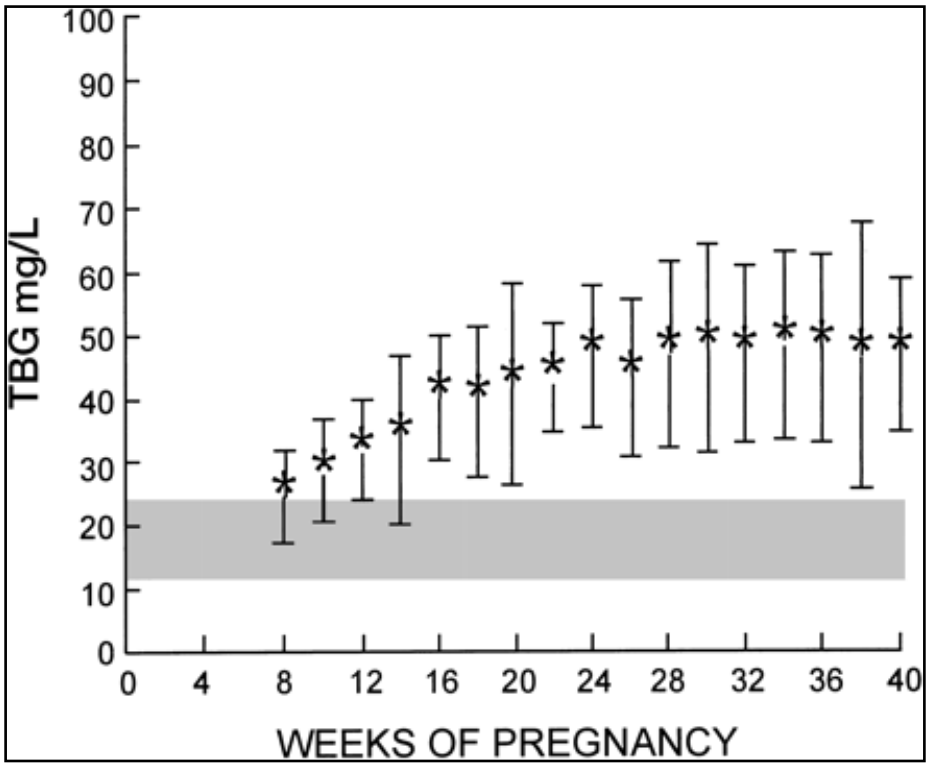
### *2. Increase in thyroid binding globulins*

Thyroid hormones are transported in serum bound to three proteins: thyroxine binding globulin (TBG), transthyretin, and albumin. Although TBG is present in low abundance in serum, it has a high

affinity for thyroid hormones and is responsible for the transport of the majority of T4-68% and T3-80% (Larsen, 1998). During pregnancy, the affinities of the three binding proteins for T4 and T3 are not significantly altered, but the circulating concentration of TBG increases two to threefold, whereas the concentrations of albumin and transthyretin remain unchanged (Ain et al, 1987; Glinoer, 1997 and Skjoldebrand et al, 1982). Serum TBG increases a few weeks after conception and reaches a plateau during mid-gestation (Figure 2.9) (Skjoldebrand et al, 1982). The mechanism for this increase in TBG involves both an increase in hepatic synthesis of TBG and an estrogen-induced increase in sialylation, which increases the half-life of TBG [from 15 min to 3 days for fully sialylated TBG (Ain et al, 1987; Glinoer, 1997 and Brent, 1997)].

**Figure 2.9: TBG during normal pregnancy (mean±2sd) in 2-week intervals**

[Shaded area-reference interval for non-pregnant fertile women]




**Source: Fantz et al, 1999**

### *3. Increase in total T4 and T3*

Plasma concentrations of total T4 and T3 are also increased during pregnancy, often outside the health-related reference interval. Total T4

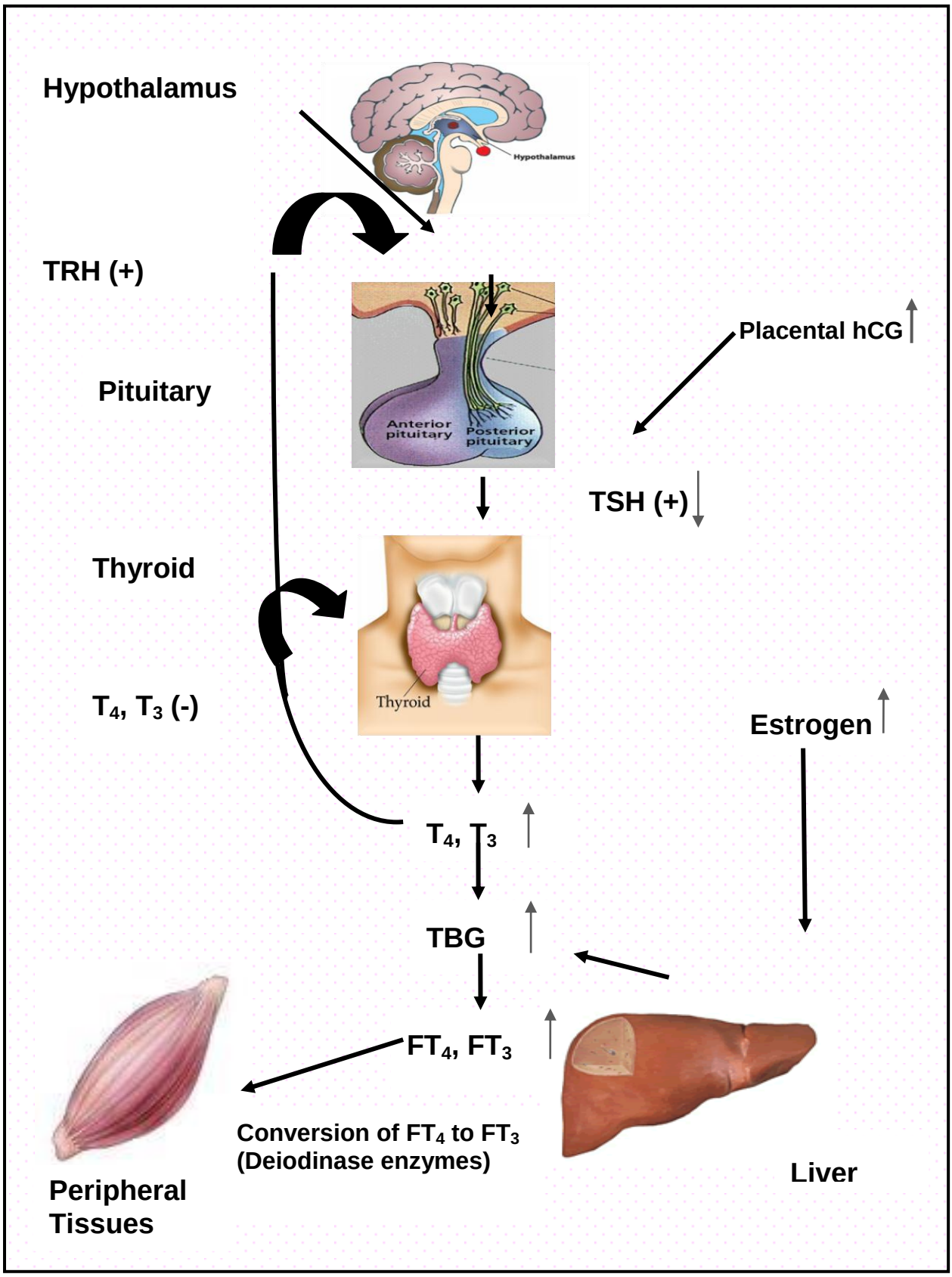
and total T3 concentrations increase sharply in early pregnancy and plateau early in the second trimester at concentrations 30–100% greater than pre pregnancy values (Skjoldebrand et al, 1982; Guillaume et al, 1985). The etiology of this increase in total circulating thyroid hormones involves, primarily, increased concentrations of plasma TBG (Glinoer, 1997; Skjoldebrand et al, 1982 and Guillaume et al, 1985). Another proposed mechanism for this increase in total thyroid hormone concentrations is production of type III deiodinase from the placenta (Glinoer, 1997). This enzyme, which converts T4 to reverse T3, and T3 to diiodotyrosine (T2), has extremely high activity during fetal life (Burrow et al and Fisher et al, 1994). Increased demand for T4 and T3 has been suggested to increase production of these hormones with, ultimately, increased concentrations in the circulation (Glinoer, 1997). Changes in free T4 and T3 concentrations during pregnancy have been controversial.

**Table 2.4: Thyroid function during pregnancy**

| Physiologic change | Resulting change in thyroid activity |
|---|---|
| ↑ Serum estrogens | ↑ Serum TBG |
| ↑ Serum TBG | ↑ Demand for T4 and T3<br>↑ In total T4 and T3 |
| ↑ hCG | ↓ TSH (in reference range unless hCG >50,000 IU/L)<br>↑ FT4 (in reference range unless hCG >50,000 IU/L) |
| ↑ Iodine clearance | ↑ In dietary requirement for iodine<br>↓ In hormone production in iodine deficient areas<br>↑ Goitre in iodine deficient areas |
| ↑ Type III deiodinase | ↑ T4 and T3 degradation<br>↑ Demand for T4 and T3 |
| ↑ Demand for T4 and T3 | ↑ Serum thyroglobulin<br>↑ Thyroid volume<br>↑ Goitre in iodine deficient areas |

**Source: Fantz et al, 1999**

**Figure 2.10: Changes in the hypothalamus-pituitary-thyroid axis during pregnancy**



***4. Increase in serum thyroglobulin***

Thyroglobulin is frequently increased during pregnancy, reflecting the increased activity of the thyroid gland during pregnancy (Glinoer, 1997). The increase in thyroglobulin can be seen as early as the first trimester, but it is more pronounced in the latter part of pregnancy (Glinoer, 1997). Increased serum thyroglobulin concentrations are also associated with an increase in thyroid volume.

***5. Increase in renal iodide clearance***

In pregnancy, the renal clearance of iodide increases substantially because of an increased glomerular filtration rate (Glinoer, 1997). The iodide loss lowers the circulating concentrations of iodide and produces a compensatory increase in thyroidal iodide clearance. In areas of the world where iodine intake is sufficient, such as the US, the iodide losses in the urine are not clinically important. In other areas of the world, however, iodine deficiency during pregnancy can lead to hypothyroidism and goiter and poses a serious public health issue. Approximately 500 million people live in areas of overt iodine deficiency (Glinoer, 1997). In the non pregnant condition, adequate iodine intake is estimated to be 100–150 mg/day.

## 2.4 DEVELOPMENT OF THE THYROID GLAND AND FETAL THYROID HORMONE SUPPLY

### Thyroid hormones and neurodevelopment

Thyroid hormones have no influence on very early developmental events, such as neural induction and establishment of polarity, but regulate later processes, including neurogenesis, myelination, dendrite proliferation and synapse formation (Bernal et al, 2003; Zoller and Rovet, 2007) (Figure 2.11). Numerous thyroid hormone responsive genes have been identified (Bernal et al, 2003) and the timing of the onset of thyroid hormone action in the developing brain is crucial (Zoller and Rovet, 2007; de Escobar et al, 2004; Obregon et al, 2007).

For example, endemic neurological cretinism is due to maternal iodine deficiency and the resulting maternal hypothyroxinemia, which is defined as thyroxine (T4) concentrations that are low for the stage of pregnancy. Low maternal T4 levels cause neurological hypothyroidism in the foetus, which results in profound mental retardation, cerebral spastic diplegia, deaf-mutism and squint in the absence of general signs of hypothyroidism (Porterfield and Hendrich, 1993). Although endemic cretinism can be prevented by public health measures, such as iodine supplementation, that prevent or correct first trimester maternal hypothyroxinemia, iodine deficiency remains the commonest endocrine disorder worldwide and the most frequent cause of preventable mental retardation (de Escobar et al, 2000). By contrast, neurological features in neonatal hypothyroidism are less severe, dependent on the severity of hypothyroidism and largely preventable by immediate thyroid hormone replacement, although deficits in memory and IQ may persist (Zoller and Rovet, 2007). Untreated neonates exhibit growth retardation and general features of hypothyroidism with mental retardation, tremor, spasticity and speech and language deficits (Zoller and Rovet, 2007; Porterfield and Hendrich, 1993). Differences between endemic cretinism and congenital hypothyroidism illustrate that the timing of thyroid hormone action is fundamental for neurodevelopment.

**Timing of thyroid hormone action in the brain**

Three stages of thyroid hormone dependent neurological development can be recognised (Figure 2.11).

***First stage:*** The first occurs before the onset of foetal thyroid hormone synthesis, which occurs at 16–20 weeks post-conception in humans or by embryonic day E17.5–18 in the rat. During this period, thyroid hormone exposure comes only from maternally synthesised hormone (de Escobar et al, 2004; Obregon et al, 2007) and influences

neuronal proliferation and migration of neurones in the cerebral cortex, hippocampus and medial ganglionic eminence (Narayanan, 1985; Lucio et al, 1997; Cuevas et al, 2005; Auso et al, 2004).

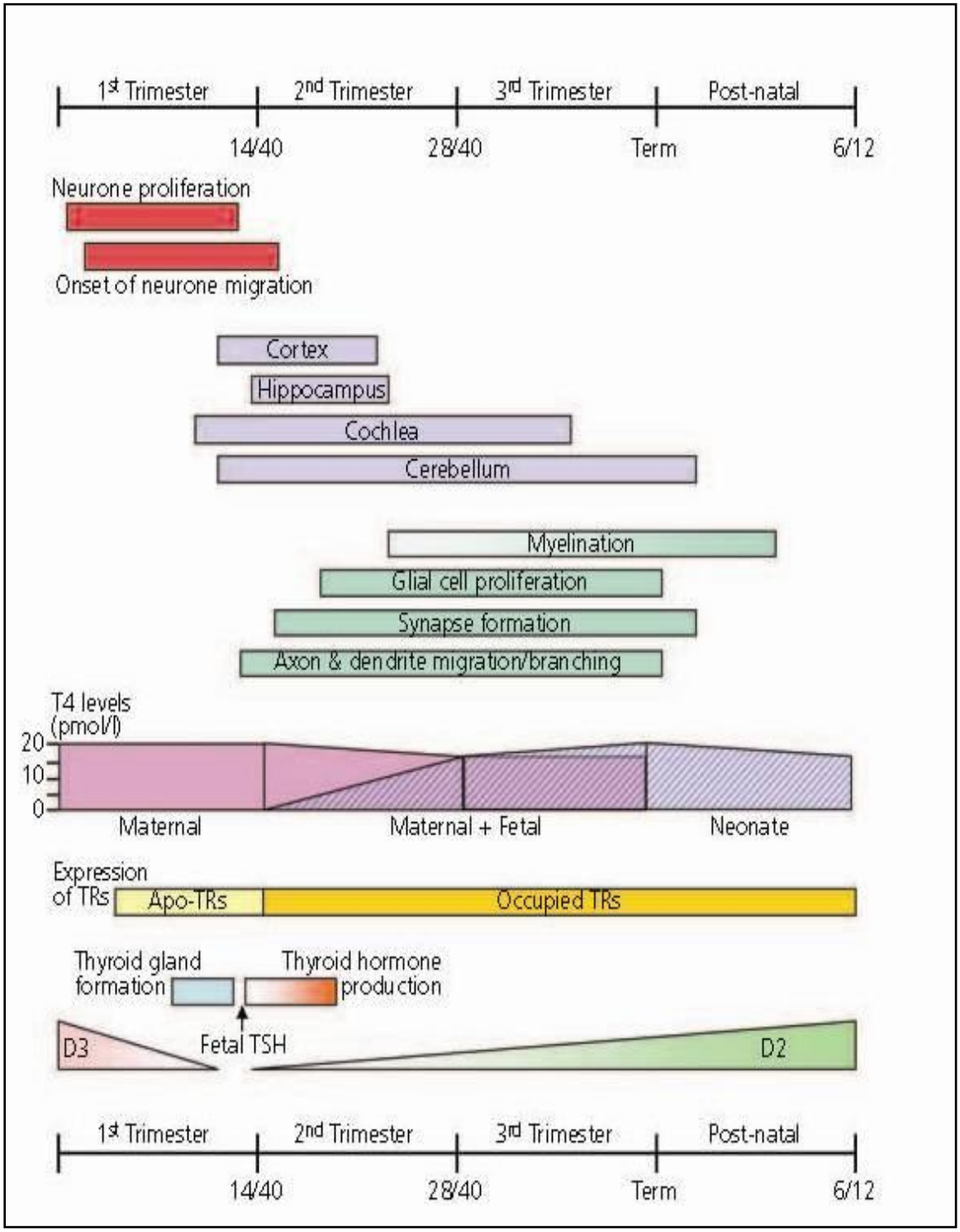
***Second stage:*** The second stage occurs during the remainder of pregnancy after the onset of foetal thyroid function when the developing brain derives its supply of thyroid hormones from both the foetus and the mother (de Escobar et al, 2004; Obregon et al, 2007). During this period, thyroid hormone dependent processes include neurogenesis, neurone migration, axonal outgrowth, dendritic branching and synaptogenesis, together with the initiation of glial cell differentiation and migration and the onset of myelination (Bernal, et al, 2003; Porterfield and Hendrich, 1993; de Escobar et al, 2000).

***Third stage****:* The third stage occurs in the neonatal and post-natal period when thyroid hormone supplies to the brain are entirely derived from the child and critical for continuing maturation. During this period, migration of granule cells in the hippocampal dentate gyrus and cerebellum, pyramidal cells in the cortex and Purkinje cells in the cerebellum are sensitive to thyroid hormones and thyroid hormone-dependent gliogenesis and myelination continues (Bernal, et al, 2003; Porterfield and Hendrich; 1993; de Escobar et al, 2004).

**Figure 2.11: Relationship between thyroid hormone action and development of the brain**

1. In the first trimester of pregnancy early neuronal proliferation and migration is dependent on maternal thyroxine (T4). In fetal tissues, inactivating type 3 deiodinase (D3) enzyme expression falls and development of the thyroid gland commences.
2. By the end of the first trimester, development of the hypothalamic-pituitary axis has occurred and a surge in thyroid-stimulating hormone (TSH) secretion results in the onset of fetal thyroid hormone production, expression of the activating type 2 iodothyronine deiodinase enzyme (D2) and increasing occupation of thyroid hormone receptors (TRs) by 3,5,3'-L-triiodothyronine (T3).

3. Continuing development of the brain in the second and third trimesters relies increasingly on T4 produced by both the fetus and mother. Continued post-natal development is entirely dependent on neonatal thyroid hormone production. Apo-TR, unliganded unoccupied thyroid hormone receptor.

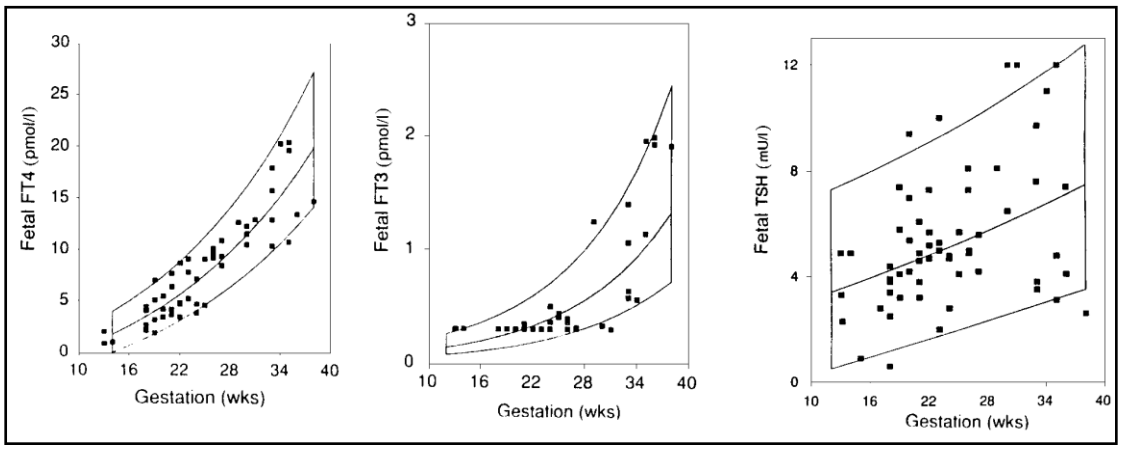



**Source: Williams, 2008**

**Circulating thyroid hormone concentration in human pregnancy**

There is a rise in fetal circulating concentrations of total T4, free T4, free triiodothyronine (T3) and thyroxine binding globulin (TBG) with gestation (Fisher, 1992; Burrow et al, 1994; Kilby et al, 1998) (Figure 2.12).

**Figure 2.12: The ontogeny of fetal thyroid hormone metabolism and concentrations of fetal TSH, T4 and T3 during gestation**




**Source: Chan and Kibly, 2000**

In the first and second trimesters there is a much higher concentration of free T4 in the maternal circulation compared with the fetal circulation. The difference decreases towards term as fetal thyroid function matures. However, even at term maternal serum free T3 concentrations are two to threefold more than those in the fetus and approximately 30% of thyroid hormones measured in cord blood are still derived from the mother (Thorpe-Beeston et al, 1992; Delbert and Fisher 1997b).

**Thyroid hormone concentrations in fetal brain**

In humans, both T3 and T4 can be detected in the first trimester brain before the fetal thyroid gland becomes active, possibly indicating that thyroid hormones transferred from the mother play an important role (Bernal and Pekonen, 1984; Sinha et al, 1997). T3 is not detectable in other fetal tissues apart from the brain at this stage, lending support to the theory that there is a specific role for thyroid hormones in very

early brain development. T4 is detected in the brain at 11–14 weeks, the level increasing 2.5 times by 15–18 weeks. Even after the fetus begins to produce its own thyroid hormones in the second trimester, maternal thyroid hormones make a significant contribution towards the supply to the fetal brain. This is indicated by positive correlations between maternal serum T4 concentrations, fetal cerebro-cortical T4 and maternal urinary iodine excretion at this stage (Sinha et al, 1997).

**Utero–placental transfer of thyroid hormones**

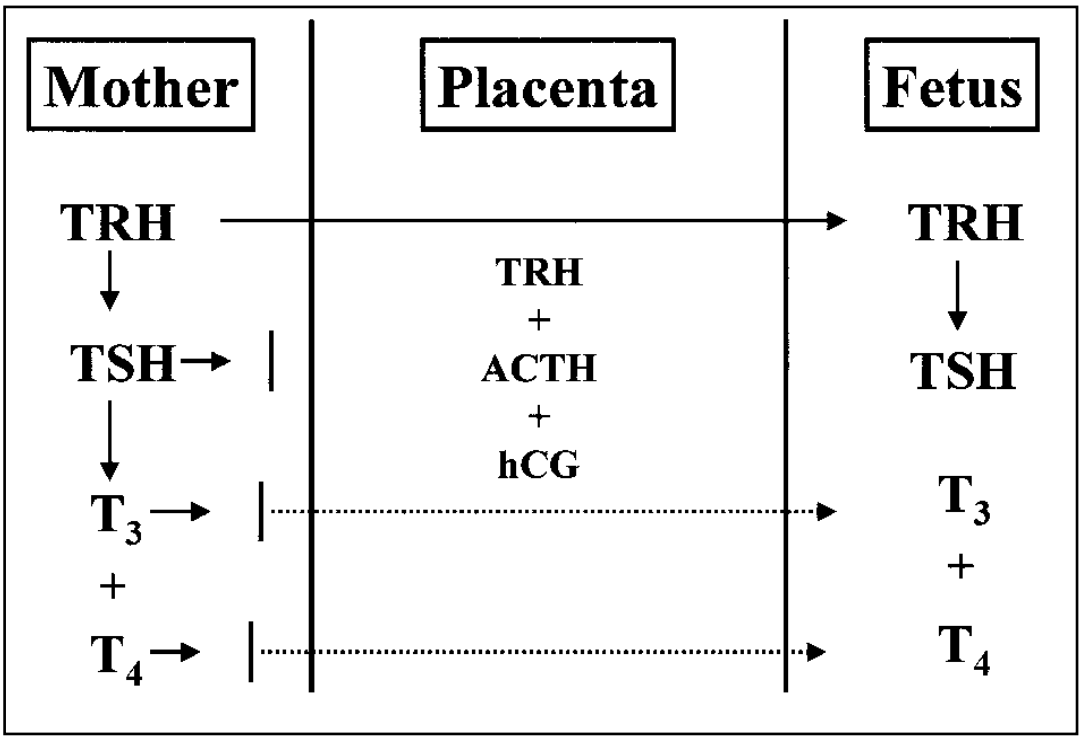
Circulating thyroid hormones in the human fetus are of both maternal and fetal origin and their presence is dependent on a functioning placenta for T4 transport and supply of iodide substrate (Figure 2.13). The placenta rapidly breaks down much of the T4 presented to it but significant amounts of T4 are still transferred (Delbert and Fisher, 1997b). The placenta is freely permeable to iodide and thyrotrophin-releasing hormone (TRH) but impermeable to TSH. Maternal TRH may have a role in controlling fetal thyroid function (Polk et al, 1991) before the maturation of the hypothalamic-pituitary-thyroid axis that occurs near term. TRH can be detected in the fetal hypothalamus by the end of the first trimester, at the same time as the thyroid begins to concentrate iodine.

TSH can be found in the pituitary at 10–12 weeks gestation, with serum levels rising towards term to values exceeding those of the adult (Fisher et al, 1977; Thorpe-Beeston et al, 1991). Chan and Kibly (2000) have indicated that the human placenta expresses all thyroid hormone receptor (TR) isoforms (both protein and mRNA) and that this expression increases with gestational age (Kilby et al, 1998). The role of these receptors in placental tissue is unknown at present.

By analogy, if the total thyroid hormone levels in the mother and fetus were similar, fetal tissues would be exposed to elevated levels of free T4 and free T3 (de Escobar et al, 2004) that are also detrimental to the critical temporal sequence of thyroid hormone responses during fetal development (Anselmo et al, 2004). These considerations underlie the

requirement for an efficient but incomplete utero–placental barrier to maternal thyroid hormone transfer that limits supplies and ensures that 'euthyroid' free hormone concentrations are maintained in fetal fluids and tissues. Following the onset of fetal thyroid hormone production, levels of total and free T3 remain very low in the fetus compared to the mother, whereas total and free T4 concentrations reach adult levels by the beginning of the third trimester (Thorpe-Beeston et al, 1991).

**Figure 2.13: Placental transfer of thyroid hormones**



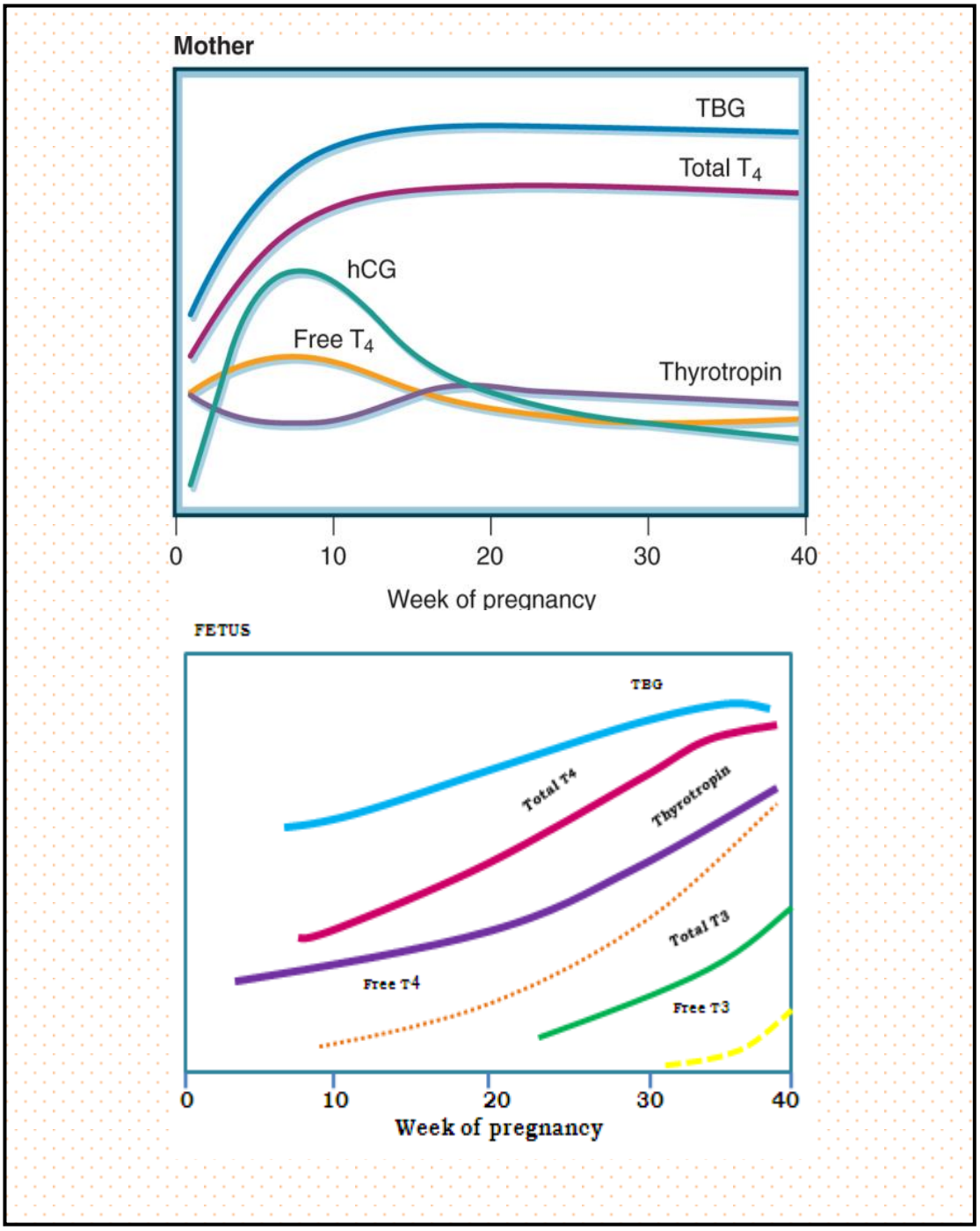

(TRH, thyroid releasing hormone; TSH, thyrotrophin; T3, triiodothyronine; T4, thyroxine; ACTH, adrenocorticotrophin; hCG, human chorionic gonadotrophin)

**Source: Chan and Kilby, 2000**

Despite the increasing concentrations of T4 in the fetus as gestation progresses, the fetal thyroid reserve remains low and the gland does not mature fully until birth (van den Hove and Vulsma et al, 1989). Thus, maternal thyroid hormones continue to contribute to fetal T4 levels until birth, as demonstrated in neonates who cannot synthesize their own thyroid hormones because of complete organification defects (Vulsma et al, 1989). Likewise, hypothyroxinemia in premature babies

results from a complete absence of maternal T4 and may account in part for their increased risk of cerebral palsy and neurological deficit (Zoller and Rovet, 2004; de Escobar et al, 2004; Lafranchi, 1999).

**Figure 2.14: Relative changes in maternal and fetal thyroid function during pregnancy**




**Source: Burrow et al, 1994**

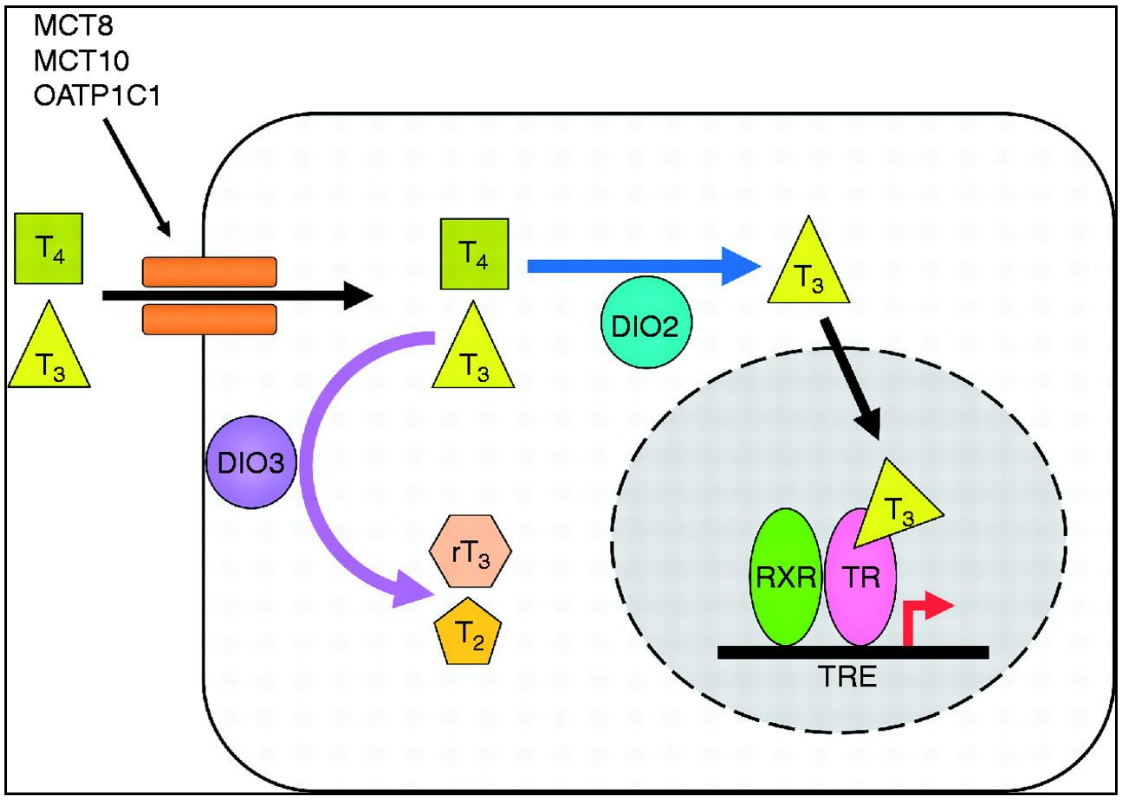
**Delivery of thyroid hormones to target tissues**

Thyroid hormones are lipophilic and poorly soluble in water. They bind reversibly to plasma transport proteins of varying affinity, which include thyroxine-binding globulin (TBG), transthyretin (previously called thyroxine-binding prealbumin), albumin and various lipoproteins. The free-fractions of circulating thyroid hormones are dependent on the concentrations and saturations of binding proteins and in normal serum free T4 represents 0.02% of the total T4 concentration, whereas free T3 is 0.3% of total T3 because of its lower affinity for TBG. As a result, total circulating concentrations of T4 are 50–60 folds higher than total T3, whereas free T4 levels are only approximately four-fold higher than free T3.

Circulating T4 is derived solely from synthesis and secretion by the thyroid gland, whereas 80% of circulating T3 is produced in peripheral tissues by enzymatic removal of an outer ring 5'-iodine atom from T4. Both T4 and T3 are transported into target tissues with equal efficiency and do not compete for uptake. Until recently, the mechanism of cellular entry of free thyroid hormones was not clear but was presumed to occur by passive diffusion because of their lipophilic nature (Friesema et al, 2005). In fact, the hormones enter target cells and cross the placenta via an energy-dependent, ATP-requiring, stereo-specific and saturable transport mechanism that is mediated by the mono-carboxylate transporter-8 (MCT8) (Friesema et al, 2003) and other transporter proteins such as OATP1c1, a member of the Na+- independent organic anion transporter protein (OATP) family (Jansen et al, 2005; Heuer, 2007).

**Figure 2.15: Regulation of intracellular supplies of T3 to the nucleus of T3 target cells**

MCT8 and MCT10, monocarboxylate transporters 8 and 10; OATP1C1, organic acid transporterprotein-1C1; DIO2 and DIO3, type 2 and 3 deiodinase enzymes; TR, thyroid hormonereceptor, RXR, retinoid X receptor; T4, thyroxine; T3, 3,5,3'-L-triiodothyronine; rT3,3,3',5'-triiodothyronine; T2, 3,3'-diiodothyronine.




**Source: Williams and Bassett, 2011**

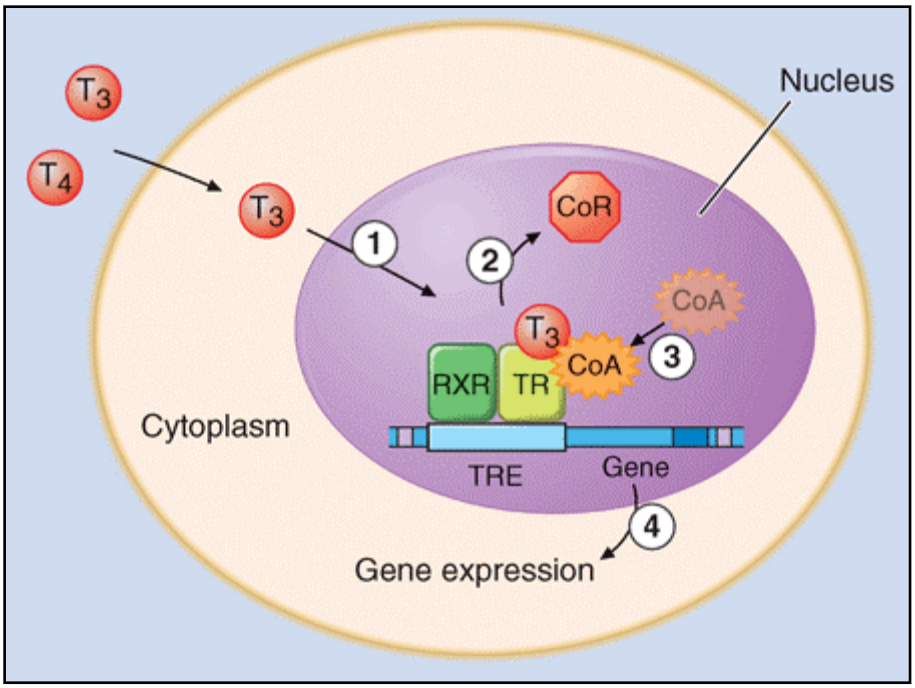
### Thyroid hormone action

Thyroid hormone receptors (TRs) bind T3 with high affinity and function as ligand-inducible transcription factors that regulate expression of T3-responsive target genes. TRα and TRβ are members of the steroid / thyroid hormone receptor super family (Sap et al, 1986; Weinberger et al, 1986). The TRα gene encodes three C-terminal variants in mammals: TRα1 binds T3 and DNA and is a functional receptor, whereas TRα2 and TRα3 do not bind T3 (Harvey and Willliams, 2002). The TRβ gene encodes two N-terminal variants in all vertebrates TRβ1 and TRβ2, both of which are functional receptors.

Unlike most nuclear receptors, unliganded apo-TRs compete with liganded TRs for DNA response elements and act as potent repressors that exert important physiological roles during the development of specific tissues including the brain (Hashimoto et al, 2001; Chassande, 2003; Venero et al, 2005; Wallis et al, 2008). Apo-TRs interact with co-repressor proteins, which recruit histone deacetylases and maintain a non-permissive chromatin structure to inhibit gene transcription. By contrast, liganded TRs bind to co-activators in a T3-

dependent manner. Co-activators possess intrinsic histone acetyl transferase activity, which facilitates formation of permissive nucleosomes and activation of gene expression. Thus, the opposing effects of unoccupied and occupied TRs result in greatly increased amplitude of transcriptional response to T3 (Harvey and Williams, 2002). Although circulating levels of free T4 are four-fold higher than free T3, the TR has at least a 15-fold greater affinity for T3 (Lin et al, 1990), indicating that T4 is a pro-hormone that must be converted to T3 prior to the onset of thyroid hormone action (Bianco et al, 2006).

**Figure 2.16: Mechanism of thyroid hormone receptor action**




**Source: Longo et al, accessible at www.accessmedicine.com**

The thyroid hormone receptor (TR) and retinoid X receptor (RXR) form heterodimers that bind specifically to thyroid hormone response elements (TRE) in the promoter regions of target genes. In the absence of hormone, TR binds co-repressor (CoR) proteins that silence gene expression.

The numbers refer to a series of ordered reactions that occur in response to thyroid hormone: (1) T4 or $T_3$ enters the nucleus; (2) $T_3$ binding dissociates CoR from TR; (3) Coactivators (CoA) are recruited to the $T_3$-bound receptor; (4) gene expression is altered.

**Thyroid hormone metabolism (iodothyronine)**

***Placenta***

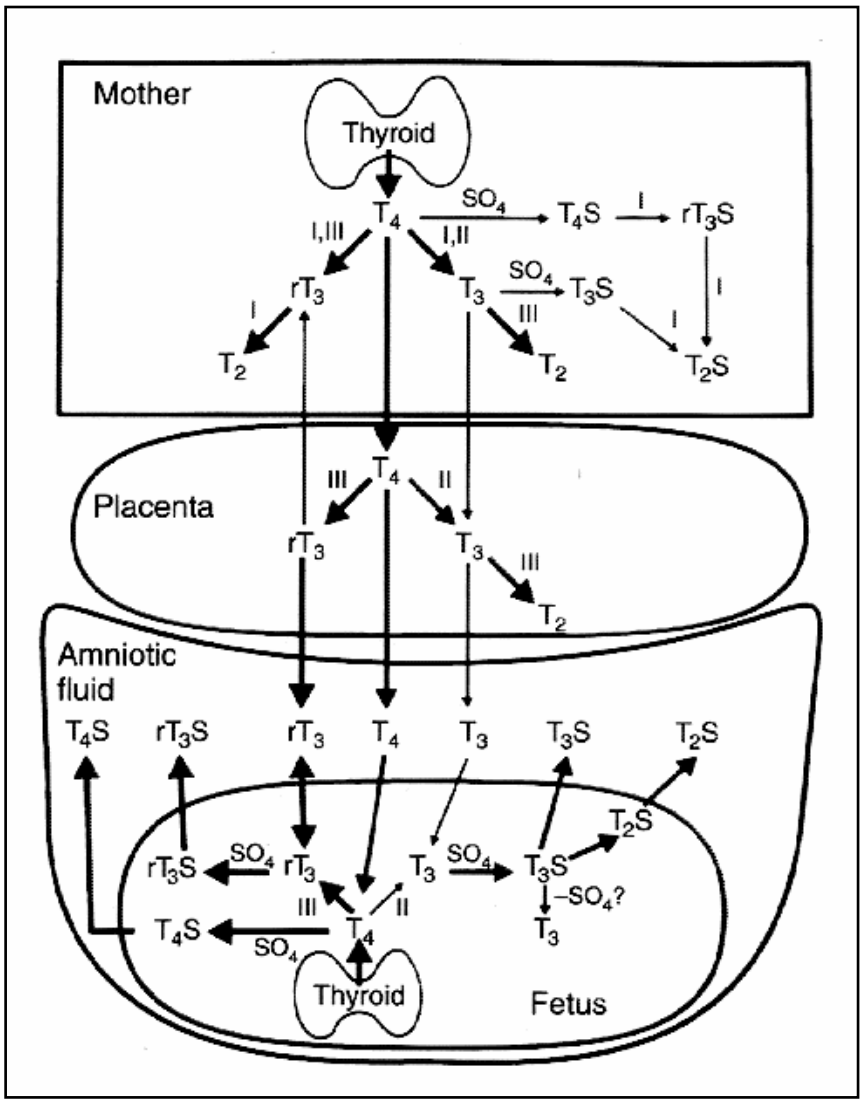
Three enzymes catalyze the deiodination of iodothyronine in human tissues (Larsen et al, 1981). Type I deiodinase, which catalyzes the deiodination of both the outer and inner rings, is a seleno-protein expressed in adults in liver, kidney, thyroid, and pituitary gland (Mandel et al, 1992). This enzyme is responsible for most of the T3 in serum. It also catalyzes the 5′ deiodination of 3,3′,5′-triiodothyronine (reverse T3), which is the rate limiting step in the clearance of reverse T3, as well as inner ring deiodination, particularly of T3 sulfate and T4 sulfate (Figure 2.17) (Otten et al, 1983; Mol and Visser, 1985).

Type II deiodinase acts only on the outer ring and prefers T4 and reverse T3. It is expressed in brain, pituitary gland, brown adipose tissue, keratinocytes, and placenta (Hidal and Kaplan, 1985; Kaplan et al, 1988; Houstek et al, 1993). Type III deiodinase, present in high amounts in placental tissue, brain, and epidermis, catalyzes the conversion of T4 to reverse T3 and T3 to 3, 3′ –di-iodothyronines (T2) (Roti et al, 1981). The activity of type II deiodinase is higher in the chorionic and decidual membranes of the placenta than in the amniotic membranes, whereas type III deiodinase is found mostly in trophoblasts (Hidal and Kaplan, 1985). The mixture of type II and type III deiodinases in the placenta provides for the conversion of T4 to T3 and of T4 and T3 to reverse T3 and T2, respectively (Figure 2.17).

As in other tissues, the activity of type II deiodinase increases when the availability of T4 decreases (Hidal and Kaplan, 1985). This suggests that deiodinase activity represents a homeostatic mechanism for maintaining T3 production in the placenta when maternal serum T4 concentrations are reduced (e.g., during hypothyroidism or iodine deficiency). Most of the beneficial effects of T3 generated by the action of maternal type II deiodinase are probably restricted to the placental cells because of the highly active placental type III deiodinase. In fact, placental type III deiodinase seems designed to maintain low serum T3

concentrations in the fetus while protecting decidual cells from hypothyroidism. The high rate of placental blood flow explains the absence of measurable difference in serum T4 and T3 concentrations between the umbilical artery and vein that might otherwise be anticipated given the high level of type III deiodinase activity in placenta (Abuid et al, 1973).

**Figure 2.17: Interaction of maternal, placental and fetal thyroid metabolism**




**Source: Burrow et al, 1994**

***Fetus***

The ontogeny of the three deiodinase that catalyze the progressive deiodination of T4 differs in the developing fetus. Type II and type III deiodinases appeared at mid-gestation, whereas type I deiodinase is

not evident until later (Fisher and Polk, 1989) (Figure 2.17). Accordingly fetal serum T3 concentrations are low (<15 ng/dl) before 30 weeks gestation; they increase gradually to about 50 ng/dl at term (Table 2.5) (Ballabio et al, 1989). By contrast maternal serum values are approximately 200 ng/dl. The preterm increase in fetal serum T3 concentration is due to an increase in type I deiodinase activity. Serum concentration of reverse T3, T4 sulfate, T3 sulfate, and reverse T3 sulfate in the umbilical cord at this time are high (Wu et al, 1993). The sulfated metabolites accumulate in fetal serum as a result of the very low type I deiodinase activity in fetal tissues and because the sulfated iodo-thyronines are not substrates for placental type III deiodinase (Santini et al, 1992*). Although T3 sulfates does not bind to nuclear T3 receptors and therefore has not biological activity, its parenteral administration in rats after thyroidectomy increases serum T3 concentrations and biological responses (Santini et al, 1993). Thus, T3 sulfate can be desulftaed in the gut. The liver, kidney, and the brain of adult rats have sulfatase activity, and desulfation of T3 sulfate to T3 occurs in the liver and brain of fetal rats (Kung et al, 1988; Santini et al, 1992). Thus, T3 sulfate could serve as a local source of T3 in fetal tissues containing sulfatase.

***The importance of iodothyronines in amniotic fluid***

In addition to being linked by the umbilical cord, the mother and fetus are linked by the amniotic cavity and amniotic fluid, providing a second pathway for fluid and molecular exchange. The amniotic fluid volume is the net balance of inflow, consisting of fetal urine and lung fluid, and outflow, consisting of fetal-maternal trans-amniotic fluid exchange and fetal swallowing (Tomoda et al, 1985). The pattern of iodothyronines in amniotic fluid reflects the effects of type III deiodinase activity in placental and fetal tissues, and the iodothyronine concentrations in amniotic fluid thus reflect both maternal and fetal thyroid hormone metabolism (Figure 2.17).

**Table 2.5: Iodothyronine concentrations in maternal and fetal serum and amniotic fluid**

| Iodothyronine | Maternal serum | Amniotic fluid (ng/dl) | | Fetal serum (ng/dl) | |
|---|---|---|---|---|---|
| | (mid-gestation) | 20 week | Term | 20 week | Term |
| T4 | 12,000 | 250 | 570 | 3,100 | 11,000 |
| T3 | 200 | 8.6 | 6.6 | 13 | 49 |
| T2 | 2.2 | 5.8 | 6.2 | - | 11 |
| rT3 | 24 | 130 | 69 | 250 | 270 |
| T4 sulfate | 1.8 | 28 | - | - | 21 |
| T3 sulfate | 2.9 | 6.6 | - | 6.7 | 12 |
| rT3 sulfate | 3.8 | 8.6 | - | - | 50 |

**Source: Burrow et al, 1994**

### Tissue thyroid status

D3 is expressed in fetal tissues and the placenta where it initially prevents maternal thyroid hormone access to the developing fetus (Wasco et al, 2003). At this time, unoccupied TRs are critical factors that generally maintain cell proliferation and prevent differentiation (Flamant et al, 2002). Increased pituitary D2 expression correlates with maturation of the hypothalamic-pituitary-thyroid (HPT) axis whereas its expression in T3-target tissues, concomitant with reduced expression of D3, results in conversion of unoccupied TRs into occupied TRs and the initiation of cell differentiation (Campos-Barros et al, 2000). Thus, the TR acts as a deiodinase-dependent developmental switch that regulates maturation of T3-dependent tissues. Expression of D2 is increased in hypothyroidism whereas D3 expression is increased in thyrotoxicosis, ensuring that the balance between D2 and D3 activities acts as a critical homeostatic regulator of T3 availability to the cell nucleus even at extremes of thyroid function. In the brain, D2 activity is markedly up-regulated in the

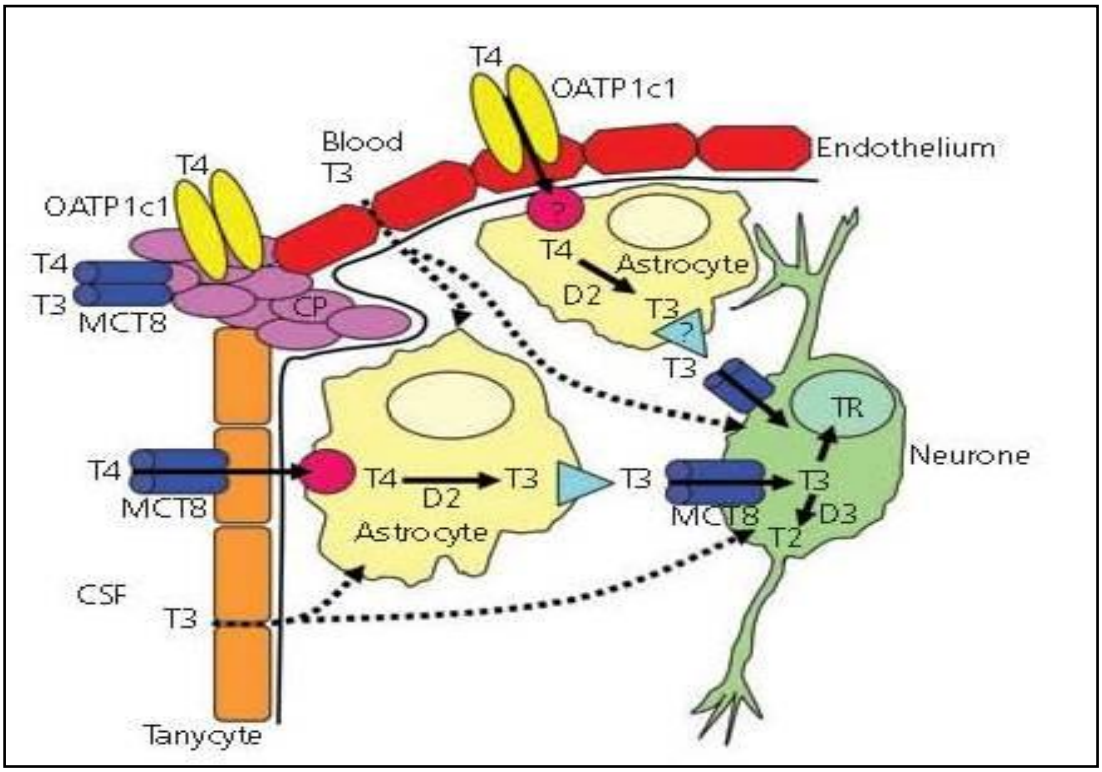
presence of low thyroid hormone levels (Burmeister et al, 1997), whereas D3 activity is strongly decreased (Friedrichsen et al, 2003).

This adaptation is thought to protect the susceptible brain from normal fluctuations in circulating thyroid hormone levels as well as to some extent protecting from the detrimental effects of hypothyroidism or hyperthyroidism, particularly during development (Guadano-Ferraz et al, 1999). Thus, local regulation of thyroid status in specific regions of the brain is achieved by the coordinated regional expression of the D2 and D3 enzymes (Kester et al, 2004) and T3 homeostasis results from compensatory reciprocal changes in activities of the D2 and D3 enzymes (Tu et al, 1997).

**Control of T3 availability and action in the brain**

The main cellular site of T3 action in the brain is the neuron, but T3 must gain access to neurons by a circuitous route that is subject to local regulation (Fliers et al, 2006) (Figure 2.18). The highly specific iodothyronine transporters OATP1c1 and MCT8 are both expressed in the central nervous system (CNS) and, in the last 5 years, our understanding of thyroid hormone uptake into the brain has progressed remarkably. Most likely, control of local T3 levels in brain tissue takes place in functional units of astrocytes and neurons (Friesema et al, 2006). T4 (and T3) must first cross the blood–brain barrier, and the T4-specific transporter OATP1c1 is ideally placed to achieve this as it is expressed at high levels in capillary endothelium throughout the CNS (Tohyama et al, 2004). Some T3 transport across the blood–brain barrier may also be mediated by MCT8 (Heuer et al, 2005). Thyroid hormones also enter via the choroid plexus–cerebrospinal fluid (CSF) barrier. OATP-1 and MCT-8 are expressed in choroid plexus and likely to mediate transport of T4 (Heuer et al, 2005), whilst MCT8 and D2 are co-expressed in tanycytes lining the third ventricle and facilitate access of thyroid hormones to hypothalamic nuclei and thyrotrophin-releasing hormone (TRH) neurons (Alkemade et al, 2005).

**Figure 2.18: Delivery of thyroid hormones to neurons**




**Source: Williams, 2008**

Circulating thyroid hormones enter the cerebrospinal fluid via the choroid plexus, which expresses both monocarboxylate transporter-8 (MCT8) and Na+-independent organic anion transporter protein 1c1 (OATP1c1) thyroid hormone-specific transporter proteins. The pro-hormone, T4 is transported across the blood–brain barrier via OATP1c1 in endothelial cells or MCT8 in tanycytes lining the third ventricle. Thyroxine (T4) enters glial cells including astrocytes via an unknown mechanism and is activated to 3,5,3'-L-triiodothyronine (T3) via the activating type 2 iodothyronine deiodinase (D2) enzyme. T3 is exported from glial cells by an unknown transporter to facilitate MCT8-dependent entry into neurons. T3 may also enter neurons directly from blood or cerebrospinal fluid (CSF) by a poorly defined route. T3 acts via thyroid hormone receptors (TRs) expressed in neurons or is metabolized by the inactivating type 3 deiodinase (D3) enzyme to inactive 3,3'-diiodothyronine (T2).

CP, choroid plexus, unknown transporter protein

Having entered the CNS, T4 is taken up by astrocytes via an unidentified transporter, where it is converted to T3 via local activity of D2 (Alkemade et al, 2005). T3 generated in astrocytes is then transported out of the cell via an unknown transporter before active uptake into neurons is mediated via MCT8 (Alkemade et al, 2005). T3 then exerts its major actions directly in neurons by regulating expression of T3-target genes (Bradley et al, 1992). T3 is finally metabolized and degraded by D3 in neurons (Alkemade et al, 2005).

**Thyroid hormone transport in the brain**

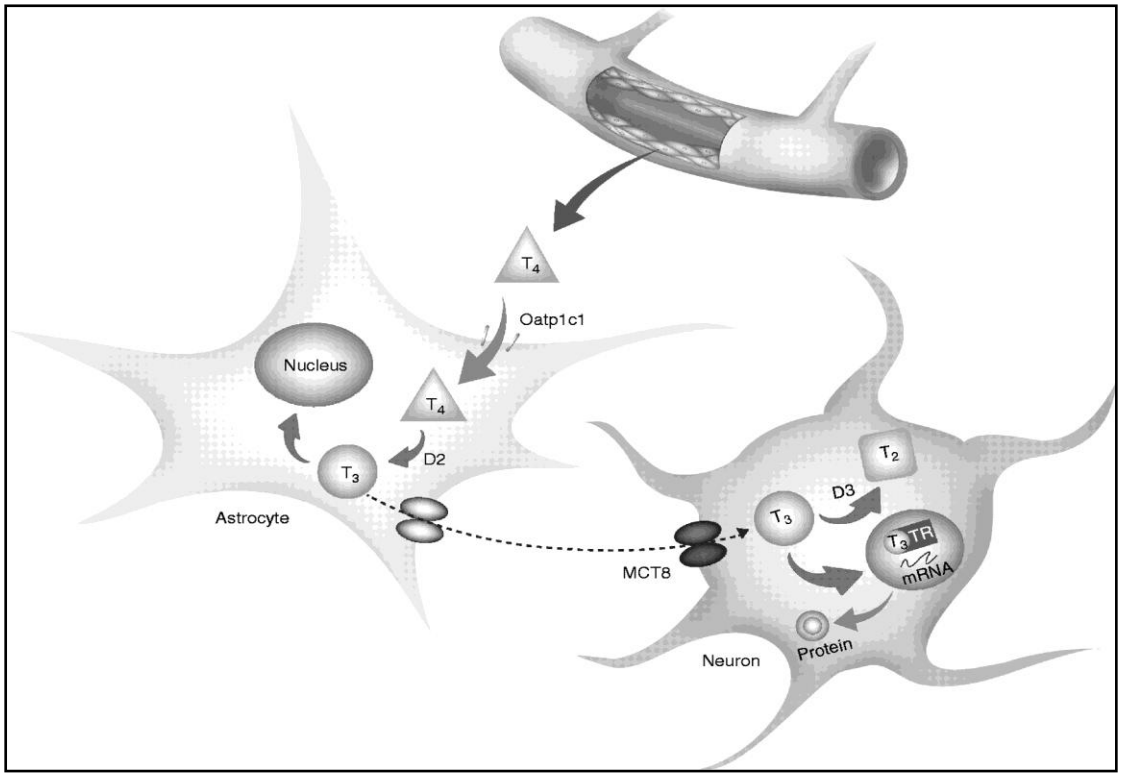
In situ hybridization, studies have shown MCT8 mRNA is expressed at high levels in choroid plexus, olfactory bulb, cerebral cortex, hippocampus and amygdala, at moderate levels in striatum and cerebellum and at low levels in some neuroendocrine nuclei. Colocalisation studies revealed that MCT8 is predominantly expressed in neurons. Together with a spatiotemporal pattern of MCT8 expression during the perinatal period, these data indicate that MCT8 plays an important role in CNS development by transporting thyroid hormones into neurons (Heuer et al, 2005). MCT8 is also expressed in pituitary folliculo-stellate cells (Alkemade et al, 2006); the same cells that express TSH receptor and may be involved in ultra-short feedback control of TSH secretion (Brokken et al, 2005). In support of this view, MCT8 protein is expressed in human hypothalamic paraventricular, supraoptic and infundibular nuclei and in the lining of ependymal cells of the third ventricle, which are all locations involved in negative feedback of TRH (Alkemade et al, 2005).

MCT8 mutations in humans

A key physiological role for thyroid hormone transport was confirmed in patients with mutations in SLC16A2 (previously MCT8) located on chromosome Xq13.2 (Friesema et al, 2004). There is global developmental delay including poor communication skills, no speech development, poor head control, mental retardation and varying degrees of truncal hypotonia, athetoid movements and motor

deficiency, which may include spastic quadriplegia in severe cases (Refetoff and Dumitrescu, 2007).

**Figure 2.19: Thyroid hormone transporters in the brain**




**Source: Patel et al, 2011**

T4 is transported to the brain thyroid hormone-binding proteins such as transthyretin (TTR), where T4 then passes out through endothelial cells lining the blood vessels. T4 is rapidly transported through the cell membrane transporter, Oatp1c1, located on the surface of astrocytes. T4 is then metabolized intra cellularly by D2 to T3, where it can then be transported out from astrocytes by an as yet unidentified cell membrane transporter. Within the brain parenchyma, T3 is then promptly uptaken by neurons and oligodendrocytes via the MCT8 cell membrane transporter. Within the cell, T3 can either translocate and bind to thyroid hormone receptors (TRs), resulting in thyroid hormone action, or be metabolized via D3 to biologically inactive T2.

## Thyroid hormone metabolism in the brain

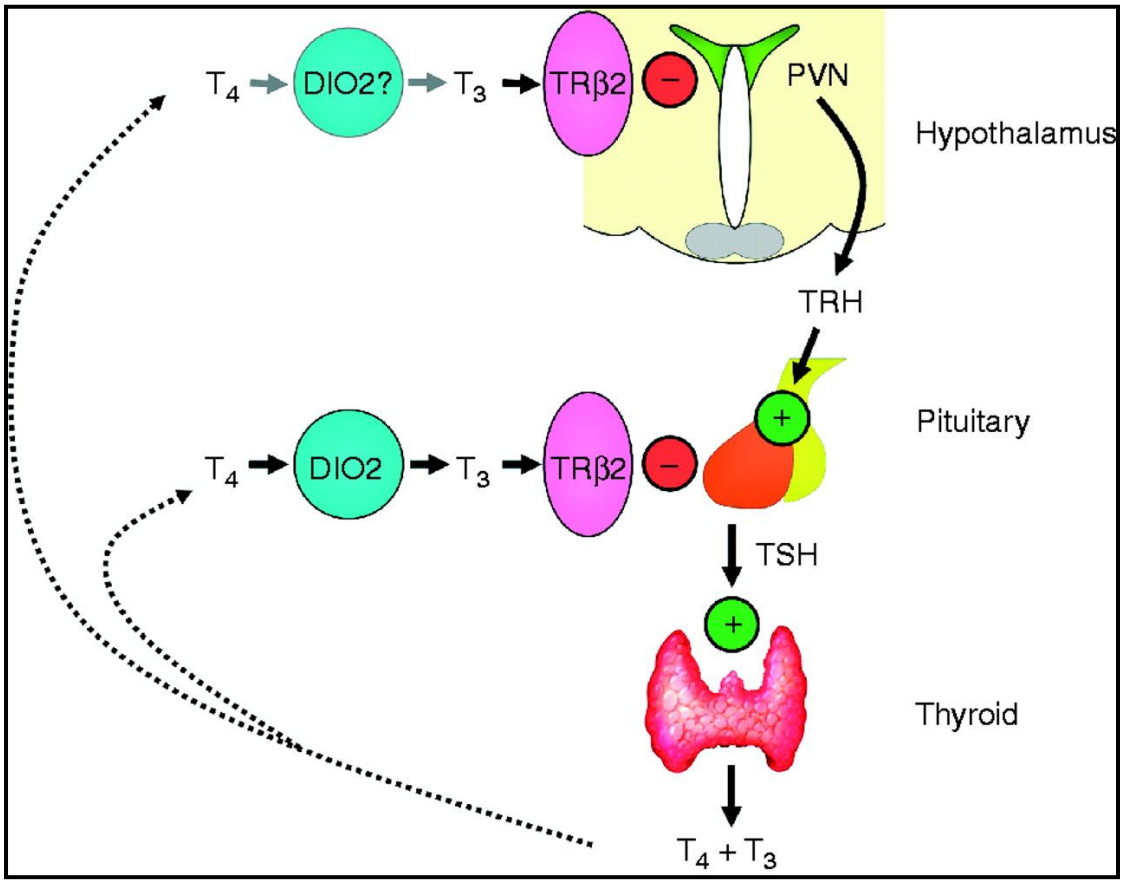
### *Type 2 deiodinase*

The activating D2 enzyme is expressed in glial cells, third ventricle tanycytes, astrocytes and some sensory neurons including nuclei within the trigeminal, auditory and visual pathways (Guadano-Ferraz et al, 1999). Understanding of key neurodevelopment roles for D2 has come from a series of elegant studies in mice. In the cochlea, D2 is expressed in periosteal connective tissue surrounding the internal sensory tissues, with enzyme activity peaking before the onset of hearing. TR expression, however, is localized to the cochlea sensory epithelium, suggesting that periosteal D2 provides a spatiotemporally regulated supply of T3 to the sensory epithelium that is necessary for correct timing of the development and maturation of the cochlea (Campos-Barros et al, 2000). This hypothesis was supported by the finding that D2-deficient mice exhibit delayed cochlea development and defective auditory function despite circulating levels of thyroid hormones that normally are permissive for development of hearing. Thus, D2-dependent local generation of T3 to the cochlea is essential for auditory function (Ng et al, 2004). In this case, the activating D2-enzyme functions as a local amplifier of T3 action to regulate sensory development.

By contrast, the inactivating D3 enzyme can influence spatiotemporal development of sensory pathways by inhibiting T3 action locally. For example, D3 regulates localized asymmetrical growth of the dorsal retina during development of the eye in Xenopus by reducing local T3 concentrations to inhibit T3-dependent proliferation of lateral projecting ganglion cells (Marsh-Armstrong et al, 1999).

D2-deficient mice also have elevated circulating T4 and TSH levels but normal T3 concentrations and display an impaired negative feedback TSH response to T4 but not T3, demonstrating that D2 is required for local T3 generation in the pituitary and essential for normal control of the HPT axis (Schnieder et al, 2001).

**Figure 2.20: Negative feedback regulation of the hypothalamic–pituitary–thyroid axis**




**Source: Williams and Bassett, 2011**

The role of DIO2 in negative feedback control of the HPT axis occurs predominantly in thyrotrophs of the anterior pituitary gland. PVN, para-ventricular nucleus; TRH, thyrotropin-releasing hormone; DIO2, type 2 deiodinase enzyme; TRb2, thyroid hormone receptor b2; T4, thyroxine; T3, 3,5,3'-Ltriiodothyronine

***Type 3 deiodinase***

The inactivating D3 enzyme is highly expressed in the developing rat brain (Bates et al, 1999) and is also present in neurons throughout the adult rat brain especially in pyramidal cells of the hippocampus, granule cells in the dentate and in cerebral cortex (Tu at el, 1999). In early studies, heterogeneous levels of D3 enzyme activity were detected throughout the brain (Kaplan et al, 1981). Studies in D3-deficient mice have also revealed a critical role for this enzyme in

maturation and activity of the HPT axis at the levels of the hypothalamus, pituitary and thyroid gland.

**Thyroid hormone receptors in the brain**

***TR expression***

In the brain, TRs are expressed prior to the onset of fetal thyroid hormone production (Forrest et al, 1991). TRα1 is the major isoform expressed during fetal life (Forrest et al, 1990) but, prior to birth, there is increased expression of TRβ1, which is distributed widely as development proceeds (Strait et al, 1990). Nevertheless, TRα has been estimated to account for 70–80% of total TR expression in the brain (Schwartz et al, 1992). TRα1 and TRβ1 also exhibit differential spatiotemporal expression in neurons throughout the post-natal and adult brain (Mellstrom et al, 1991), suggesting discrete roles for the two isoforms during development and in the mature CNS. For example, in cerebellum, TRα1 is expressed in granular cells whereas both TRα1 and TRβ1 are present in Purkinje cells. Accordingly, T3 acts via TRα1 to regulate granular cell migration and via both TRα1 and TRβ1 to control Purkinje cell differentiation (Morte et al, 2002). Similarly, differences in levels of TRα1 and TRβ1 expression in GABAergic interneurons in cerebral cortex and hippocampus correlate with behavioral phenotypes characterized in TR mutant mice (Guadano-Ferraz et al, 2003).

Furthermore TRα1, but not TRβ, has been shown to regulate the onset of oligodendrocyte precursor cell differentiation and control the timing of oligodendrocyte maturation and migration in the optic nerve (Bilion et al, 2002). The actions of TRα1 and TRβ1 in the brain, however, are not necessarily discrete; recent studies have revealed cooperative interactions between the two isoforms during astrocyte maturation (Morte et al, 2004). Expression of the TRβ2 isoform, by contrast, is localized and restricted to the hypothalamus (Cook et al, 1992), anterior pituitary (Wood et al, 1991; Hodin et al, 1989), developing cochlea (Bradley et al, 1994) and neural retina (Sjoberg et al, 1992).

***Regulation of the HPT axis by TRs***

In the hypothalamus and pituitary, TRβ controls the HPT axis. Mice lacking all TRβ isoforms or harboring a dominant negative knocking mutation of Thrβ display the biochemical features of resistance to thyroid hormone (RTH) seen in patients with mutations in THRB (Weiss and Refetoff, 2000). TRβ knockout mice or mice expressing dominant-negative TRβ proteins display defective HPT axis regulation in both hypothalamus and pituitary resulting in elevated T3 and T4 concentrations and inappropriately normal or elevated TSH (Abel et al, 2003).

## 2.5 SCREENING FOR THYROID DISORDERS ASSOCIATED WITH PREGNANCY

There are no recommendations for universal screening for thyroid dysfunction in women before or during pregnancy. As the overall benefits of screening for thyroid dysfunction (primarily hypothyroidism) have not yet been universally justified by current evidence-based medicine, recent international guidelines have recommended 'aggressive' case finding among the following groups of women who are at high risk, preferably already prior to pregnancy or during early gestation (Abalovich et al, 2007).

High risk women for whom screening is recommended:

- Women with a history of hyperthyroid or hypothyroid disease, postpartum thryoiditis, thyroid lobectomy, and women who already take thyroxine prior to conception.
- Women with a family history of thyroid disease.
- Women with a goiter.
- Women with thyroid antibodies (when known).
- Women with symptoms or clinical signs suggestive of thyroid under-function or over-function, including anemia, elevated cholesterol, and hyponatremia

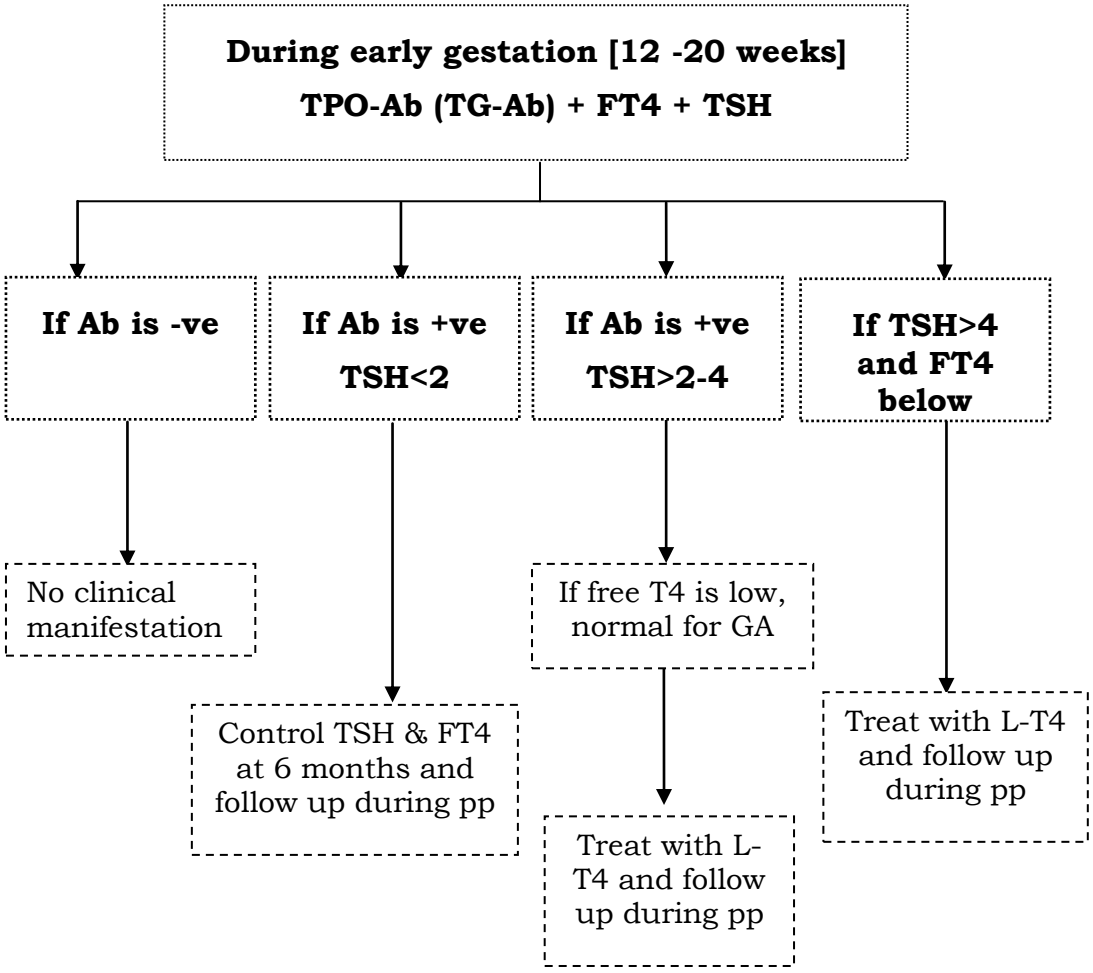
- Women with type I diabetes
- Women with other autoimmune disorders
- Women with a prior history of head and neck irradiation
- Women with infertility should have screening with TSH as part of their infertility work-up
- Women with a prior history of miscarriage and preterm delivery

Among possible screening algorithms, the following scheme has been proposed (Figure 2.21). The first step in the algorithm is to measure serum TSH and thyroid antibodies in early gestation. Because isolated hypothyroxinemia may occur in some women (without concomitant rise in serum TSH), it is reasonable to include systematically a free T4 determination. Ideally, both TG-Ab and TPO-Ab should be determined; however, if for economic reasons only one antibody can be measured, then it is preferable to measure TPO-Ab because it yields the best diagnostic score. When serum TSH is elevated or free T4 clearly below normal, and irrespective of the presence (or absence) of thyroid autoimmunity (TAI), women should be considered to have thyroid under-function and treated with thyroxine.

The next step concerns those women with TAI and normal thyroid function. When serum TSH is <2.5 mIU/L (most frequently associated with low antibodies titers and normal free T4 levels), thyroxine treatment is not systematically warranted, and serum TSH and free T4 should be monitored later during gestation. For women with TAI and a serum TSH that lies within the normal range in early gestation, but is already slightly shifted to higher 'normal' values, i.e. between 2.5-4.0 mIU/L (most frequently associated with higher antibody titers and low-normal free T4 levels), obstetric care providers should consider thyroxine treatment.

**Figure 2.21: An algorithm for systematic screening of thyroid autoimmunity and hypothyroidism during pregnancy based on the determination of thyroid antibodies (Ab)**

[serum TSH and free T4 concentrations during the first half of gestation, GA = gestational age; NL = normal limits; PP = postpartum]




**Source: Glinoer, 1998**

It is important to keep in mind that serum TSH is down-regulated under the influence of peak hCG values in the 1st half of gestation, and also that the thyroid deficit tends to deteriorate as gestation progresses in TAI-positive women. Because the potential deleterious effects, for both mother and progeny, are not due to high serum TSH per se but to low free T4 concentrations, clinical judgment should be based on serum free T4. If low or low-normal for gestational age, thyroxine treatment is probably justified. In daily practice, when such a scheme is systematically applied, most-if not all-of the pregnancies followed are successful and uneventful (Abalovich et al, 2002).

Even though more prospective studies are needed to assess the final clinical relevance of the proposed scheme, the recent study of Negro et al, provided strong arguments in favor of early thyroxine administration in women with AITD and normal thyroid function during early gestation (Negro et al, 2006).

Systematic screening for AITD during early pregnancy also allows to delineating women who are prone to developing thyroid dysfunction after parturition. Thus, even when no specific treatment is warranted during gestation, systematic screening is useful to clinicians for organizing the monitoring of potential postpartum thyroid dysfunction (Kamijo et al, 1990).

**Guidelines for treatment of thyroid disorders (Banerjee, 2011)**

**Hypothyroidism during pregnancy**

1. Both maternal and fetal hypothyroidism exerts serious adverse effects on the fetus, so maternal hypothyroidism should be avoided by early diagnosis at the first prenatal visit or at diagnosis of pregnancy.
2. In cases of hypothyroidism diagnosed before pregnancy, adjust the preconception T4 dose to reach a TSH level not higher than 2.5 μIU/ml before pregnancy.
3. By 4-6 weeks of gestation, the T4 dosage needs to be increased by about 30-50%.
4. If overt hypothyroidism is diagnosed during pregnancy, thyroid function should be normalized as rapidly as possible. The target is to achieve and maintain TSH concentrations below 2.5 μIU/ml in the first trimester (or 3 μIU/ml in the second and third trimesters) or to trimester specific normal TSH ranges. This can be achieved by rapidly titrating the T4 dosage to reach and maintain the target TSH levels. A re-assessment of the thyroid function should be carried out within 30 to 40 days.
5. Women who have thyroid antibodies in the early stages of their pregnancy but are otherwise euthyroid should be monitored for

elevations of TSH above the normal range because they are risk of developing hypothyroidism.

6. Sub clinical hypothyroidism: Recommend T4 replacement as T4 treatment has been shown to improve obstetrical outcome, though do not modify long-term neurological development in the offspring.
7. After delivery, dose of T4 need to be decreased in most hypothyroid women.

**Autoimmune and thyroid disease and miscarriage**

Universal screening for anti-thyroid antibodies and possible treatment cannot be recommended as there are very few reports regarding positive association between the presence of thyroid antibodies and pregnancy loss.

**Iodine nutrition during pregnancy**

Increase daily iodine intake to 250 μg on an average during pregnancy and breastfeeding by encouraging the use of iodized salt.

**Postpartum thyroiditis (PPT)**

1. TSH estimation at 3 and 6 months in women known to be thyroid peroxidase antibody positive, for women with type 1 diabetes mellitus (PPT 3-fold greater).
2. Women with a history of PPT have a markedly heightened risk of developing permanent primary hypothyroidism within 5 to 10 years, should undergo annual TSH assessments.
3. Asymptomatic women with PPT who have a TSH above the reference range but less than 10 μIU/ml and who are not planning a subsequent pregnancy do not necessarily require intervention but should, if untreated, be re-monitored in 4–8 weeks. Symptomatic women and women with a TSH above normal and who are attempting pregnancy should be treated with levothyroxine.
4. Women with postpartum depression should be screened for hypothyroidism and appropriately treated.

## 2.6 SCREENING OF NEONATES

Neonatal screening programs for detection of CH in neonatal period are widespread in the developed countries for the last three decades (Dussault, 1999) and are fast gaining momentum in the developing world as well (Wu et al, 1999). In most screening programs blood samples are collected at 5-6 days age, but with large number of babies being discharged early, cord blood samples are being used as well (Ordookhani et al, 2003).

In our country, it is very difficult to call back babies once discharged. Also, an effective social system whereby babies could be reached at home is practically non-existent. Thus cord blood remains a very practical alternative for screening purposes, and thus is the practice in some Asian countries (Ordookhani et al, 2003). The Indian Academy of Pediatrics recommends the use of cord blood samples for screening for CH.

## 2.7 GESTATIONAL AGE-SPECIFIC REFERENCE INTERVALS

Laboratory reference intervals for thyroid function tests have traditionally been derived from non-pregnant subjects who are free from thyroid disease. Their validity in pregnant women is debatable as pregnancy produces profound physiological changes in the mother, which in turn complicate the interpretation of maternal thyroid function tests. Therefore, a local reference range for thyroid hormones in pregnant women is needed (Price et al, 2001) along with international reference ranges.

Table 2.6 and 2.7 presents trimester specific reference intervals for thyroid hormones, developed worldwide using percentile method and mean/median method respectively. For India we have trimester specific reference intervals (thyroid hormone) given by Marwah et al (2008) using percentile method (table 2.6) and Kumar et al (2003) using mean/median method (table 2.7). However, in these two studies, trimester specific reference intervals for urinary iodine were not exercised.

**Table 2.6: Worldwide trimester specific reference intervals [percentiles- 5th & 95th, 2.5th & 97.5th]**

| **Reference intervals based on INDIAN population** *Source: Marwah et al, 2008* | | | |
|---|---|---|---|
| **Thyroid Hormone** | **Trimester** | **5th Percentile** | **95th Percentile** |
| TSH [mIU/L] | First | 0.6 | 5.0 |
| | Second | 0.435 | 5.78 |
| | Third | 0.74 | 5.7 |
| FT4 [pM/L] | First | 12 | 19.45 |
| | Second | 9.48 | 19.58 |
| | Third | 11.3 | 17.71 |
| FT3 [pM/L] | First | 1.92 | 5.86 |
| | Second | 3.2 | 5.7 |
| | Third | 3.3 | 5.18 |
| **Reference intervals based on UAE and ASIAN population** *Source: Dhatt et al, 2006* | | | |
| **Thyroid Hormone** | **Trimester** | **2.5thPercentile** | **97.5thPercentile** |
| | | **UAE** | |
| TSH [mIU/L] | First | 0.06 | 8.3 |
| | Second | 0.17 | 5.9 |
| | Third | 0.21 | 6.9 |
| FT4 [pM/L] | First | 8.9 | 24.6 |
| | Second | 8.4 | 19.3 |
| | Third | 8.0 | 18.0 |
| | | **ASIAN** | |
| TSH [mIU/L] | First | 0.12 | 7.4 |
| | Second | 0.3 | 5.5 |
| | Third | 0.3 | 4.85 |
| FT4 [pM/L] | First | 11.3 | 21.9 |
| | Second | 9.7 | 18.5 |
| | Third | 8.9 | 16.6 |
| **Reference intervals based on CHINESE population** *Source: Yu et al, 2010* | | | |

| Thyroid Hormone | Trimester | 2.5thPercentile | 97.5thPercentile |
|---|---|---|---|
| TSH [mIU/L] | First | 0.02 | 3.65 |
| | Second | 0.36 | 3.46 |
| | Third | 0.44 | 5.04 |
| FT4 [pM/L] | First | 11.85 | 21.51 |
| | Second | 9.45 | 16.25 |
| | Third | 9.30 | 17.14 |
| TPO-Ab [IU/mL] | First | 5 | 19.69 |
| | Second | 5 | 19.62 |
| | Third | 5 | 21.96 |

**Reference intervals based on CHINESE population**

*Source: Panesar et al, 2001*

| Thyroid Hormone | Pregnancy Status (wk) | 2.5thPercentile | 97.5thPercentile |
|---|---|---|---|
| TSH [mIU/L] | 7 | 0.05 | 2.3 |
| | 11 | 0.03 | 2.3 |
| | 15 | 0.03 | 2.8 |
| | 19 | 0.03 | 3.1 |
| | 23 | 0.03 | 3.7 |
| | 27 | 0.08 | 3.5 |
| | 31 | 0.46 | 3.8 |
| | 35 | 0.13 | 3.4 |
| | 39 | 0.03 | 3.56 |
| FT4 [pM/L] | 7 | 11.8 | 20.8 |
| | 11 | 11.1 | 22.9 |
| | 15 | 10.1 | 17 |
| | 19 | 9.5 | 15.4 |
| | 23 | 8.1 | 16.7 |
| | 27 | 8.7 | 15.1 |
| | 31 | 7.8 | 13.7 |
| | 35 | 8.5 | 14.4 |
| | 39 | 9.1 | 15.6 |

| Thyroid Hormone | Pregnancy Status (wk) | 2.5$^{th}$Percentile | 97.5$^{th}$Percentile |
|---|---|---|---|
| FT3 [pM/L] | 7 | 3 | 5.7 |
| | 11 | 3 | 5.7 |
| | 15 | 2.8 | 4.9 |
| | 19 | 2.5 | 4.9 |
| | 23 | 2.8 | 4.2 |
| | 27 | 2.5 | 4.1 |
| | 31 | 2.3 | 3.9 |
| | 35 | 2.4 | 4.1 |
| | 39 | 2.4 | 4.2 |

**Reference intervals based on AUSTRALIAN population**

*Source: Gilbert et al, 2008*

| Thyroid Hormone | Pregnancy Status (wk) | 2.5$^{th}$Percentile | 97.5$^{th}$Percentile |
|---|---|---|---|
| TSH [mIU/L] | 9 | 0.05 | 2.20 |
| | 10 | 0.02 | 2.13 |
| | 11 | 0.02 | 2.16 |
| | 12 | 0.06 | 2.00 |
| | 13 | 0.06 | 2.54 |
| FT4 [pM/L] | 9 | 11.0 | 17.4 |
| | 10 | 10.6 | 17.6 |
| | 11 | 10.4 | 18.6 |
| | 12 | 10.3 | 17.9 |
| | 13 | 9.7 | 16.7 |
| FT3 [pM/L] | 9 | 3.3 | 5.5 |
| | 10 | 3.3 | 5.6 |
| | 11 | 3.3 | 5.6 |
| | 12 | 3.3 | 5.9 |
| | 13 | 3.2 | 6.0 |

**Reference intervals based on SPANISH population**

*Source: Bocos-Terraz et al, 2009*

| Thyroid Hormone | Pregnancy Status (wk) | 2.5th Percentile | 97.5thPercentile |
|---|---|---|---|
| TSH [mIU/L] | <11 | 0.10 | 2.65 |
| | 11-20 | 0.03 | 2.57 |
| | 21-30 | 0.12 | 2.64 |
| | 31-36 | 0.23 | 3.56 |
| | >36 | 0.36 | - |
| FT4 [ng/dl] | <11 | 0.83 | 1.38 |
| | 11-20 | 0.77 | 1.34 |
| | 21-30 | 0.70 | 1.14 |
| | 31-36 | 0.66 | 1.17 |
| | >36 | 0.17 | - |
| FT3 [pg/mL] | <11 | 2.34 | 4.34 |
| | 11-20 | 2.24 | 4.43 |
| | 21-30 | 2.47 | 4.18 |
| | 31-36 | 2.25 | 4.16 |
| | >36 | 2.59 | - |
| TPO-AB [UI/mL] | <11 | 0.0 | 0.68 |
| | 11-20 | 0.0 | 1.04 |
| | 21-30 | 0.0 | 0.89 |
| | 31-36 | 0.0 | 1.27 |
| | >36 | 0.0 | - |
| TG-Ab [UI/mL] | <11 | 0.41 | 3.11 |
| | 11-20 | 0.34 | 3.31 |
| | 21-30 | 0.37 | 2.60 |
| | 31-36 | 0.38 | 2.45 |
| | >36 | 0.46 | - |

**Table 2.7: Worldwide trimester specific reference intervals [mean/median]**

| Country (year) | Analyte | Trimester | | | PP |
|---|---|---|---|---|---|
| | | I | II | III | |
| UK Asians (2001) | TSH (mIU/L) | 0.9 | 1.3 | - | 1.3 |
| | FT4 (pmol/mL) | 12.6 | 11.5 | - | 13.1 |
| | UI (µg/L) | 125 | 170 | - | - |

| Country (year) | Analyte | Trimester | | | PP |
|---|---|---|---|---|---|
| | | I | II | III | |
| UK whites (2001) | TSH (mIU/L) | 0.9 | 1.3 | | 1.7 |
| | FT4 (pmol/mL) | 12.4 | 11.5 | | 13.2 |
| | UI (µg/L) | 125 | 170 | 147 | - |
| Belgium (1990) | TSH (mIU/L) | 0.75 | 1.05 | 1.29 | - |
| | T4 (nmol/L) | 138 | 148 | 148 | - |
| | FT4 (pmol/mL) | 17.9 | 14.5 | 13.4 | - |
| | UI (µg/L) | 58 | 58 | 53 | - |
| Italy (2002) | TSH (mIU/L) | 1.1 | - | - | - |
| | Tg (ng/mL) | 25 | - | - | - |
| | FT4 (pmol/mL) | 10.4 | - | - | - |
| | UI (µg/L) | 116 | - | - | - |
| ***India (2003)*** | ***TSH (mIU/L)*** | ***1.20*** | ***2.12*** | ***3.3*** | - |
| | ***T4 (nmol/L)*** | ***164*** | ***165*** | ***159*** | - |
| | ***T3 (nmol/L)*** | ***1.85*** | ***2.47*** | ***1.82*** | - |
| Nigeria (2005) | TSH (mIU/L) | 2.7 | 2.12 | 2.29 | 3.1 |
| | T4 (nmol/L) | 129 | 157 | 173 | 139 |
| | T3 (nmol/L) | 2.6 | 7.9 | 8.2 | 7.4 |
| | UI (µg/L) | - | - | - | 79 |
| Japan (2005) | TSH (mIU/L) | 1.05 | 1.51 | 1.23 | 2.96 |
| | FT3 (pg/mL) | 3.60 | 3.39 | 3.17 | 3.57 |
| | FT4 (ng/dL) | 1.43 | 1.11 | 1.02 | 1.08 |
| UAE (2006) | TSH (mIU/L) | 0.71 | 1.04 | 1.20 | 1.32 |
| | FT4 (pmol/L) | 14.6 | 12.7 | 12.0 | 13.7 |
| Other Arabs (2006) | TSH (mIU/L) | 0.63 | 1.1 | 1.30 | - |
| | FT4 (pmol/L) | 14.9 | 13.3 | 12.4 | - |
| Asian (Ind) (2006) | TSH (mIU/L) | 0.95 | 1.30 | 1.1 | - |
| | FT4 (pmol/L) | 15.7 | 13.4 | 12.1 | - |
| Sweden (2004) | TSH (mIU/L) | 0.89 | 1.17 | 1.16 | 1.06 |
| | FT4 (pmol/L) | 12.3 | 10.5 | 10.5 | 13.7 |
| | Tg (ng/mL) | 15.48 | 14.92 | 18.55 | 13.95 |
| | UI (µg/L) | 180 | 170 | 145 | - |
| Singapore (2001) | TSH (mIU/L) | 0.65 | 1.2 | - | - |
| | UI (µg/L) | 107 | 116 | 124 | 105 |
| Sudan (2000) | TSH (mIU/L) | 1.1 | 1.2 | 1.0 | - |
| | FT4 (pmol/L) | 11.4 | 9.6 | 10.2 | - |

**Source: Soldin et al, 2007** [PP- postpartum]

## 2.8 Repercussions of hypothyroidism on pregnancy outcome

Despite the known association between decreased fertility and hypothyroidism, the latter condition does not preclude the possibility to conceive. This is probably the main reason why, until a few years ago, hypothyroidism had been considered wrongly to be relatively rare during pregnancy (Krassas, 2000). In a study by Abalovich and team, 34% of hypothyroid women became pregnant without thyroxine treatment, 11% of them had OH and 89% SCH (Abalovich et al, 2002). When hypothyroid women become pregnant and maintain the pregnancy, they carry an increased risk for early and late obstetrical complications (Table 2.8 and Table 2.9).

**Table 2.8: Pregnancy outcome associated with hypothyroidism: maternal aspects**

| MOTHER | Frequency | % | Type of Hypo† | Reference |
|---|---|---|---|---|
| Anemia | Increased | 31 % | OH ** | Davis, 1988 |
| PP hemorrhage | Increased | 4 % | SCH ** | Leung, 1993 |
| PP hemorrhage | Increased | 19 % | OH | Davis, 1988 |
| Cardiac dysfunction | Increased | n. a. | OH | Davis, 1988 |
| Pre-eclampsia | Increased | 15 % | SCH | Leung, 1993 |
| Pre-eclampsia | Increased | 22 % | OH | Leung, 1993 |
| Pre-eclampsia | Increased | 44 % | OH | Davis, 1988 |
| Pre-eclampsia | Increased | n. a. | OH | Mizgala, 1991 |
| Placenta abruption | Increased | 19 % | OH | Davis, 1988 |

† Hypothyroidism; ** SCH: subclinical hypothyroidism; ** OH: overt hypothyroidism, PP: postpartum

**Source: Glioner, 2008**

Table 2.8 and 2.9 clearly shows that both obstetrical and fetal complications occur with an increased frequency in pregnant women with hypothyroidism. As expected, these complications are both more frequent and more severe in women with OH than SCH. Most

importantly, adequate treatment with thyroid hormone greatly reduces risks of a poorer obstetrical outcome (Tan et al, 2006).

**Table 2.9: Pregnancy outcome associated with hypothyroidism: fetal and neonatal aspects**

| FETUS-NEWBORN | Frequency | % | Type of Hypo† | Reference |
|---|---|---|---|---|
| Fetal distress in labour | increased | 14 % | OH ** | Wasserstrum, 1995 |
| Prematurity /LBW* | increased | 31 % | OH | Davis, 1988 |
| | increased | 9 % | SCH ** | Leung, 1993 |
| | increased | 22 % | OH | Leung, 1993 |
| | increased | 13 % | OH | Abalovich 2002 |
| | increased | R.R.: 1.8 *** | SCH | Casey, 2005 |
| | increased | O.R.: 3.6 *** | OH | Idris, 2005 |
| Breech presentation | increased | O.R.: 4.7 *** | Early hypo-T4 | Pop, 2004 |
| Cesarean section | increased | 29 % | OH | Idris, 2005 |
| Impaired intra-uterine growth | increased | n. a. | OH | Blazer, 2003 |
| Congenital malformations | increased | 4 % | OH | Leung, 1993 |
| | increased | 6 % | OH | Abalovich, 2002 |
| Fetal death | increased | 4 % | OH | Leung, 1993 |
| | increased | 12 % | OH | Davis, 1988 |
| | increased | 3 % | OH | Abalovich 2002 |
| | increased | 8 % | OH | Allan, 2000 |
| Perinatal death | increased | 9-20 % | OH | Montoro, 1981 |
| | increased | 3 % | OH | Allan, 2000 |

† Hypothyroidism; * LBW: low birth weight; ** SCH: subclinical hypothyroidism; ** OH: overt hypothyroidism; *** O.R.: Odds Ratio; *** R.R.: Relative Risk

**Source: Glioner, 2008**

## 2.9 IODINE PROPHYLAXIS

Salt is the most widely used food vehicle for iodine fortification. USI, that is iodization of all salt for human (food industry and household) and livestock consumption, is the strategy recommended by WHO for the control of iodine deficiency (WHO, 2008). Adequate iodization of all salt will deliver iodine in required quantities to the population on a continuous and self-sustaining basis. In 1994, a special session of the WHO and UNICEF joint committee on health policy recommended USI as a safe, cost effective and sustainable strategy to ensure sufficient intake of iodine in all individuals (UNICEF/2005).

In nearly all countries where iodine deficiency occurs, it is now well recognized that the most effective way to achieve the virtual elimination of IDD is through USI. Salt iodization programmes are currently implemented in over 70 countries around the world where IDD is a public health problem (WHO, 2008). The additional cost of iodine fortification in the process of salt production should eventually be borne by the consumer, but is negligible. This will greatly assist sustainability.

National salt iodization programmes are now well implemented worldwide and have followed a common pattern of evolution, which includes the following phases:

- **Decision phase:** This phase involves making the decision for USI supported by industry, backed by standards and regulation, and supported by an implementation plan.
- **Implementation Phase:** This phase ensures the infrastructure for iodization and packaging of all human and livestock salt, and supports that infrastructure with quality assurance measures, communication and demand creation, regulation and enforcement.
- **Consolidation Phase:** Once the goal of USI is achieved, it needs to be sustained and assessed through on-going process and impact monitoring, as well as periodic evaluations, the latter may include international multidisciplinary teams.

A successful salt iodization programme depends upon the implementation of a set of activities at the national level by various sectors:

- Government ministries (legislative and justice, health, industry, agriculture, education, communication and finance.
- Salt producers, salt importers and distributors, food manufacturers.
- Concerned civic groups, including consumer associations.
- Nutrition, food and material scientists, and other key opinion makers.

USI has been remarkably successful in many countries. Over 30 countries have achieved the goal of USI (>90 % of households using iodized salt), and many others are on track (WHO/UNICEF/ICCIDD, 2007).

***The choice of salt as the preferred vehicle for the delivery of iodine is based on the following factors:***

1. Salt is one of the few commodities consumed by everyone
2. Consumption is fairly stable throughout the year
3. Salt production is usually limited to a few geographical areas, which facilitates its quality control
4. Salt iodization technology is easy to implement and available at a reasonable cost throughout the developing world
5. The addition of iodine to salt does not affect its colour, taste or odour
6. The quality of iodized salt can be monitored at the production, retail and household levels (Allen L et al, 2006).

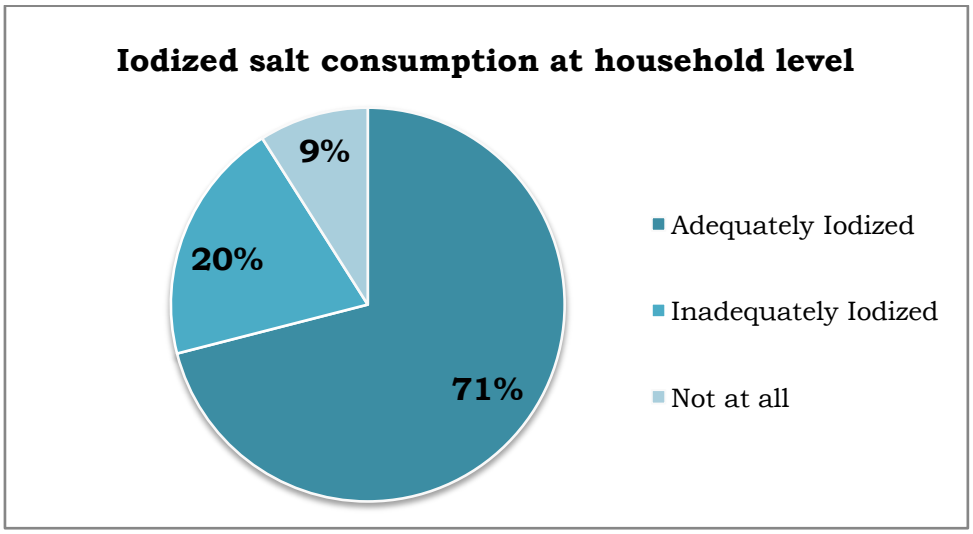
***Some obstacles to the implementation of USI are:***

1. Difficulties in enforcing legislation on iodized salt.
2. Problems caused by having a high number of small-scale salt producers and the absence of an effective operational monitoring system.
3. The lack of monitoring of the salt quality may result in variations in the iodine content of salt, inadequate supply of fortificants, inadequate supply and use of iodized versus non-iodized salt, and population levels of salt intake (measured versus estimated).

**Status of iodized salt in INDIA**

A lift on the ban of sale of non-iodized edible salt between 2000 and 2005 left iodized salt use suspended at 51% of households as per the NFHS-3 (National Family Health Survey). The ban's 2005 re-instatement combined with heightened consumer awareness, effective monitoring, and improvements in iodization practices and packaging helped to boost the use of iodized salt from 51% of households in 2006, to 71% by 2009 (Figure 2.24). Amidst this impressive increase in coverage, equitable distribution remains a challenge: consumption of iodized salt is much higher in urban areas (83%) than in rural areas (66%) leaving the most vulnerable population at a greater risk (Figure 2.23).

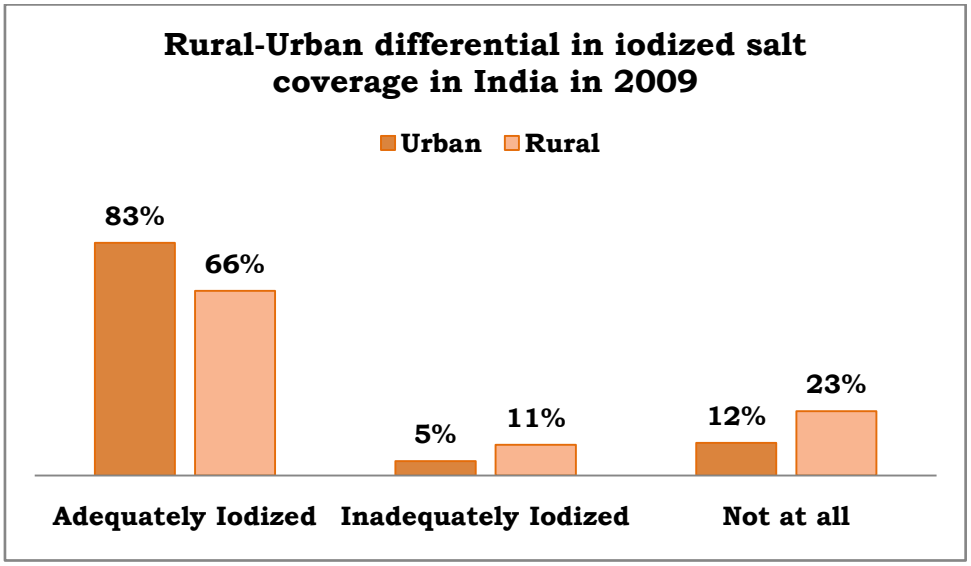
**Figure 2.22: Iodized salt consumption at household level in INDIA**




**Source: IDD Newsletter, May 2011**

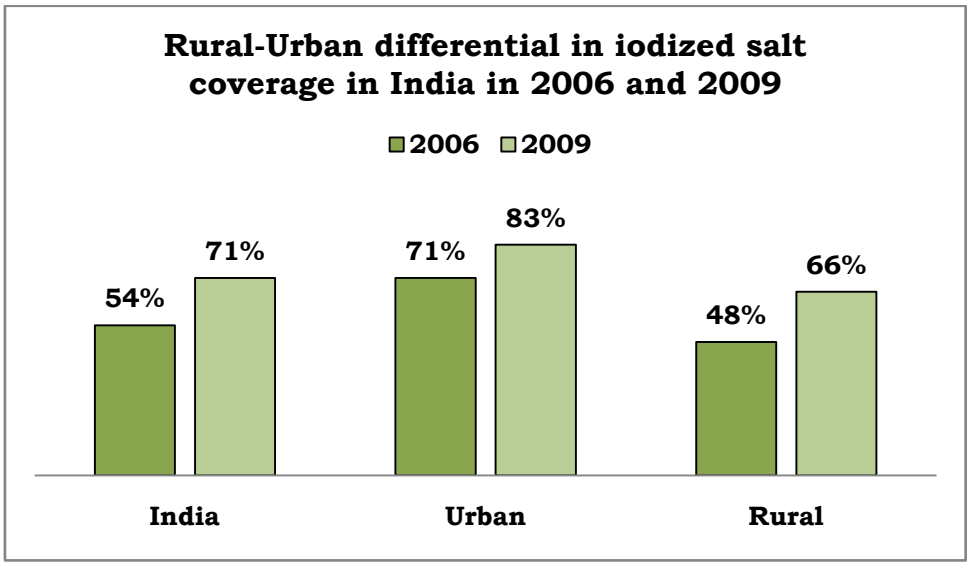
As per UNICEF CES (2009), overall 71% of the households were using cooking salt which was iodized at the recommended level of 15 ppm or more (Figure 2.22). Only 9% of the households used salt that was not iodized at all and 20% used salt that was iodized inadequately (<15 ppm). The rural-urban differential in salt iodization was found to be pronounced (Figure 2.23). Around 83% of households in urban areas used salt with 15 ppm or more iodine content compared with 66% of households in rural areas.

**Figure 2.23: Difference in iodized salt coverage**




**Source: IDD Newsletter, May 2011**

**Figure 2.24: Comparison of difference in IS coverage [2006-09]**




**Source: IDD Newsletter, May 2011**

The proportion of households using non-iodized salt was greater in rural areas (12%) than in urban areas (5%). The use of iodized salt was high in northeastern States and in States of Delhi, Goa, Haryana, Himachal Pradesh, Jammu and Kashmir, Punjab and all UTs, ranging from 80% to 94% (Figure 2.24). In the States of Karnataka, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Uttar Pradesh, Orissa, and Jharkhand use of iodized salt was low as compared to other States (Figure 2.25).

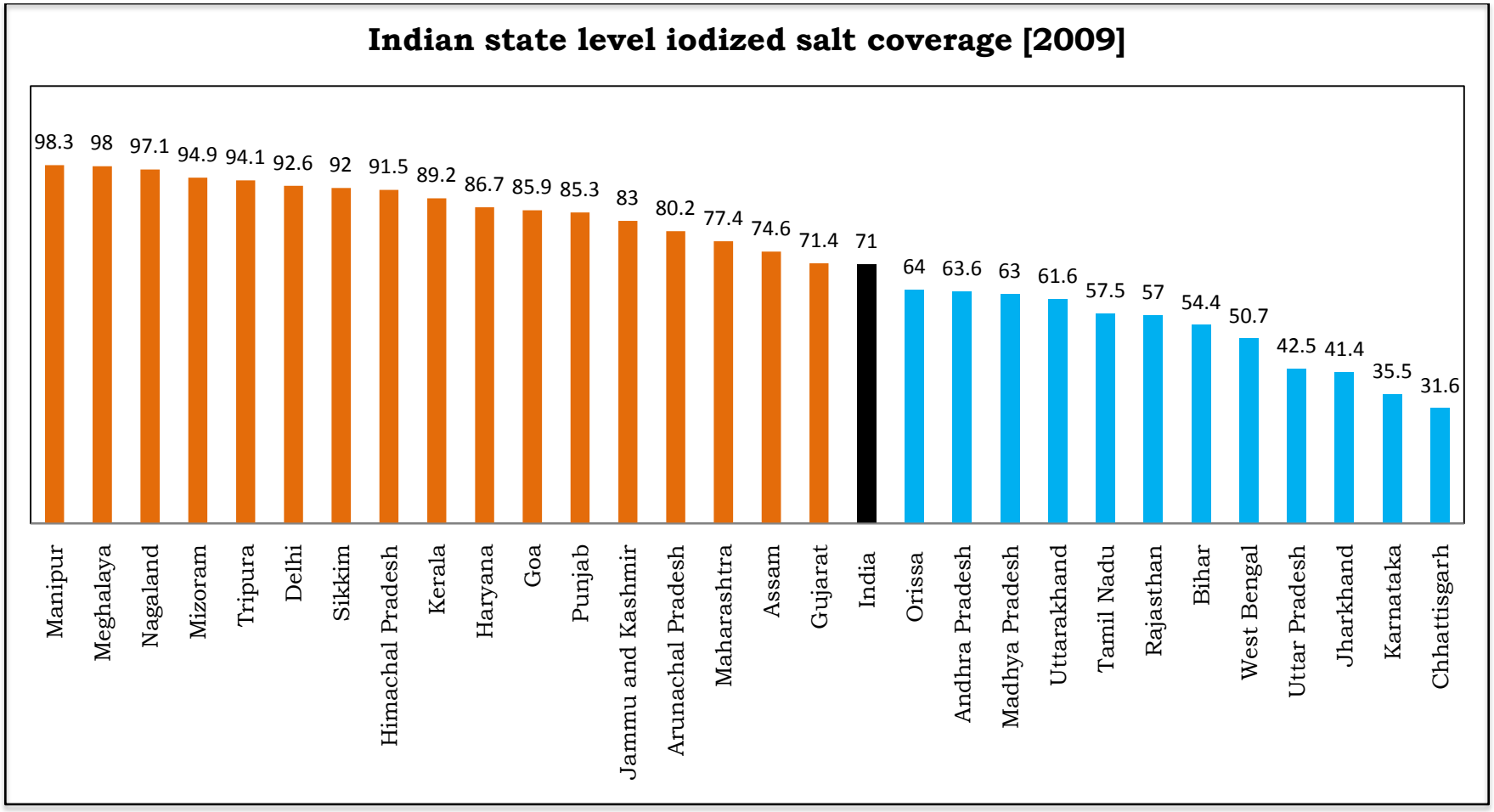



**Figure 2.25: State level iodized salt coverage in Indian in 2009**

**Source: IDD Newsletter, May 2011**

**Harvesting of salt**

**Figure 2.26: Glimpses of production of iodized salt in India**

The results of the UNICEF CES 2009 shows that tremendous progress was made towards achieving USI in India in recent years. In the last national level survey conducted in 2005-06 (National Family Health Survey 3) the consumption of adequately iodized salt at household level was only 51%. No increase in iodized salt coverage was seen between the two national level surveys in 1998-99 (NFHS 2) and 2005-2006 (NFHS 3). The stagnation in the household level coverage of adequately iodized salt between 1998 and 2006 was primarily because of the lifting of the ban on the sale of non-iodized salt in India in year 2000.

The remarkable progress made in iodized salt coverage in the country was driven by a multitude of factors and by bringing together all stakeholders of USI at National and State level.

The results of the UNICEF CES (2009) survey are extremely encouraging and with further acceleration of the efforts to eliminate IDD in India, the country may achieve Universal Salt Iodization (USI) soon. Keys to achieving USI in India is better understanding of changing consumer preferences, and the trend towards an increasing share of refined/branded/packaged edible salt in India.

The Indian salt industry has made rapid progress over last decade with an increase in both quantity and quality of iodized salt produced in the country. In year 2008-2009, for the first time, the iodized salt production (5.37 million MT in year 2008-09) was greater than the national requirement of edible salt (5.2 million MT) (IDD Newsletter, May 2011). There has been a trend in recent years towards an increasing share of refined salt and packaged in edible salt category. The share of refined edible salt in total salt production in India is increasing by approximately10% annually (currently approximately 50% of salt production in India consists of refined salt).

**2.10 IODINE DEFICIENCY**

Iodine deficiency (ID) used to be a major public health problem. It is the leading cause of mental retardation during childhood. Concerted

international action taken since 1990 has aimed at the sustainable elimination of IDDs using salt iodization as main strategy (WHO/ UNICEF/ICCIDDD, 1996).

**GLOBAL progress**

A worldwide effort has dramatically raised the proportion of people consuming iodized salt from less than 20% in 1990 to about 70% by 2000. Thirty-four countries have achieved the elimination of iodine deficiency through universal salt iodization. By 2006, more than 120 countries were implementing salt iodization programmes, an increase of one third in just six years compared to the 90 countries with such programmes in 2000 (UNICEF, 2008).

***Sustainable elimination of iodine deficiency***

- Countries that have met the goal-34
- Countries on track -38
- Countries declining or lagging-24
- Countries with low coverage (<20%) and no progress-12

**NATIONAL progress**

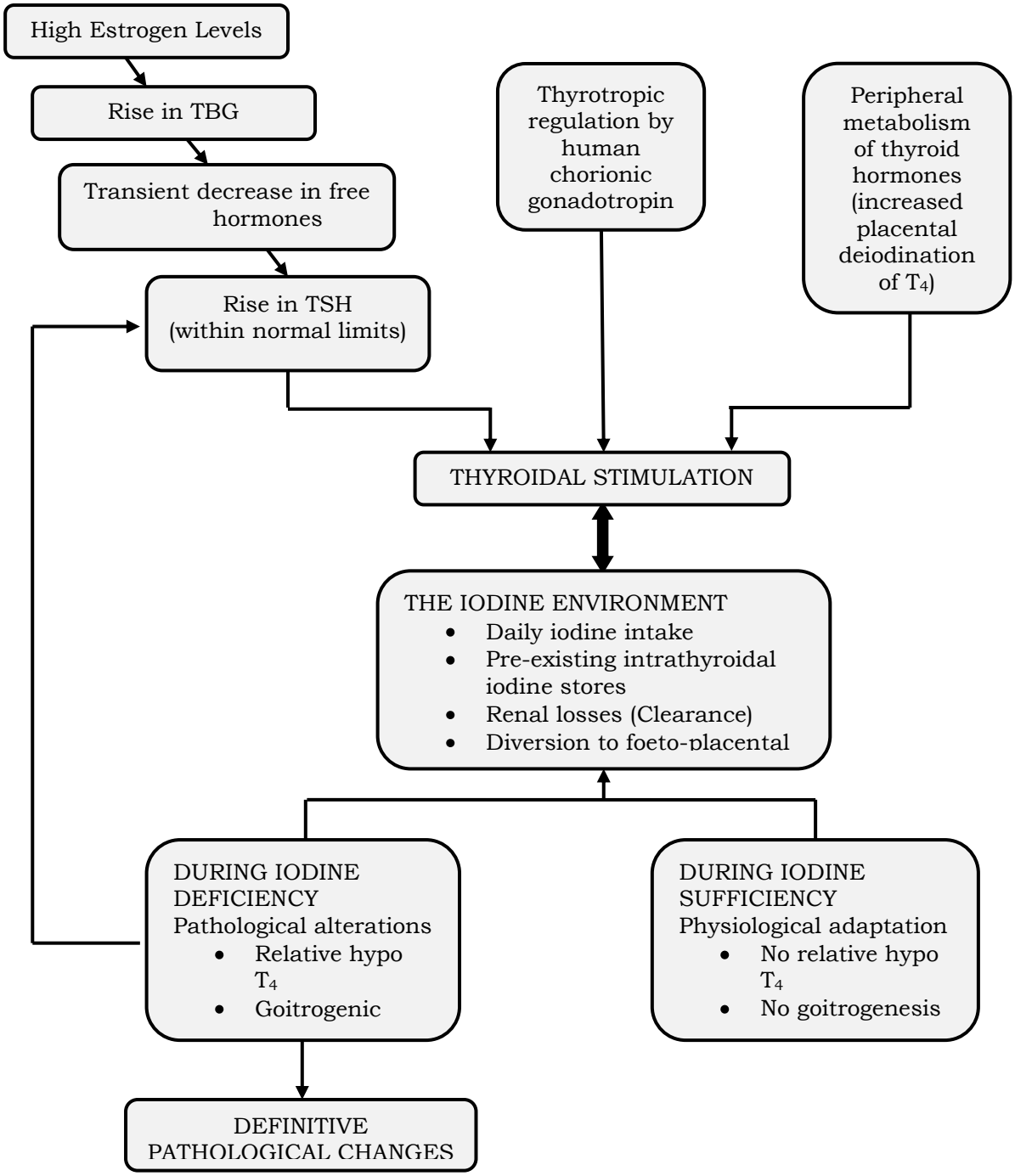
Since the United Nations Special Session on Children in 2002, many countries have reported continued progress towards universal salt iodization. However, some countries have made little tangible progress. In 2006, UNICEF identified 16 'make or break' countries that need additional support to accelerate their efforts. These are major salt-producing countries with high numbers of unprotected newborns, low levels of salt iodization, and a need for special advocacy and technical support to renew national IDD elimination programmes. If these 16 countries achieve universal salt iodization, the global average of households consuming adequately iodized salt will be about 85%. These countries are India, Pakistan, China, Russian Federation, Ethiopia, Indonesia, Ukraine, Philippines, Sudan, Bangladesh, Afghanistan, Egypt, Ghana, Angola, Niger and Senegal (UNICEF, 2008).

## 2.11 PREGNANCY AND IODINE DEFICIENCY

Physiologic adaptation of the thyroidal economy associated with normal pregnancy is replaced by pathologic changes when pregnancy

takes place in conditions with iodine deficiency or even only mild iodine restriction.

**Figure 2.27: From physiological adaptation to pathological alterations of the thyroidal economy during pregnancy**




**Source: Glinoer, 1997***

Globally, the changes in maternal thyroid function that occur during gestation can be viewed as a mathematical fraction, with hormone requirements in the numerator and the availability of iodine in the denominator. When availability of iodine becomes deficient during gestation, at a time when thyroid hormone requirements are increased, this situation presents an additional challenge to the maternal thyroid (Glinoer, 1997*). Figure 2.27 illustrates the steps through which pregnancy induces a specific challenge for the thyroid gland and the profound difference between glandular adaptation in conditions with iodine sufficiency or deficiency.

Thus during pregnancy, the physiologic changes that take place in maternal thyroid economy lead to an increase in thyroid hormone production of ~50% above preconception baseline hormone production. In order to achieve the necessary increment in hormone production, the iodine intake needs to be increased during early pregnancy.

**Metabolism of iodine during normal pregnancy**

After reduction to iodide, dietary iodine is rapidly absorbed from the gut. Then, iodide of dietary origin mixes rapidly with iodide resulting from the peripheral catabolism of thyroid hormones and iodothyronines by deiodination, and together they constitute the extra-thyroidal pool of inorganic iodide (PII). This pool is in a dynamic equilibrium with two main organs, the thyroid gland and the kidneys.

A normal adult utilizes ~80 μg of iodide to produce thyroid hormones (TH) and the system is balanced to fulfill these daily needs. When the iodine intake is adequate (150 μg/day) in non-pregnant conditions, a kinetic balance is achieved with a 35% uptake of the available iodine by the thyroid (Panel A Figure 2.28). From the 80 μg of hormonal iodide produced each day by TH catabolism, 15 μg of iodide is lost in feces, leaving 65 μg to be redistributed between the thyroid

compartment (hence, providing 25 µg for daily TH production) and irreversible urinary losses. In such conditions, the metabolic balance is in equilibrium, with 150 µg of iodide 'in' and the same amount 'out', and 80 µg available for daily hormone production. Thus, with an iodine intake level of 150 µg/day (or above) in non-pregnant healthy adults, the system is able to maintain plentiful intra-thyroidal stores, in the order of 15-20 mg of iodine.

In contrast, when the iodine intake level is restricted to only 70 µg/day, the system must up-regulate the glandular iodine trapping mechanisms and increase the relative iodine intake to 50% (Panel B Figure 2.28). The higher uptake allows to recover 35 µg of iodine from dietary intake and 33 µg from TH catabolism but, in these conditions in a non-pregnant healthy adult, this is no longer strictly sufficient to sustain requirements for the production of TH, since 80 µg of iodide is still required daily. To compensate for the missing amount (i.e. ~10-20 µg), the system must use the iodine that is stored in the gland, which therefore becomes progressively depleted to lower levels (~2-5 mg of stable iodine). Over time, if the nutritional situation remains unchanged and despite some adaptation of urinary iodine losses, the metabolic balance becomes negative. The thyroid gland tries to adapt by an increased uptake, glandular hypertrophy, and a higher setting of the pituitary thyrostat.

During pregnancy, two fundamental changes take place. There is a significant increase in the renal iodide clearance (by ~1.3 to ~1.5 fold) and, concomitantly, a sustained increment in TH production requirements (by ~1.5 fold), corresponding to increased iodine requirements, from 80 to 120 µg iodide/day. Since the renal iodide clearance already increases in the first weeks of gestation and persists thereafter, this constitutes a non-avoidable urinary iodine loss, which tends to lower circulating PII levels and, in turn, induce a

compensatory increase in the thyroidal clearance of iodide. These mechanisms underline the increased physiologic thyroidal activity during pregnancy. Panel C in Figure 2.28 indicates that when the daily iodine intake is only 70 μg during pregnancy, despite an increase in glandular uptake to 60%, the equilibrium becomes more or less rapidly unbalanced, since the iodide entry resulting from both uptake and recycling is insufficient to fulfill the increased requirements for TH production.

Calculations show that in such conditions, ~20 μg of iodine are missing daily and, in order to sustain TH production, the glandular machinery must draw from already depleted intra-thyroidal iodine stores. Thus in about one trimester after conception, the already low intra-thyroidal iodine stores become even more depleted and, when iodine deprivation prevails during the first half, it tends to become more severe with the progression of gestation to its final stages.

A second mechanism of iodine deprivation for the mother occurs later in gestation, from the passage of a part of the available iodine from maternal circulation to the fetal-placental unit. The extent of iodine passage has not yet been precisely established. At mid-gestation, the fetal thyroid gland has already started to produce TH, indispensable for the adequate development of the fetus.

In summary, augmentation of iodine trapping is the fundamental mechanism by which the thyroid adapts to changes in the iodine supply, and such mechanism is the key to understanding thyroidal adaptation to iodine deficiency. During pregnancy, increased hormone requirements and iodine losses alter the preconception steady-state. When the iodine supply is restricted (or more severely deficient), pregnancy triggers a vicious circle that leads to excessive glandular stimulation (Glinoer, 1999).

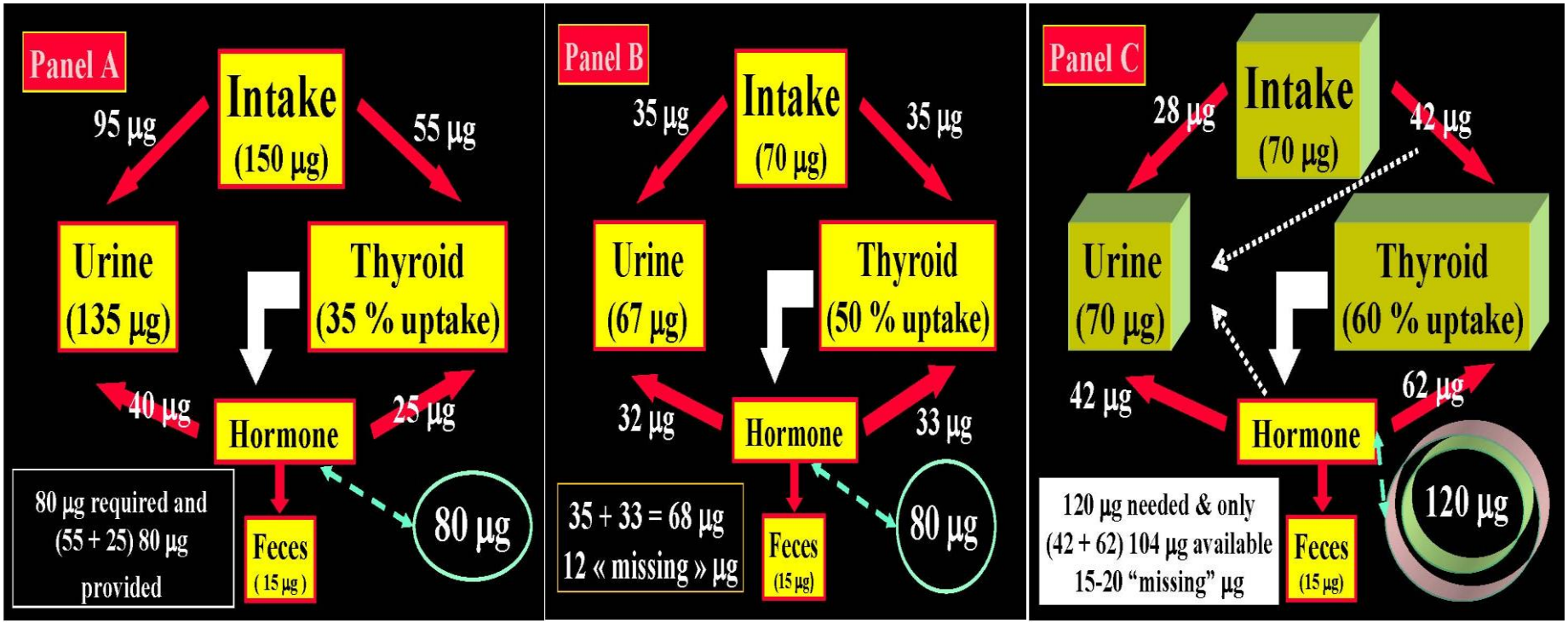



**Figure 2.28: Schematic representation of the kinetics of iodide in healthy non-pregnant and pregnant adults**

Panel A: non-pregnant adult with an adequate iodine intake of 150 µg/day. Panel B: non-pregnant adult with a restricted iodine intake, corresponding to 70 µg/day. Panel C: the latter condition is compared with an identically restricted level of iodine intake (i.e. 70 µg/day) in a pregnant woman. Daily TH production was set at 80 µg of iodine/day (in non-pregnant) and increased by 1.5-fold to 120 µg/day during pregnancy

**Source: Glinoer, 1999**

**Iodine nutrition of pregnant women in India**

Very limited data is available regarding the iodine nutritional status of pregnant women in India. Review (systematic review) of published literature assessing the iodine nutrition status of pregnant women in India clearly shows a significant iodine deficiency in pregnant women (Yadav et al, 2012). There is a need to conduct national level representative survey to better quantify the iodine nutrition of pregnant women in India.

Even in countries that have achieved iodine sufficiency, the status of iodine nutrition in pregnant and lactating women may still be inadequate. For example, in the United States of America, where the status of iodine nutrition is adequate in the general population, with a median UI concentration of 145 μg/L, 6.7% of pregnant women are nevertheless affected by moderate to severe ID and have a UI concentration below 50 μg/L. This is probably largely due to the fact that women are recommended to limit their intake of salt during pregnancy, which includes iodized salt, but also because of the metabolic changes that occur during pregnancy and lactation that result in an increased requirement for iodine (Beckers and Reinwein, 1991). Yet pregnant women are the most sensitive group in the population to the effects of ID, maternal hypothyroxinemia due to ID occurring early during gestation, even before the onset of fetal thyroid function, is the cause of irreversible brain damage in the fetus resulting in mental deficiency in the offspring (Zoller, 2003).

Hence, iodine status of the pregnant women needs to be addressed on priority.

**Iodine requirements during pregnancy, lactation and Infancy: new recommendations**

The thyroid economy undergoes a series of metabolic changes during pregnancy and lactation (Glinoer, 1997*). One of the factors involved in these changes is the increased requirement of iodine in the mother due to the transfer of thyroxine (T4) and of iodide from mother to fetus during pregnancy and to the loss of iodide in breast milk during

lactation. These two processes are required in order to ensure normal brain development and prevention of mental retardation in the offspring (de Escobar et al, 2000; IOM, 2001). Because of these factors, the recommended dietary intake of iodine during pregnancy is higher than the value of 150 µg/day recommended for non-pregnant adults and adolescents (WHO/UNICEF/ICCIDD, 2001; IOM, 2001) (Table 2.10). Below this critical threshold of 150µg/day, the iodine balance is negative during pregnancy (Dworkin et al, 1966).

**Table 2.10: Iodine requirements during pregnancy, lactation and neonatal period**

| Age or Population group | IOM EAR | AI or RDA | Age or Population group | WHO RNI |
|---|---|---|---|---|
| Infant 0-12 m | - | 110-130 | Children 0-5 y | 90 |
| Children 1-8 y | 65 | 90 | Children 6-12 y | 120 |
| Children 9-13 y | 73 | 120 | | |
| Adults > 14 y | 95 | 150 | Adults >12 y | 150 |
| Pregnancy | 160 | 220 | Pregnancy | 250 |
| Lactation | 200 | 290 | Lactation | 250 |

**Source: Marwah and Gopalkrishnan, 2011**

## 2.12 METHODS TO ASSESS IODINE STATUS

Four methods are generally recommended for assessment of iodine nutrition in populations: urinary iodine concentration (UI), the goitre rate, serum TSH, and serum Tg. These indicators are complementary, in that UI is a sensitive indicator of recent iodine intake (days) and Tg shows an intermediate response (weeks to months), whereas changes in the goitre rate reflect long-term iodine nutrition (months to years).

### Thyroid size

Two methods are available for measuring goitre:

1. Neck inspection and palpation
2. Thyroid ultrasonography

By palpation, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined.

In the classification system of WHO (2007):

- Grade 0 is defined as a thyroid that is not palpable or visible
- Grade 1 is a goitre that is palpable but not visible when the neck is in the normal position (i.e., the thyroid is not visibly enlarged)
- Grade 2 goitre is a thyroid that is clearly visible when the neck is in a normal position.

**Urinary iodine concentration**

More than 90% of dietary iodine eventually appears in the urine therefore urinary iodine (UI) is an excellent indicator of recent iodine intake. UI can be expressed as a concentration (mg/l), in relationship to creatinine excretion (mg iodine/g creatinine), or as 24-h excretion (mg /day).

For populations, because it is impractical to collect 24-h samples in field studies, UI can be measured in spot urine specimens from a representative sample of the target group and expressed as the median, in mg/L. Although the median UI does not provide direct information on thyroid function, a low value suggests that a population is at higher risk of developing thyroid disorders.

There is no information about iodine nutrition for pregnant and lactating women in the WHO assessment table, and the upper limits of the median UI for lactating women and children less than two years of age were not specified (Table 2.11).

1. The term excessive means in excess of the amount needed to prevent and control iodine deficiency.
2. In lactating women, the numbers for median UI are lower than the iodine requirements because of the iodine excreted in breast milk.

However, the median UI is often misinterpreted. Individual iodine intakes and, therefore, spot UIs are highly variable from day to day and a common mistake is to assume that all subjects with a spot UI less than 100 µg/L are iodine deficient. Daily iodine intake for

population estimates can be extrapolated from UI, using estimates of mean 24-h urine volume and assuming an average iodine bioavailability of 92% using the formula: urinary iodine (µg/L) x 0.0235 x body weight (kg) = daily iodine intake. Using this formula, a median UI of 100µg/l corresponds roughly to an average daily intake of 150 µg.

**Table 2.11: Epidemiological criteria from the WHO for assessment of iodine nutrition in a population based on median or range of UI**

| UI | Iodine intake | Iodine Nutrition |
|---|---|---|
| School-aged children | | |
| <20 | Insufficient | Severe iodine deficiency |
| 20-49 | Insufficient | Moderate iodine deficiency |
| 50-99 | Insufficient | Mild iodine deficiency |
| 100-199 | Adequate | Optimum |
| 200-299 | More than adequate | Risk of iodine-induced hyperthyroidism in susceptible group |
| >300 | Excessive | Risk of adverse health consequences |

| Pregnant women | | Lactating women | | Children <2 yr of age | |
|---|---|---|---|---|---|
| <150 | Insufficient | <100 | Insufficient | <100 | Insufficient |
| 150-249 | Adequate | ≥100 | Adequate | ≥100 | Adequate |
| 250-499 | More than adequate | | | | |
| ≥500 | Excessive | | | | |

**Source: WHO/UNICEF/ICCIDD, 2007**

### Thyroid stimulating hormone

Serum TSH is determined mainly by the level of circulating thyroid hormone, which in turn reflects iodine intake, TSH can be used as an indicator of iodine nutrition. However, in older children and adults, although serum TSH may be slightly increased by iodine deficiency, values often remain within the normal range. TSH is therefore a relatively insensitive indicator of iodine nutrition in adults. In contrast, TSH is a sensitive indicator of iodine status in the newborn period.

**Thyroglobulin**

Tg is synthesized only in the thyroid and is the most abundant intra thyroidal protein. In iodine sufficiency, small amounts of Tg are secreted into the circulation, and serum Tg is normally less than 10 µg/L. In areas of endemic goitre, serum Tg increases due to greater thyroid cell mass and TSH stimulation. Serum Tg is well correlated with the severity of iodine deficiency as measured by UI. Commercially available assays measure serum Tg, which requires vein puncture, centrifugation, and frozen sample transport, which may be difficult in remote areas.

**Thyroid hormone concentrations**

In contrast, thyroid hormone concentrations are poor indicators of iodine status. In iodine-deficient populations, serum T3 increases or remains unchanged, and serum T4 usually decreases. However, these changes are often within the normal range, and the overlap with iodine-sufficient population is large enough to make thyroid hormone levels an insensitive measure of iodine nutrition

**Assessment during pregnancy**

According to the WHO/ICCIDD/UNICEF for evaluating the status of iodine nutrition in pregnant women, evaluating the median urinary iodine concentration (UI) is recommended (WHO/UNICEF/ICCIDD, 2007). The recommended daily iodine intake can be used to extrapolate the expected UI in µg/L. This assumes the median 24-h urine volume for girls in the age group of 7-15 years to be 0.9 ml/h/kg; for adult women to be 1.5 L; and the mean iodine bioavailability to be 92%. Using this model, the UI during pregnancy is derived to be approximately 135-150 µg/L, corresponding to the recommended daily iodine intake of 220-250 µg for the period of pregnancy. It may also be necessary to take into account the variations in age of pregnant women, particularly in developing countries, where adolescent pregnancy in not uncommon. For

example, for a15-yr-old weighing 50 kg, the UI value comes about to be approximately 185-215µg/L, considering the recommended daily intake of 220-250 µg. However, given the physiological alterations during pregnancy, an increase in the glomerular filtration rate and possibly renal iodine clearance (RIC), this estimation of UI may stand less valid. These uncertainties were reflected in the recent WHO expert group report, which recommended an adequate iodine intake in pregnancy to be 150-249 µg, and suggested that the UI be extrapolated from this value.

**Assessing status during lactation**

Since the mammary gland is able to concentrate iodine, iodine supply to the newborn via the breast milk may be maintained even in the face of maternal iodine deficiency. Iodine supply to the infant may be maintained even in cases of maternal iodine deficiency because of the ability of the mammary glands to concentrate iodine. This may explain the higher values of BMICs than expected (based on UI) that are observed in lactating mothers in areas of iodine deficiency. The full-term infant's iodine requirement is approximately 7µg/kg body weight.

Assuming that the iodine in breast milk is 95% absorbed, and the mean breast milk excretion of iodine is 0.78L in the first six months post-childbirth, the minimum BMIC required to fulfill the infant's iodine requirement (of 50µg/day) is 80µg/L until introduction of food. Although maternal iodine requirement during lactation is high (200-290 µg/day), the median UI that indicates adequate iodine nutrition status in a lactating woman is the same as that of a non-pregnant, non-lactating woman, keeping in mind the amount of iodine lost in breast milk (WHO/UNICEF/ICCIDD, 2007).

**Assessing status during infancy**

WHO recommendations state, a median UI of atleast 100µg/L in infants is sufficient. At the same time, they recommend an iodine intake of 90µg/d during infancy and suggest extrapolating from this

to a median UI assuming a urine volume of 300–500 ml/d, but this would produce a higher cut-off of at least 180 µg/L (Zimmerman, 2009*).

## 2.13 IODINE DEFICIENCY, THYROID DYSFUNCTION AND INFANT DEVELOPMENT

In areas of iodine sufficiency, healthy women maintain iodine stores of 15–20 mg in the thyroid. During pregnancy, to help meet the approximately 50% increase in maternal iodine requirements; women may draw on this significant iodine store (Zimmerman, 2009*). In areas of chronic iodine deficiency, women enter pregnancy with already depleted iodine stores. With little thyroidal iodine to draw on to meet the increased maternal iodine requirement, pathological changes goiter and hypothyroidism may occur that can adversely affect maternal and fetal health.

Whether mild-to-moderate maternal iodine deficiency causes more subtle impairment of cognitive and/or neurological function in the offspring is uncertain. Two case-control studies in iodine-sufficient women with mild thyroid hypofunction have reported developmental impairment in their offspring. In the United States (Haddow et al, 1999), the IQ scores of 7- to 9-yr-old children of mothers with subclinical hypothyroidism during pregnancy (an increased TSH in the second trimester) were 7 points lower compared with children from mothers with normal thyroid function during pregnancy. In The Netherlands (Pop et al, 1999), infant development to 2 yr was impaired in children of women with a free $T_4$ (FT4) below the 10th percentile at 12 wk gestation. These studies suggest that cognitive deficits may occur in the offspring even if maternal hypothyroidism is mild and asymptomatic. However, the maternal thyroid dysfunction in these studies was presumably not due to iodine deficiency because they were done in iodine-sufficient populations. Table 2.12 gives psycho-neurological outcome in the progeny associated with maternal hypothyroidism due to iodine deficiency.

**Table 2.12: Neuropsychiatric and intellectual deficits in infants and schoolchildren born to mothers residing in conditions with mild to moderate iodine deficiency**

| REGION | TESTS | MAIN FINDINGS | AUTHOR |
|---|---|---|---|
| Spain | Locally adapted:<br>- BAYLEY<br>- McCARTHY<br>- CATTELL | Lower psychomotor and mental development | Bleichrodt (1989) |
| Italy (Sicily) | BENDER-GESTALT | Low perceptual integrative motor ability & neuromuscular and neuro-sensorial abnormalities | Vermiglio (1990) |
| Italy (Tuscany) | WECHSLER<br>RAVEN | Low verbal IQ, perception, motor and attentive functions | Fenzi (1990) |
| Italy (Tuscany) | WISC<br>Reaction time | Lower velocity of motor response to visual stimuli | Vitti (1992)<br>Aghini Lombardi (1995) |
| **India** | **Verbal, pictorial learning tests, Tests of motivation** | **Lower learning capacity** | **Tiwari (1996)** |
| Iran | BENDER-GESTALT<br>RAVEN | Retardation in psychomotor development | Azizi (1993) |

**Source: Glinoer and Delange, 2000**

**Infant development testing scales (Spreen and Strauss, 1998)**

*The Cattell Infant Intelligence Scale-* The Cattell was designed to assess infants and toddlers from 2 through 30 months old.

*The Standard Raven Progressive Matrices-* Drawing on Spearman's theory of general ability consists of 60 matrix problems, which are

separated into five sets of 12 designs each. Within each set of 12, the problems become increasingly difficult. Each individual design has a missing piece. The participant's task is to select the correct piece to complete the design from among six to eight alternatives. Correct responses are based on various organizing principles, such as increasing size, reduced or increased complexity, and number of elements. The SPM uses nonverbal stimuli, and it is assumed that it does not require a specific knowledge base.

*The Wechsler Intelligence Scale for Children-* This test is an individually administered clinical instrument for assessing the intellectual abilities of children aged 6 years through 16 years, 11 months. The instrument consists of three main composite scores: Verbal IQ (comprising Information, Similarities, Arithmetic, Vocabulary, Comprehension, and Digit Span subtests), Performance IQ (comprising Picture Completion, Coding, Picture Arrangement, Block Design, Object Assembly, Symbol Search, and Mazessubtests), and Full Scale IQ.

*The Denver Developmental Screening Test-* The Denver Developmental Screening Test is one of the most popular developmental screening tests (with an age range of 1 month to 6 years). The driving factor of the Denver is its brevity-it takes 15-20 minutes to administer. The content of the test includes personal-social, motor, language, and adaptive domains. The scoring is based on parent reports, direct child assessment, and observation. The assessment results expressed by a single score assigning the child to one of the four descriptive categories: Pass, Questionable, Abnormal, or Un-testable.

*The McCarthy Scales of Children's Abilities-* The McCarthy Scales of Children's Abilities form a well standardized and psychometrically sound measure of the cognitive abilities of young children (ages 2 1/2 to 8 1/2 years). The test is individually administered and takes about 45 to 60 minutes to administer, depending on the age of the child. The McCarthy Scales have some unique features valuable for the assessment of young children with learning problems or other

exceptionalities. The test produces a general measure of intellectual functioning called the General Cognitive Index (GCI), as well as a profile of abilities that includes measures of verbal ability, nonverbal reasoning ability, number aptitude, short-term memory, coordination, and hand dominance.

*Bayley Scales of Infant Development-* This scale is arguably the most widely used measure of the development of infants and toddlers. In addition, the BSID has an extensive psychometric history and a very respectable track record. The BSID-II is applicable to children from 1 through 42 months of age. The administration takes about 25 to 35 minutes for infants under 15 months of age and up to 60 minutes for children over 15 months.

**Baroda Development Screening Tests for Infants (BDSTI) (Pathak and Khurana, 1991)**

A screening test for the assessment of the motor-mental development of infants was developed by selecting items from the Bayley Scales of Infant Development (BSID). Many items of the BSID use standardized equipment (cubes, pegboard, form-board, etc.) and standardized techniques (certain performances timed by stop-watch). The age-placements cannot be presumed to be the same when non-standardized tools and techniques are used. For instance, it was reported that the size, weight and surface of the cubes affects the performance of the child in building a tower of cubes and using any cubes or boxes is wrong. The BSID is a detailed test and has many items for testing the development of one skill-some of them very close in the developmental sequence. For instance, there are ten items related to the skill of sitting. Items like: sits with support, effort to sit, sits alone momentarily; sits alone 30 sec or more and sits down require some experience and good judgment on the part of the health worker. For instance one must be sure that the child sat down and did not slump down.

**Table 2.13: Screening test items (BDSTI)**

| Age group | Sr. No. | Items |
|---|---|---|
| 1 | 1 | Arms and legs thirst in play * |
| | 2 | Momentary regard |
| | 3 | Lateral head movement (prone) * |
| 2 | 4 | Responds to sound |
| | 5 | Follows moving person |
| | 6 | Free inspection of surroundings |
| 3 | 7 | Social smile/vocalizes |
| | 8 | Eye co-ordination |
| | 9 | Head erect and steady* |
| 4 | 10 | Holds head steady* |
| | 11 | Recognizes mother |
| | 12 | Elevates on arms* |
| 5 | 13 | Play with rattle/hand play |
| | 14 | Reaches for dangling ring |
| | 15 | Sits with slight support* |
| 6 | 16 | Turns head to sound |
| | 17 | Turns from back to side* |
| | 18 | Exploitive paper play |
| 7 | 19 | Discriminates strangers |
| | 20 | Pulls to sit* |
| 8 | 21 | Bangs to play |
| | 22 | Sits alone steadily* |
| 9 | 23 | Retails two things in two hands |
| | 24 | Pulls to stand* |
| | 25 | Playful response to mirror images |
| | 26 | Sits with good coordination* |
| 10 | 27 | Pulls string-secures toy |
| | 28 | Co-operates in play |
| | 29 | Crawling (pre-walking) * |
| 11 | 30 | Rings bell purposefully |
| | 31 | Fine prehension* |
| | 32 | Raises to sit* |
| | 33 | Stands by furniture* |
| 12 | 34 | Adjusts to words |
| | 35 | Says da-da |

| | | |
|---|---|---|
| **13-15** | **36** | Inhibits on command |
| | **37** | Midline skills * |
| | **38** | Walks with help* |
| | **39** | Turns pages |
| **16-18** | **40** | Imitates words |
| | **41** | Stands alone* |
| | **42** | Spontaneous scribble |
| | **43** | Throws ball* |
| | **44** | Aufstein I* |
| | **45** | Walks alone* |
| | **46** | Gestures for wants* |
| **19-24** | **47** | Shows shoes, etc. |
| | **48** | Two words |
| | **49** | Walks up and down stairs with* help |
| | **50** | Words for wants |
| **25-30** | **51** | Two word sentences |
| | **52** | Name three objects |
| | **53** | Stands on one foot* |
| | **54** | Walks up and down stairs without help* |

**Source: Pathak and Khurana, 1991** *Motor scale items

Only those items which were simple and easy to administer and to assess and not requiring any special training, experience and equipment were selected. Further duplication of items with similar age-placement was avoided. While some items of similar nature like eye co-ordination were grouped together. In this way a total of 54 items 22 motor and 32 mental were selected for the screening test (Table 2.13).

## 2.14 IRON DEFICIENCY DURING PREGNANCY

The overall iron requirement during pregnancy is significantly greater than that in the non-pregnant state despite the temporary iron losses incurred during menstruation.

### Iron requirements during pregnancy

If the demand for iron were spread evenly throughout gestation, iron requirements could be met more easily by a sustained rise in the rate

of iron absorption. The need for iron, however, varies markedly during each trimester of pregnancy.

***First trimester:*** Iron requirements decrease during the first trimester because menstruation stops, which represents a median saving of 0.56mg Fe/d (160 mg/pregnancy) (Hallberg and Rossander-Hulten, 1991). The only iron losses that must be met during this period are the obligatory ones from the body via the gut, skin, and urine, which accounts to ~0.8 mg/d in a 55-kg woman (14g/kg/d or 230 mg/pregnancy) (Green et al, 1968). Early hemodynamic changes include generalized vasodilation, some increase in the plasma volume, and an increase in red blood cell 2, 3- diphosphoglycerate concentrations (Hallberg and Hulten, 1996). There is also some evidence that erythropoietic activity may be reduced during this period, with a slight reduction in red blood cell mass (Taylor and Lind, 1979), a reduction in the number of reticulocytes, and a rise in the serum ferritin concentration (Kaufer and Casanueva, 1990).

***Second Trimester:*** During the second trimester, iron requirements begin to increase and continue to do so throughout the remainder of pregnancy. The increase in oxygen consumption by both mother and fetus is associated with major hematologic changes.

Most studies in women supplemented with iron show a change in total blood volume of ~45%, with an increase in plasma volume of ~50% and an increase in red blood cell mass of ~35%. The rise in hemoglobin mass is similar at ~30% (de Leeuw et al, 1966). There has been some difficulty in establishing the normal hemoglobin concentration in pregnancy because of both the disproportionate increases in the plasma volume and the frequent occurrence of iron deficiency anemia.

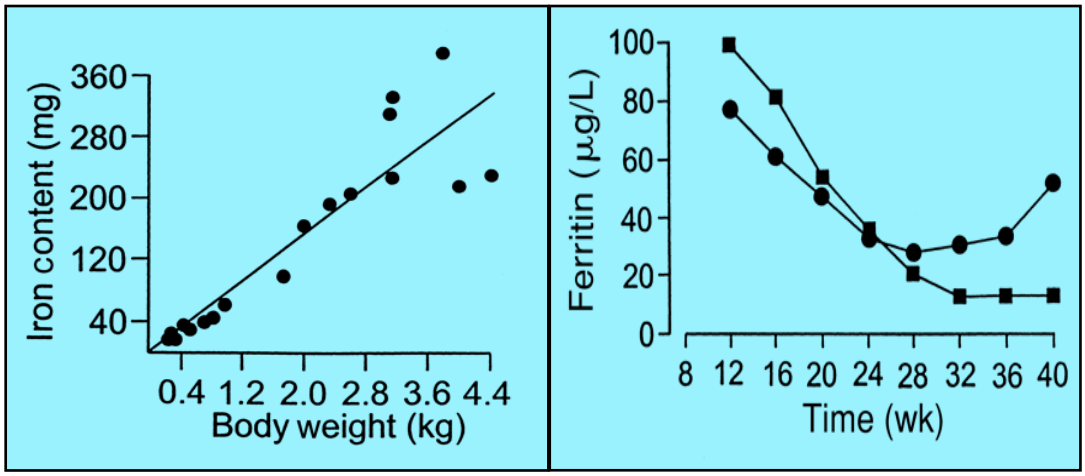
In studies in which iron deficiency was prevented, however, the average decrease in hemoglobin concentration appeared to be <10g/L (Bonner and Goldberg, 1969). Translated into iron requirements, the increase in red blood cell mass that occurs in a normal pregnancy

amounts to ~450 mg Fe in a 55-kg woman (de Leeuw et al, 1966). Whereas this represents a significant drain during the later part of the pregnancy, it does not affect long-term iron balance because the iron is returned to the body's stores at the end of pregnancy, when the red blood cell volume gradually reverts to normal.

***Third Trimester:*** As pregnancy progresses, iron requirements for fetal growth rise steadily in proportion to the weight of the fetus, with most of the iron accumulating during the third trimester (Figure 2.29a). The average iron content of a fetus weighing >3kg is ~270mg (Widdowson and Spray, 1951).

In determining iron requirements during pregnancy, the losses incurred during parturition must also be added. These include an average maternal blood loss equivalent to 150 mg Fe and a further 90 mg present in the placenta and umbilical cord.

**Figure 2.29: Left (a)- Relation between body weight and body iron content in the fetus and newborn child (de Leeuw et al, 1966) & Right (b)- Effects of iron supplementation on serum ferritin concentration in pregnancy (Fenton et al, 1977)**




[Supplemented group- ● , un-supplemented group- ■]

***Period after delivery:*** In the period after delivery, there is a small additional iron loss of ~0.3mg/d through lactation (Fransson and Lonnerdal, 1980), but this is offset by the absence of menstruation, except when breast-feeding is continued long after the return of menstruation.

To summaries, the total iron requirement during pregnancy for a 55-kg woman is ~1,040 mg (Table 2.14). At delivery, there is a further loss of maternal blood, which raises the total cost of pregnancy to ~1,190 mg iron. The net cost, however, is only 580 mg because the iron used to increase the red blood cell mass is returned to stores and overall losses are further offset by the absence of menstruation during pregnancy.

**Table 2.14: Iron requirements in pregnancy**

| **Total cost of pregnancy** | **Amount (mg)** |
|---|---|
| Fetus | 270 |
| Placenta | 90 |
| Expansion of red blood cell mass | 450 |
| Obligatory basal losses | 230 |
| Sum | 1040 |
| Maternal blood loss at delivery | 150 |
| Total cost | 1190 |
| **Net cost of pregnancy** | |
| Contraction of maternal red blood cell mass | -450 |
| Absence of menstruation during pregnancy | -160 |
| Subtotal | -610 |
| Net cost | 580 |

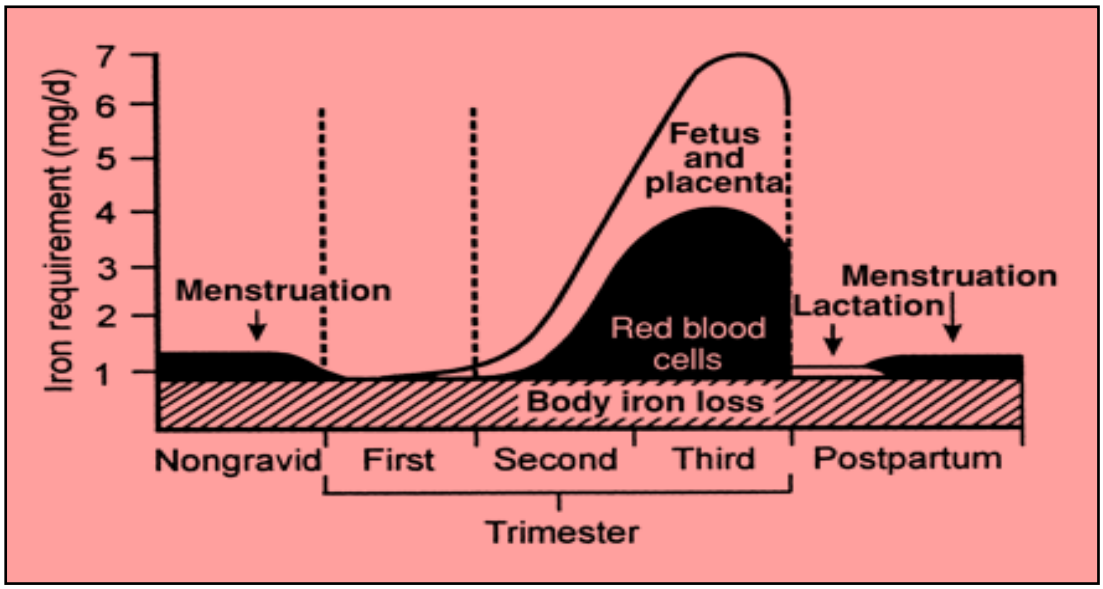
**Source: Bothwell, 2000**

**Iron balance in pregnancy**

When total iron requirements during pregnancy are translated into increased daily needs, it is apparent that there is an unequal distribution over time (Figure 2.30). Although reduced during the first trimester, iron requirements rise to between 4 and 6 mg in the second and third trimesters, respectively (FAO, 1988). Because major changes in the red blood cell mass start occurring only in the middle of the second trimester (Lund and Donovan, 1967), iron requirements may reach as much as 10 mg/d during the last 6–8 wk of pregnancy (Hallberg, 1992).

Irrespective of the exact value, it is apparent that daily iron requirements cannot be met from dietary absorption alone in the latter part of pregnancy, even from the most optimal diet. In diets containing large quantities of bio-available iron diets in which there are generous quantities of meat, poultry, fish, and foods containing high amounts of ascorbic acid, overall iron absorption is usually 3–4 mg/d and, at most, 5 mg/d. The amount of iron absorbed is much lower when the diet contains only small amounts of bio-available iron (FAO, 1988), as is often the case in many developing countries where the staple food is cereal and the intake of meat and ascorbic acid is limited.

**Figure 2.30: Estimated daily iron requirements during pregnancy in a 55-kg woman**




**Source: Bothwell, 2000**

### Importance of iron stores

Iron balance can be maintained in pregnancy only when there are adequate iron stores at the start of pregnancy. If a woman routinely eats a diet high in bio-available iron, a pre-pregnancy iron store of 300 mg is probably sufficient to carry her through pregnancy, although a higher amount of stored iron is needed when the diet is less than optimal.

**Assessment of iron status in pregnancy**

Assessing iron status during pregnancy is filled with difficulties because the profound hemodynamic changes associated with pregnancy affect several indexes of iron status. During pregnancy, hemo-dilution leads to a reduced hemoglobin concentration, whereas both serum iron and ferritin concentrations decrease and total iron-binding capacity increase (Fenton et al, 1977).

The relative contributions of pregnancy per se and a pregnancy induced negative iron balance in bringing about these changes can be assessed by measuring the changes in hemoglobin, serum iron, serum ferritin, and total iron-binding capacity that occur during pregnancy in women rendered iron replete after adequate iron supplementation during pregnancy (Fenton et al, 1977). The disproportionate increase in plasma volume during pregnancy leads to a drop in the hemoglobin concentration of <10g/L. Although hemoglobin concentrations <110 g/L have been occasionally reported in iron-replete women, this concentration has proved to be a useful cutoff for defining anemia in pregnancy (FAO, 1988). There is a moderate drop in the concentration of serum iron that stabilizes in the middle of pregnancy (Fenton et al, 1977). More striking than the changes in either hemoglobin or serum iron concentrations is the steady rise in total iron-binding capacity during pregnancy to <50% above normal, which reflects an increase in the concentration of transferrin in plasma (Fenton et al, 1977). As a result, there is a drop in transferrin saturation. As discussed previously, there is some evidence that serum ferritin rises modestly early in pregnancy, presumably because of reduced erythropoietic activity; thus, iron is diverted to stores (Kaufer and Casanueva, 1990). Thereafter, however, the serum ferritin concentration drops steadily to <50% of normal at midterm (Figure 2.29b) (Milman et al, 1991). These changes reflect hemo-dilution and the mobilization of iron from stores to meet the increased demands of pregnancy. It is, therefore, apparent

that all the indexes associated with iron deficiency including hemoglobin, transferrin saturation, and serum ferritin concentrations are reduced during pregnancy even in iron-replete women. In contrast, the concentrations of circulating transferrin receptor have been found to be normal in pregnancy, only being raised if iron deficiency is present. This suggests that serum transferrin receptor concentrations may prove to be a useful tool for diagnosing iron deficiency in pregnancy.

**Strategies to combat iron deficiency in pregnancy**

***Daily supplementation***

Iron supplementation regimens in pregnancy vary depending on the characteristics of the population. In developed countries most women enter pregnancy with normal hemoglobin concentrations and variable amounts of stored iron. In contrast, large numbers of women in developing countries are anemic at the onset of pregnancy (WHO, 1992). Prenatal iron supplementation is not compulsory in many industrialized countries and the recommended dose is often small (30 mg ferrous iron daily), but has been as high as 240 mg/d in developing countries. In India (Sood et al, 1975) the World Health Organization (WHO) recommended universal supplementation of all pregnant women with 60 mg ferrous iron twice daily in populations where gestational anemia is common and once daily in populations where overall iron nutrition is better (de Maeyer et al, 1989). This recommendation was subsequently modified to a single daily dose of 60 mg Fe for 6 month in pregnancy or 120 mg Fe if 6 month duration cannot be achieved (Stoltzfus and Dreyfus, 1998). Keeping the dose as low as is compatible with unimpaired effectiveness is an important principle because the side effects of iron therapy, which can seriously limit compliance, are dose-dependent phenomena (Solvell, 1970).

Two strategies that merit consideration are programs to modify dietary habits and iron fortification of foods (Bothwell and MacPhail, 1992). The second has the advantage that it can be applied to large population groups at low cost and the identification and cooperation of deficient or potentially deficient individuals is not a prerequisite, as it is with supplementation.

## 2.15 DOUBLE FORTIFIED SALT (DFS)

Salt has been the vehicle for the world's most successful food fortification initiative to date - Universal Salt Iodization. The fortification of salt with iodine has been hailed as one of the world's great public health advancements. Now breakthrough technology that allows salt to be double fortified with iron as well as iodine has created an exciting new opportunity to reach the world with supplemental iron easily and inexpensively, without having to change people's habits.

**Iodine deficiency** is the world's leading cause of preventable intellectual disability

**Iron deficiency anemia** is the most common and wide-spread nutritional disorder in the world

**Iodine and Iron**

Previously incompatible in food fortification, they can now be combined through new technology in Double Fortified Salt.

Billions of people are affected by the hidden hunger of micronutrient deficiencies. Double Fortified Salt is an innovative new fortified food product - delivering crucial amounts of iodine and iron to human beings through their diet. Double Fortified Salt presents one of the most cost-effective opportunities to deliver two of the most critical micronutrients for mental capacity, maternal and infant survival and human productivity.

Fortification of common salt with iron has been developed by the National Institute of Nutrition (NIN) as a public health strategy for the

control of IDA on the lines of iodization of salt for the effective control of IDD (Working group report, 1982). However, with the advent of universal iodization of edible salt as a National policy in 1988, NIN undertook research studies aimed at development and testing of double fortified salt (DFS) containing iodine and iron for reducing the deficiencies of both these micronutrients (Narasinga Rao, 1994).

In view of their antagonistic chemical properties, the incorporation of iron and iodine in salt requires a stabilizer. NIN developed a DFS formulation using sodium hexa-metaphosphate (SHMP) as a stabilizer. SHMP is intended to protect iodine and prevent the interaction between the iron and iodine and also with the other constituents of the salt. The stability, bioavailability and acceptability of DFS were determined and found to be good (Narasinga Rao, 1994).

The Micronutrient Initiative (MI) in Canada (MI report, 1999) and a company in Chennai with the trade name of the salt "Nutrisalt" (Rajagopalan and Malvika, 2000) have developed two other formulations of DFS, in which physical separation of iodine was achieved by barrier methods. Parallel to these developments, studies were continued at NIN to explore other formulations. On the suggestion and support from the International Life Sciences Institute (ILSI, Washinton D.C.), NIN also tested other DFS formulations containing sodium ferric EDTA as a source of iron along with Iodine and also SHMP and EDTA as promoters of iron absorption (NIN, 2002). Some formulations were identified as promising second-generation preparations. DFS containing encapsulated iron salt was found to have good stability characteristics and iron bioavailability, even with powdered common crystal salt. Working with powdered common salt reduces the cost of salt but increases the cost of iron incorporation, so that at the final product level there may not be any major difference in cost between these different approaches.

**Table 2.15: Comparative Physico-Chemical Features of different DFS Formulations**

| **Characteristics** | **NIN formulation** | **MI formulation** | **Nutrisalt** |
|---|---|---|---|
| Clinical constituents | 30-40 ppm I, KIO3 or KI<br>Ferrous sulphate SHMP Stabilizer and promoter | 50 ppm I, KI–Ferrous fumarate Encapsulation of iodine by dextrin | 30 ppm I, KIO3, Iron salt Barrier |
| Stability | Stable up to 9 months | Stable for 12 months. | Report claims good stability |
| Acceptability | Full-fledged acceptability described | Not acceptable with some foods | Report claims good acceptability and stability during cooking |
| Bioavailability of iron and iodine | Demonstrated (Iron absorption 6.1%)<br>Urinary iodine increased like within IS | Demonstrated (Iron absorption variable 13.5%)<br>Urinary iodine equal to IS | Not reported |
| Pilot scale/ plant scale production tested | Plant scale production | Not tested | Not known |

**Source: Shivkumar, 2004**

**Table 2.16: Comparative bio-impact features of different DFS formula (Source: Shivkumar, 2004)**

| **Characteristics** | **NIN formulation** | **MI formulation** | **Nutrisalt** |
|---|---|---|---|
| Study Populations | Tribal villages in (AP) single blind, placebo Residential school children, Hyd. Double blind, placebo | Mothers and children in Ghana, double blind, placebo | Tea estate labourers, Valpari, South India Double blind, placebo |
| De-worming treatment | No treatment | No treatment | Simultaneously de-wormed |
| Stability of iodine at the Location | Done | Done | Data not given |
| Impact on iron and iodine Status | Goitre prevalence decreased and urinary iodine improved. Iron status benefited only in some groups of the tribals. Benefited residential school children both in iron status and iodine status. The impact on iron status was mainly on control of anemia. | Maintain good iodine levels in children and mothers. Children showed small reduction in prevalence of anemia with DFS while the prevalence of anemia increased in controls. Mothers too showed improvement with DFS, though the anemia prevalence at baseline was not comparable to control. | Benefited only in females. De-wormingis an important requisite for response. |
| Cost | About Rs. 4.50/kg | Worked out | Worked out (Rs. 4.50/kg) |
| Productivity | Not worked out | Not Worked out | Measured Plucking of tea leaves |
| Safety issues, if any, due to components | Safety of SHMP evaluated in rats as well as in children | Perhaps issues are not involved | Not known, if any safety issues are involved |

The NIN also carried out extensive studies on the safety and impact of its DFS (both experimental and community situations) on both iron and iodine statuses (Sivakumar and Nair, 2002). Trials of large-scale production, operational feasibility of distribution and acceptability in the community have been repeatedly demonstrated with the NIN DFS (Table 2.15).

Limited studies dealing with the acceptability and impact were reported on the other two formulations. The MI formulation, though stable, developed an unacceptable colour with some recipes (MI report, 1999). No detailed studies are available on the organoleptic properties of Nutrisalt.

Both the NIN and MI formulations demonstrated a limited, but significant impact on both iron and iodine status of different population groups. Nutrisalt was found to have an impact on iron status (as measured by an increase in blood hemoglobin) at a lower probability than the other formulations of DFS (Rajagopalan and Malavika, 2000) (Table 2.16).

It has to be understood that the impact of DFS on hemoglobin increase will not be very striking since providing iron through DFS is a preventive measure and not a therapeutic one. However, it is a sustainable method of doubling iron intake of one billion plus population for several decades.

**Iron fortified Iodized Salt to be promoted to battle malnutrition in the country (www.pmindia.nic.in)**

Public health experts from various part of our country have different opinion regarding promotion of double fortified salt (table 2.17). However, our Prime Minister has agreed to promote DFS in ICDS and MDM programmes.

A meeting was held in the Prime Minister's Office on the promotion of consumption of Iron fortified Iodized Salt as a measure to deal with malnutrition in the country. The meeting on 18.4.2011 was chaired by

the Principal Secretary to the Prime Minister and was attended by the officers of Ministries of Health, Women and Child Development, Department of Industrial Policy and Promotion, Director of National Institute of Nutrition and a Member of the Prime Minister's National Council on India's Nutrition Challenges.

Actions on the following lines were agreed upon:

- To begin with, Ministries dealing with food and nutrition programmes like ICDS and Mid-day Meal Programme will make the use of iron fortified iodized salt (double fortified salt) mandatory in those programmes, in an appropriate manner;
- The Dept. of Food and Public Distribution will examine the possibility of supplying DFS through the PDS;
- A major mass media campaign will be taken up to promote the use of iron fortified iodized salt (DFS) by the Ministries of Women and Child Development and Health and Family Welfare;
- The communication campaign and the decision to use DFS in Government programmes needs to be associated with efforts to increase the supply of DFS in the country. The Department of Industrial Policy and Promotion will work with the private industry and cooperatives to promote manufacture of iron fortified iodized salt (DFS). The Department will also explore the possibility of taking up a scheme to promote capital investment and technology up-gradation so that the installed capacity for producing iron fortified iodized salt (DFS) in the country is substantially stepped up.
- The Departments of Health and Family Welfare and Health Research under the Ministry of Health and Family Welfare will take necessary measures for promoting the use of DFS in the country, including advising the Ministries of Women and Child Development, Human Resource Development and Consumer Affairs, Food and Public Distribution on use of DFS in Government programmes.

**Table 2.17: Responses from various Public health experts/ scientists towards double fortification of salt**

| Public Health Expert | Response on use of DFS |
|---|---|
| ***Umesh Kapil (AIIMS, New Delhi)** | DFS developed by NIN needs to be tested under public health conditions to demonstrate its effectiveness both on iodine and iron status. The results of limited controlled field studies undertaken in residential school children do show the effectiveness of the DFS. However, before DFS is introduced as a National Programme or in to a National Programme like ICDS, we need to have effectiveness studies of DFS under public health conditions. |
| ***R. K. Bakshi (GMC, Baroda)** | Experience of even single fortification - Iodized salt utilization has been far from satisfactory. Apparently, there is no reason for its low consumption. Gujarat has followed up the history of low utilization in face of very significant production of the edible salt. A lot of organized effort is on to improve. It has been the priority of the state as well as many international agencies. |
| ***Prakash Kotecha (GMC, Baroda)** | In a culturally diverse country like India, across the country a common food that can be fortified and would work is salt. It is with this background that we are trying to reach out to explore double fortified salt. ICMR has been in to systemic review currently with committees working on this and data are favourable though not satisfactory to absolute scientific standards.<br><br>We need to generate more data on this and to do this at various levels we will have to try out rather than wait for a green signal from high bodies to start use of DFS at national level. Studies have not very convincingly shown the efficacy but have shown its acceptability and overall recommendation is favourable for its use.<br><br>More trial to study both efficacy and effectiveness |

| | |
|---|---|
| | and simultaneously acceptability are required. If we need to control anemia effectively supplementation alone will be main approach but by itself would not be enough as global experience documents. Fortified salt is a big hope and let us hope it becomes a reality in near future. |
| ***Subadra Seshadri (MSU, Baroda)** | There are two ways of getting more and better bio available iron through diets. One is dietary diversification, improving access to foods that are good sources of iron and ascorbic acid, for a predominantly vegetarian population. The other is to fortify foods with iron. In India, since salt is universally consumed, salt has been chosen as a vehicle for fortification. As pointed out by others, quantity of iron added to salt is small, 1mg per g. Given the low bioavailability when consumed with typical vegetarian meals of India, we cannot expect the fortified salt to produce any dramatic reduction in anemia prevalence even over a period of a year or two.<br><br>However, what the iron-fortified salt will do is to add small but useful amounts of iron to our diets daily (because we consume salt every day) and possibly prevent a decline in HB that seems to occur under many conditions in our day-to-day lives. There is some good evidence to support this from the NIN studies. So fortified salt should be considered as one of the several approaches for adding some iron to our diets. This will have to be combined with diet diversification, nutrition awareness promotion and other approaches, for a more lasting solution to the problem of anemia. |
| ***H S Sharma (Consultant, Gurgaon)** | We are discussing only fringe subject like DFS while the malady lies somewhere else. The DFS would be of use when the people get adequate amount of nutrition in terms of carbohydrate and protein. We are going the wrong way why should poverty line be pegged at 1700 K cal in India while it is 3.000 K cal in China? |

| Public Health Expert | Response on use of DFS |
|---|---|
| ****S Nair & K Joshi** **(MSU, Baroda)** | Our experiences with fortification are suggestive that, it is highly beneficial in urban as well as rural scenario. Major success remains when we are able to convince the consumers who are using it, especially it is their right to know what deficiency do they have, thus they need to consume the fortified items. We have used Double fortified salt as a replacement from the daily salt though minor, the changes are playing a contributory role when it is used in long term strategies and programmes mainly because of sustained release of Iron and Iodine. Using it in a pregnant mother's diet did not bring any significant change in iron, but contributory roles in Iodine levels were observed. In children, along with dietary counseling and improved food intake, mothers opined that they have become more active and fatigue is not seen. This is suggestive that we need to promote consumer education before introducing the fortificant. It would add more meaning to our contributions. |
| ****B Sesikaran** **(NIN, Hyderabad)** | Double-fortified salt (DFS) which is fortified with both iron and iodine is identified as a potential public health tool for delivering nutritional iron to population at large. National Institute of Nutrition (NIN), Hyderabad has carried out extensive studies on stability, bioavailability, acceptability, safety and impact (including in community) of DFS. Feasibility both at factory level production and community level implementation have been worked out. It has been tried and distributed through a state school feeding program in the state of Tamil Nadu. |
| ****Deepika Anand** **(RA, Chhattisgarh)** | Around 50 percent of women in Chhattisgarh were anemic. Iron Fortified Salt Distribution (IFSD) Scheme was launched wherein iron fortified salt was distributed to the beneficiaries through ICDS programme. The results indicated that this salt was beneficial in reducing the level |

| | |
|---|---|
| | of anemia among pregnant women, lactating mothers and under-6 years children. Thus, the programme should be continued regularly without any interruption. |
| ****Mehtab B Bamji (Dangoria Charitable Trust, Hyderabad)** | It is a pity that a promising technology to address a major nutrition problem i.e. iron deficiency, is allowed to languish for some reasons. No one is questioning the need for dietary diversification. However, with the price of vegetables, pulses and even millets, besides animal foods, which are rich sources of iron, so high, our field experience shows that even the farmers who grow vegetables prefer to sell them to earn money, this despite heavy dose of nutrition education. It is not the case of either or, but both or all strategies that would help to increase body iron stores. Hemoglobin increase is the end result. Even improvement in body store has impact on functions of iron like brain function.<br>DFS is meant to build iron stores and not treat anemia. In a country where the burden of anemia is high, treatment strategies have to accompany food- based strategies. DFS is also afoot based strategy. |
| ****Prema Ramachandran, (Nutrition Foundation of India, New Delhi)** | All the studies on dietary intake of iron in Indians have shown that the current intake is very low. Any and every method of increasing iron intake is therefore needed. Dietary diversification is one method. Use of iron and iodine fortified salt is another. These would improve iron intake and overtime improve the iron status, iron stores and Hb levels across the population groups. Relationship between high salt consumption and hypertension are well documented and nutrition education to reduce salt consumption is underway in all countries of the world. Added iron or iodine in the salt does not in any way increase the risk of hypertension and therefore the worry that DFS may increase risk of hypertension is not correct.<br>Currently majority of Indians are anemic; DFS |

| | |
|---|---|
| | intake and dietary diversification alone cannot correct anemia. Therefore detection and treatment of anemia will have to continue to receive attention. |
| ****Sheila C. Vir (Public Health Nutrition and Development Centre, New Delhi)** | I have been following the DFS responses and totally agree that we have the technology and supportive research studies which indicate benefits of DFS and establish the stability, acceptability, bioavailability and safety of DFS. There is no doubt that we need to consider DFS as one of the strategies for addressing the problem of iron deficiency and anemia. However, we need to be extremely cautious prior to rolling out the programme in the country. We need to accept the fact that public health strategy should be evidence based in terms of effectiveness as well as capacity for sustained quality production of DFS. |

**Source: Solution Exchange, 2006*; Solution Exchange, 2012****

# MATERIALS AND METHODS

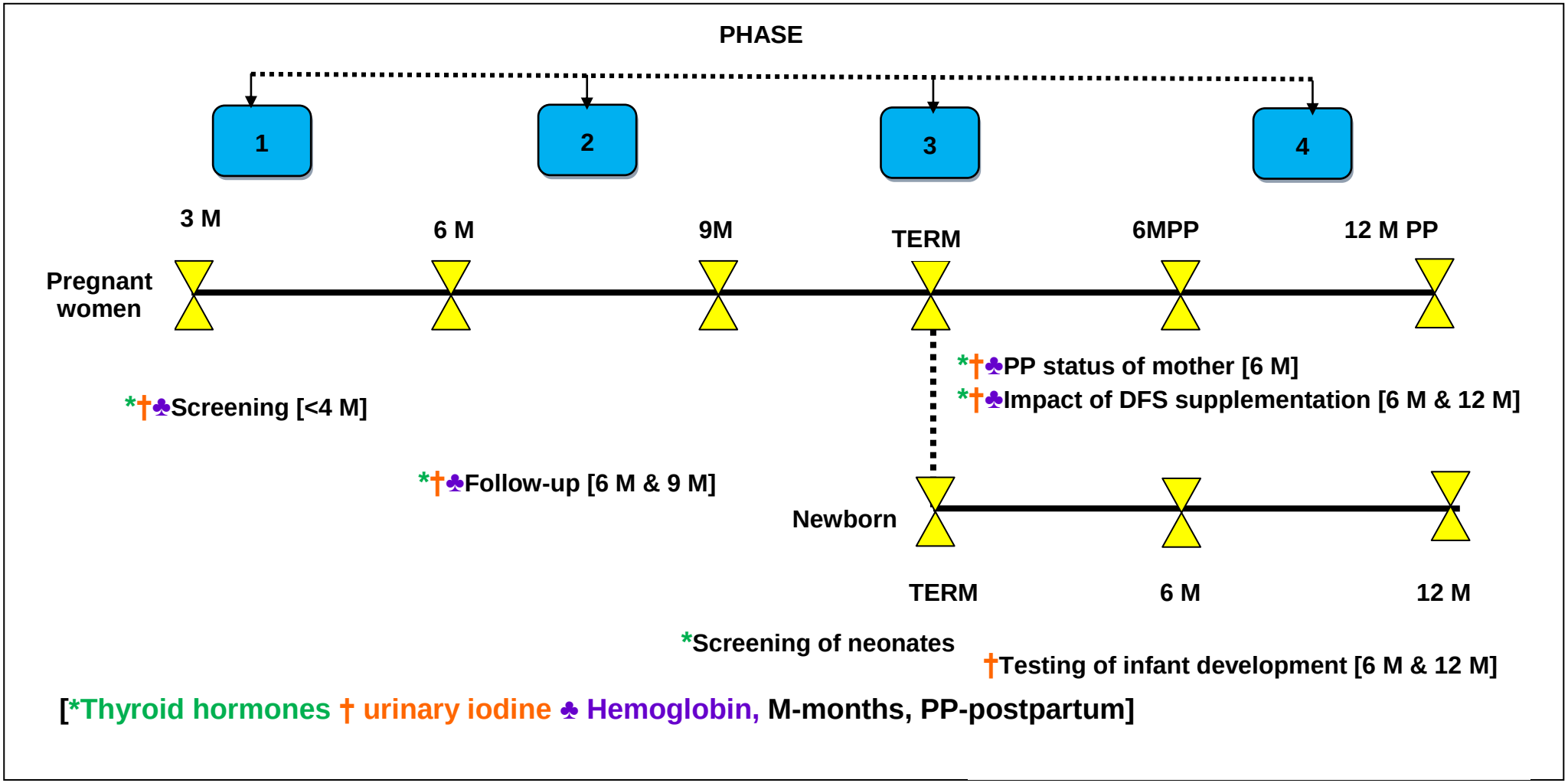
# MATERIALS AND METHODS

Present study is carried out in Vadodara district of Gujarat. Iodine deficiency disorder (IDD) is a public health problem in Gujarat and Vadodara district was considered as a new pocket of IDD (Brahmbhatt et al, 2001). Iodine Deficiency was identified as a public health problem in Porbandar district in 2011 (Kotecha et al, 2011). Many studies have been conducted in Different states of Gujarat to study the prevalence of iodine deficiency and thyroid function in school aged children. However, no data is available on prevalence of iodine deficiency and thyroid dysfunction in pregnant women and infants of Vadodara.

**The study is divided into four phases:**

| | |
|---|---|
| **Phase I** | |
| [Jan. to Mar. 2010] | Screening of pregnant women for thyroid dysfunction during early pregnancy |
| **Phase II:** | |
| [Apr. to Sept 2010 ] | Follow-up of pregnant women,<br>Nutrition Health Education (NHE) |
| **Phase III:** | |
| [Sept. Oct. Nov. 2010] | Screening of neonates |
| **Phase IV:** | |
| [Jan. Feb. Mar. 2011 ]<br><br>[Apr. May. June 2011] | Postpartum status of mother,<br>Testing of Infant Development<br>Impact of double fortified salt (DFS) supplementation on maternal iodine and iron status |

## STUDY TIMELINE

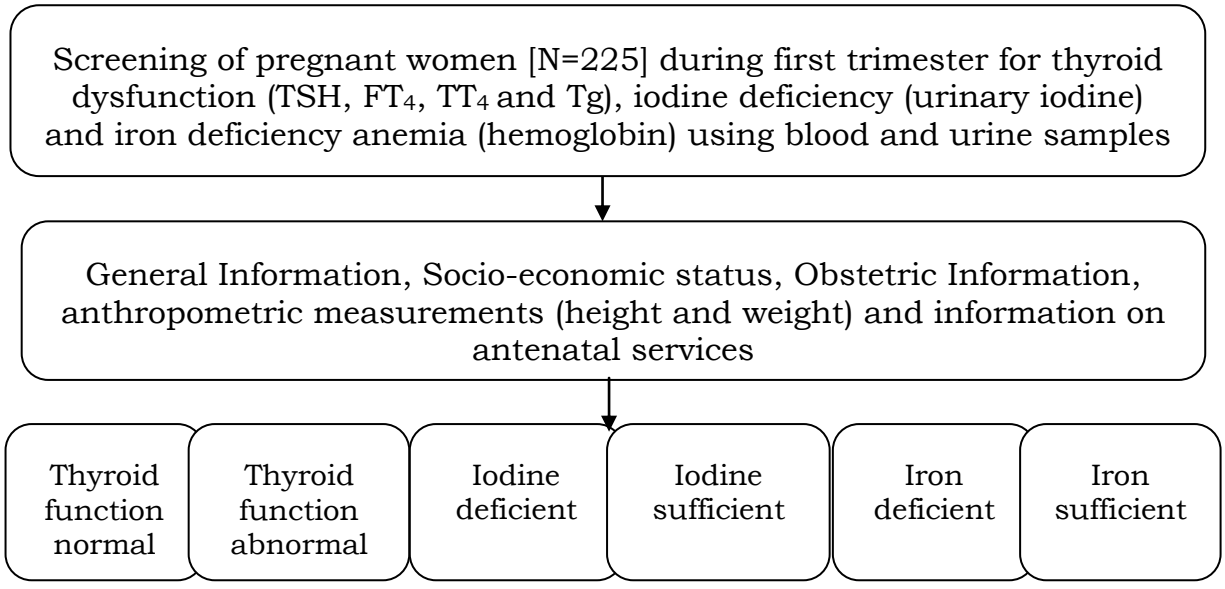


**Phase I: Screening of pregnant women during early pregnancy**

In December 2009, a survey was carried out in all (low budget) government/general hospitals of Vadodara for identifying a hospital which is having highest delivery rate and where most of the low income group (LIG) pregnant women go for delivery. Hence the study was focused using a single hospital enrolment of pregnant women, who represents the general population of pregnant women of urban Vadodara.

Between January to March 2010, pregnant women who checked in for antenatal assessment in Jamnabai General Hospital (JGH) were enrolled for the study. JGH is Vadodara's most popular hospital among LIG people. It has a delivery rate of 250-300/month, which is highest among all hospitals in Vadodara. People from different parts of Vadodara and nearby villages, come to JGH for availing antenatal services, immunization and delivery.

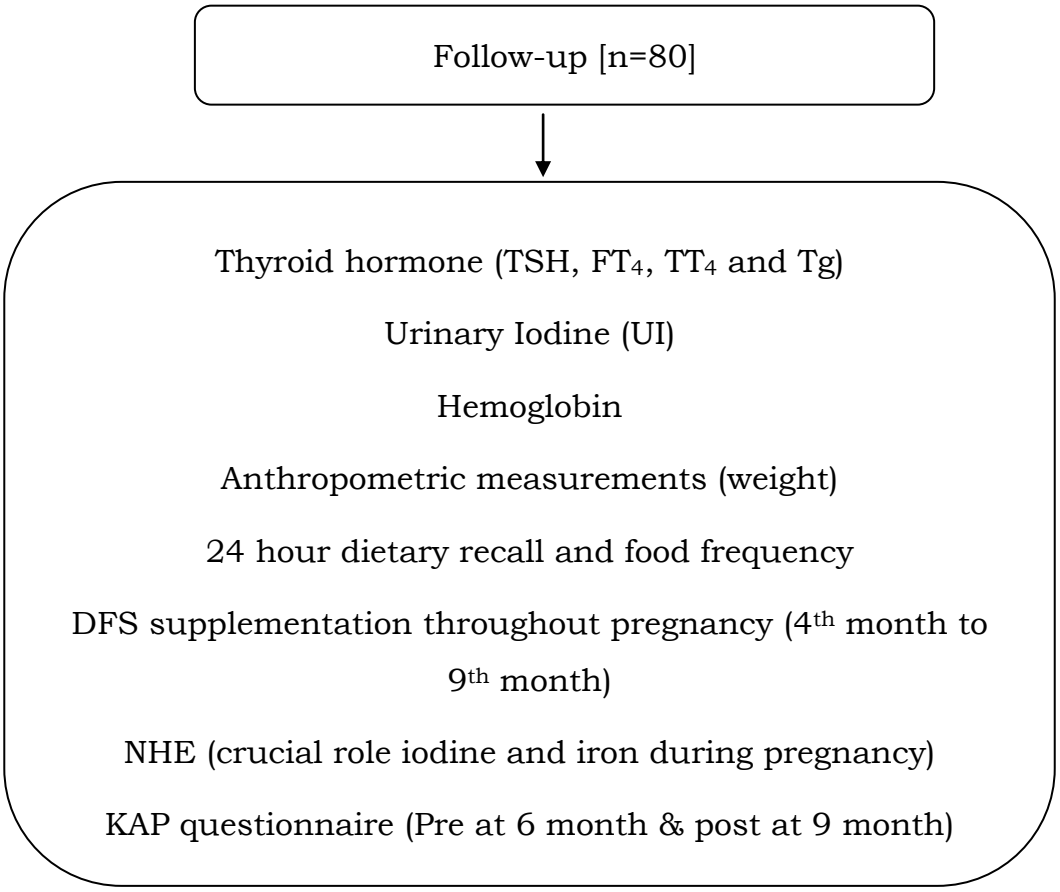
A total of 225 pregnant women during first trimester were screened for thyroid dysfunction, iodine deficiency and iron deficiency anemia. All pregnant women were given a consent form in local language (Gujarati) and the purpose of the study was explained to them. After obtaining consent from them, general information, socio-economic status, obstetric history, anthropometric measurements, etc. were recorded (Appendix- i).



**Phase II: Follow-up of pregnant women and Nutrition Health Education (NHE)**

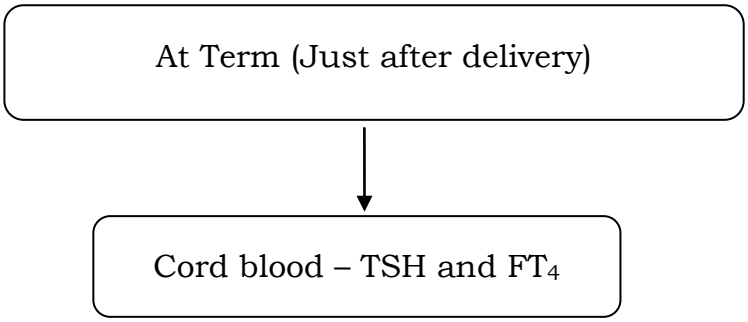
In the second phase, follow-up of pregnant women during second trimester and third trimester was carried out on a sub sample (n=100). Due to various reasons (change in address, change in contact number or not interested in further participating in the study) all 100 pregnant women were not continued in the follow-up. All three trimesters data was collected from 80 pregnant women.

During this phase NHE was provided to all the pregnant women irrespective of their screening result. Again blood and urine samples were collected for thyroid hormone status, iodine deficiency and iron deficiency anemia. Additional information was also availed like-dietary information, anthropometric measurements and testing their knowledge regarding importance of iodine and iron during early pregnancy. Each component mentioned in Mamta card was explained to them (TT injection, BP check-up, regular weight check-up, danger signs etc.).



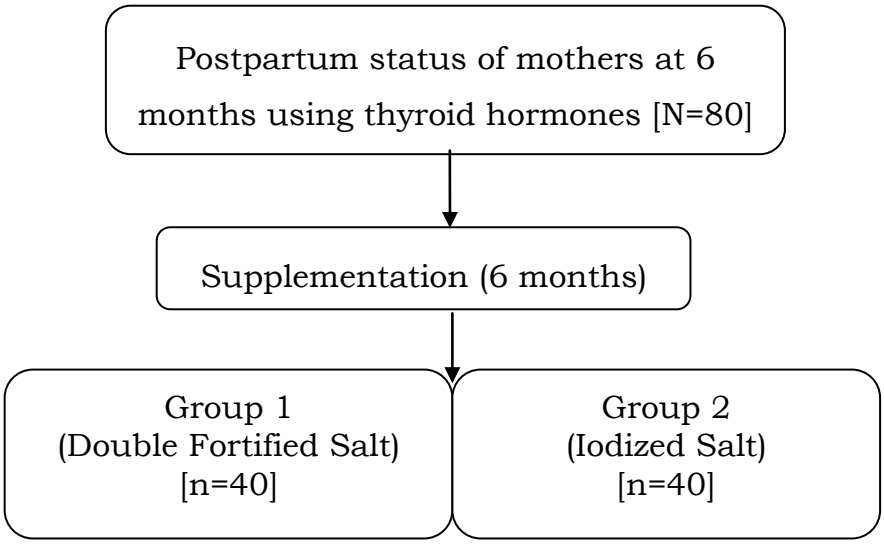
**Phase III: SCREENING of neonates**

Screening of neonates was carried out using cord blood samples. During this phase delivery care information of mothers was also recorded. NHE was given to them using Manta Card regarding- care of newborn and care of herself, importance of colostrum and exclusive breastfeeding. Anthropometric measurements (birth weight, length and head circumference) of the neonates were also recorded.




**Phase IV: Postpartum status of mother**

After 6 months postpartum, mothers were again called up with the baby for checking their thyroid status. Blood samples were collected from mothers for testing thyroid hormones. Weight of the mothers was also recorded. NHE was provided to mothers using Mamta card regarding initiation of complementary feeding along with breastfeeding. Next immunization details for their child were explained to mothers.

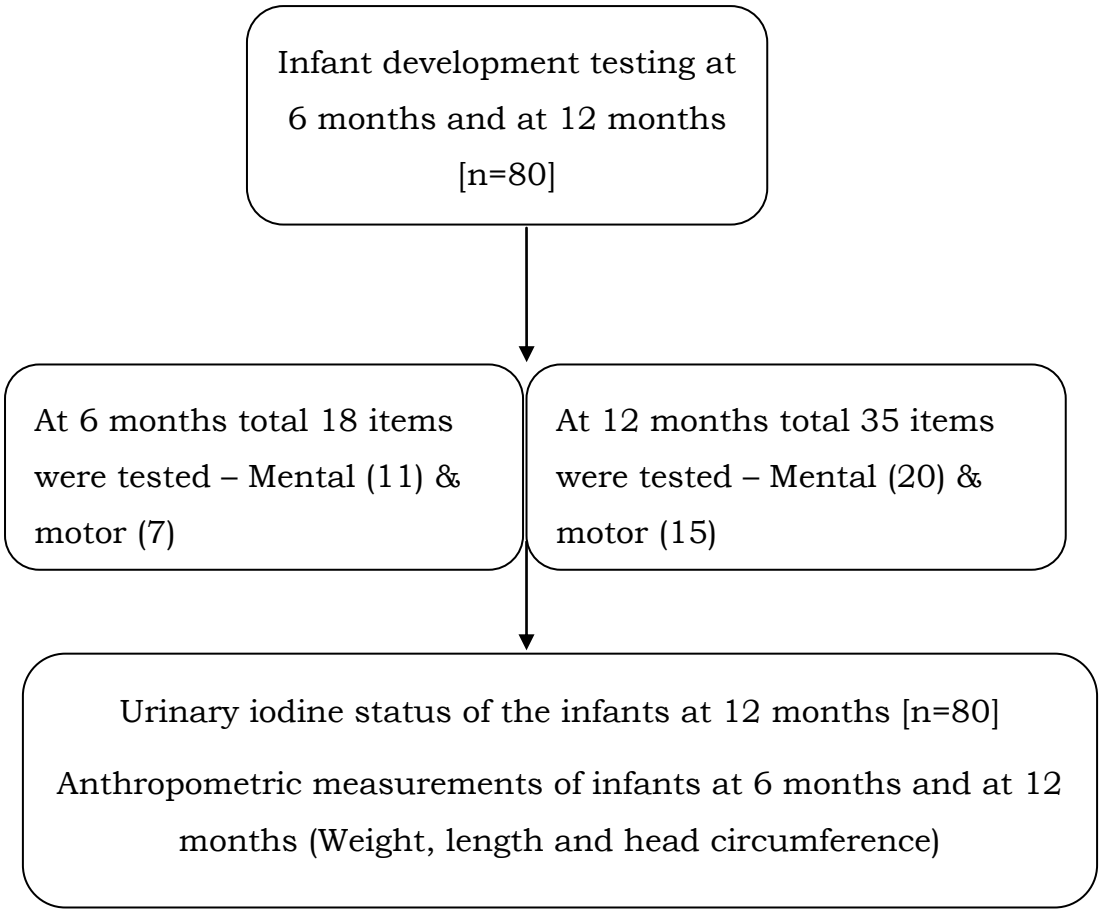


**Impact of double fortified salt (DFS) supplementation on maternal iodine and iron status**

Mothers were provided DFS for 6 months along with control group. Urine and blood samples were collected from mothers at 6 months (pre data) and at 12 months (post data) for urinary iodine and hemoglobin.

**Testing of Infant Development**

Infant development was tested twice, first at 6 months and second at 12 months using BDSTI (Baroda Norms). Anthropometric measurements of the infants were also recorded at 6 months and at 12 months along with immunization details. Urine samples were collected from infants at 12 months for checking their iodine status. Additional information was also recorded regarding health of the child.



**Methods Used**

3.1 Collection of urine samples and storage

3.2 Collection of blood samples and storage

3.3 Anthropometric Measurements

3.4 Dietary Assessment Methods

3.5 Nutrition Health Education (NHE) and Knowledge Attitude and Practices (KAP)

3.6 Double Fortified Salt supplementation

3.7 Determination of Urinary Iodine

3.8 Thyroid Hormone Analysis

Determination of Thyroid Stimulating Hormone (TSH)

Determination of Free Thyroxine ($FT_4$)

Determination of Thyroglobulin (Tg)

Determination of Total Thyroxine ($TT_4$)

3.9 Determination of Hemoglobin (Hb) Concentration

Method: Sahli (Acid Hematin) method

Method: Cyanmethemoglobin method

3.10 Infant Development Testing

Ethical approval

Statistical analysis

### 3.1 Collection of Urine samples and storage

Urine samples were collected in sterile 50 ml bottles (Tarson). After collection of urine samples in sterile bottles, they were transferred to ependof tubes of 1.5 ml (Tarson) rest of the sample was stored in the bottle with addition of toluene till analysis. A total of 625 bottles were utilized for the study.

### 3.2 Collection of blood samples and storage

Hemoglobin: Blood samples were collected in EDTA coated tubes (BD) of 4ml capacity. Hemoglobin was estimated on the same day. A total of 160 tubes were used.

Thyroid hormones: Blood samples were collected in plain tubes (BD) of 4 ml capacity and for cord blood 7 ml tubes were used. Sample was allowed to clot, after 15 minutes samples were centrifuged and serum was separated. Serum was transferred in small vial of 1.5 ml (Tarson) capacity and stored at -18$^0$C in deep freezer. A total 515 of plain tubes were used.

### 3.3 ANTHROPOMETRIC MEASUREMENTS

**Weight**

**Mother:** Weight of pregnant women was measured in kg (to the nearest 100 gm) using a simple weighing scale (standardized).

**Baby:** Weight of the baby was measured in kg (to the nearest 10 gm) using electronic infant weighing scale. Infants were weighed with minimum clothing and excessive infant movements were avoided during measurement.

**Height/Length**

**Mother:** Height of pregnant women was measured in cm (to the nearest 0.1 cm) using a tape mounted on wall. While measuring height following things were taken care off-

- Subject was bare footed
- Was standing with heels together, arms to the side, legs straight, shoulders relaxed
- Position the head in the Frankfort horizontal plane (“look straight ahead”)

- Heels, buttocks, scapulae (shoulder blades), and back of the head should be against the wall.

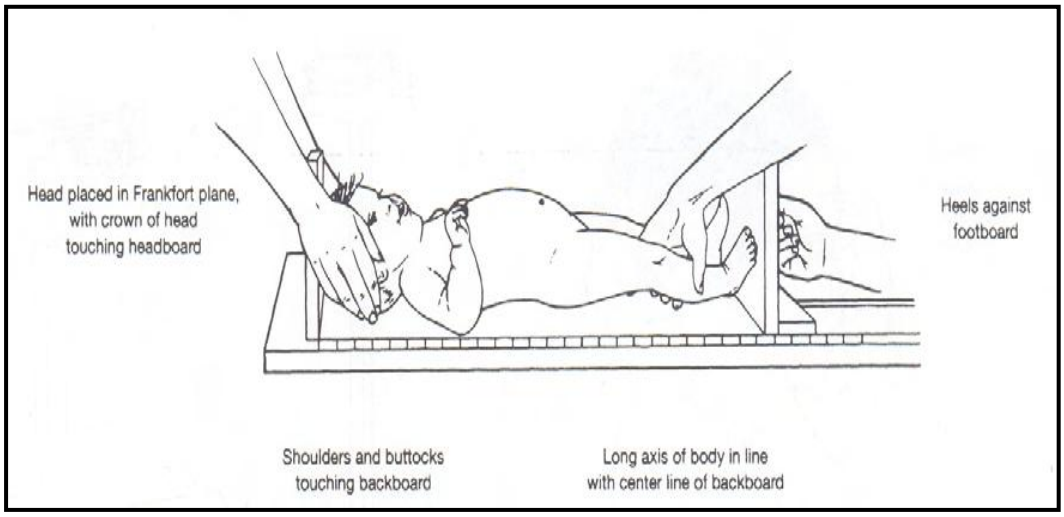



**Figure 3.1: Measurement of length of infant**

**Baby:** Length of the baby was measured in cm (to the nearest 0.1 cm) using an infantometer. Two persons were required for taking the measurement. One person holds the child's head against the backboard with the crown securely against the headboard and with Frankfort plane perpendicular to the backboard. This person also keeps the long axis of the child's body aligned with the center line of the backboard, the child's shoulders and buttocks securely touching the backboard, and the shoulders and hips at right angles to the long axis of the body. The other person keeps the child's legs straight and against the backboard, slides the footboard against the bottom of the feet (without shoes or socks) with the toes pointing upward, and reads the measurement. The footboard should be pressed firmly enough to compress the soft tissues of the soles but without diminishing the vertebral column length.

**Head Circumference**

Head circumference of the baby was measured (to the nearest 0.1 cm) using a flexible tape. It was used as a measure of brain development.

**Body Mass Index (BMI)**

BMI was used as an indicator of nutrition status.

BMI = $Weightinkg/Heightin(m)^2$

**Table 3.1: BMI reference for adults**

| Classification | BMI(kg/m²) | |
|---|---|---|
| | **Principal cut-off** | **Additional cut-off** |
| **Underweight** | **<18.50** | **<18.50** |
| Severe thinness | <16.00 | <16.00 |
| Moderate thinness | 16.00 - 16.99 | 16.00 - 16.99 |
| Mild thinness | 17.00 - 18.49 | 17.00 - 18.49 |
| **Normal range** | **18.50 - 24.99** | **18.50 - 22.99** |
| | | **23.00 - 24.99** |
| **Overweight** | **≥25.00** | **≥25.00** |
| Pre-obese | 25.00 - 29.99 | 25.00 - 27.49 |
| | | 27.50 - 29.99 |
| **Obese** | **≥30.00** | **≥30.00** |
| Obese class I | 30.00 - 34.99 | 30.00 - 32.49 |
| | | 32.50 - 34.99 |
| Obese class II | 35.00 - 39.99 | 35.00 - 37.49 |
| | | 37.50 - 39.99 |
| Obese class III | ≥40.00 | ≥40.00 |

**Source: WHO, 2004**

**Child growth standards for infants**

Weight, length and head circumference were used to assess the growth of infant and size of their brain. Four anthropometric index were used which are- weight-for-age, length-for-age, weight-for-length and head circumference-for-age.

1. **Weight-for-age** reflects body weight relative to age and is influenced by recent changes in health or nutritional status. It is not used to classify infants, children and adolescents as under or

overweight. However, it is important in early infancy for monitoring weight and helping explain changes in weight-for-length.

2. **Length-for-age** describes linear growth relative to age. Length-for-age is used to define shortness or tallness.
3. **Weight-for-length** reflects body weight relative to length and requires no knowledge of age. It is an indicator to classify infants and young children as overweight and underweight.
4. **Head circumference-for-age** is critical during infancy. Head circumference measurements reflect brain size. Very small and very large are both indicators of health or developmental risk.

Each of these indices was expressed in standard deviation units (Z-scores) from the median of the reference population. These were calculated using WHO anthro plus software (version 3.1 2011).

**Table 3.2: Cut-offs used for classifying nutritional status of infants based on WHO reference standards (2006)**

| Classification | Cut-off points |
|---|---|
| **Weight-for-age** | |
| Underweight | <-2SD |
| Normal | -2SD to +2SD |
| Overweight | >+2SD |
| **Length-for-age** | |
| Under length/short | <-2SD |
| Normal | -2SD to +2SD |
| Above average/tall | >+2SD |
| **Weight-for-length** | |
| Thin | <-2SD |
| Normal | -2SD to +2SD |
| Overweight | >+2SD |
| **Head circumference-for-age** | |
| Very small | <-2SD |
| Normal | -2SD to +2SD |
| Very large | >+2SD |

## 3.4 DIETARY ASSESSMENT METHODS

### 24-hour dietary recall

For the 24-hour dietary recall, the respondents were asked to remember and report all the foods and beverages consumed in the preceding 24 hours or in the preceding day (Appendix-iv).

### Food Frequency

For food frequency respondents were asked to report their usual frequency of consumption of each food, mainly foods rich in iron and vitamin C (Appendix-v).

## 3.5 NUTRITION HEALTH EDUCATION (NHE) AND KNOWLEDGE ATTITUDE AND PRACTICES (KAP)

NHE was provided as intervention to pregnant women using posters. Key messages included in the poster were

- Importance of iodine during early brain development.
- Role of iron in reducing risk of maternal and child mortality
- How to recognize iodized salt
- Healthy cooking and storage practices to minimize iodine loss from iodized salt

The idea was to provide knowledge regarding the importance of iodine and iron nutrition during early pregnancy and to change their incorrect practices of cooking and storing iodized salt.

For measuring the outcome of NHE, a KAP questionnaire was used (before and after NHE intervention).

## 3.6 DOUBLE FORTIFIED SALT SUPPLEMANTATION

Iodine (30 ppm) and iron (1 ppm) fortified salt was given to pregnant women throughout pregnancy and 6 months postpartum. This was procured from ANKUR Chem. Foods Ltd., Gandhidham. A total of 720 kg of DFS was used. However, a total of 1,000 kg of salt was procured from Ankur. This was ordered in split, 500 kg during pregnancy and 500 kg during postpartum period. This was done to avoid loss of iodine due to storage.

## 3.7 DETERMINATION OF URINARY IODINE

UIC estimation was performed at Molecular Diagnostics Laboratory, Lucknow. Prior to that 7 days practical training was availed from ICCIDD laboratory AIIMS, New Delhi under the guidance of Prof. M. G. Karmarkar.

**Method: Simple micro plate method (Ohashi et al, 2000)**

- Urine iodine estimation include initial step in which urine is digested in strong acid, ashed at high temperature or use of chemical agents such as ammonium per sulphate. Following digestion step, iodine is measures by its catalytic action on the reduction of Ceric ion ($Ce^{4+}$) to cerous ion ($Ce^{3+}$ ) coupled to oxidation of arsenite ($As^{3+}$) to ($As^{5+}$), this reaction is called Sandell-Kolthoff reaction.
- Ceric ion has yellow colour, while cerous is colourless. *(The colour disappearance is directly proportional to amount of iodine present in the system)*
- The initial step of digestion is necessary as it removes substances like nitrite, thiocyanate or ferrous ions which may interfere by reducing or oxidizing the ceric or arsenite reactants.

**Principle**

Urine is digested with ammonium per sulphate. Iodine is the catalyst in the reduction of ceric ammonium sulphate (yellow) to cerous form (colourless), and is detected by rate of colour disappearance (Sandell-Kolthoff reaction).

**Reaction**

$$2Ce^{4+}\ (\text{ceric}) + 2I^- \longrightarrow 2Ce^{3+}\ (\text{cerous}) + I_2$$

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

**Equipments and apparatus required**

1. Polypropylene SEROCLUSTER 96-well micro plates (Corning Costar Japan)

2. Standard oven (which can maintain temperature of 95$^{o}$C)
3. Stainless steel cassette (sealing cassette, specially designed by Hitachi Chemical Techno-plant)
4. Microplate reader (ELISA reader)
5. Measuring devices (Micropipettes 0-100 µl, multichannel 8 plate micropipette 0-100 µl, standard pipettes- 10 ml, 5 ml, 1 ml)
6. Vortex mixer
7. Weighing machine
8. Micro tips

**Chemicals Required**

Ammonium Per Sulphate ($H_8N_2O_8\ S_2$)

Arsenic Trioxide ($As_2O_3$)

Ceric Ammonium Sulphate [ $Ce(NH_4)_4\ (SO_4)4\ .2H_2O$ ]

Concentrated Sulphuric Acid ($H_2SO_4$)

Sodium Hydroxide (NaOH)

Sodium Chloride (NaCl)

Potassium iodate ($KIO_3$)

*(Glass distilled deionized water was used for preparation of reagent solution and dilution procedures)*

**Preparation of reagents**

***Ammonium per sulphate solution (1.31 mol/l)*** **-** Ammonium per sulphate (30 g) was dissolved in water to a final volume of 100 ml.

*(Prepare fresh every day)*

***Arsenious acid solution (0.05 mol/l)*** **-** Arsenic trioxide (5 g) was dissolved in 100 ml of 0.875 mol/1 sodium hydroxide solution. Concentrated sulphuric acid (16 ml) was then added slowly to the solution in an ice bath. After cooling, 12.5 g of sodium chloride was added to the solution, and the mixture was diluted to 500 ml with cold water and filtered.

*(Filter and store in amber coloured reagent bottle, store at 4°C)*

***Ceric ammonium sulphate solution (0.019 mol/l)***-Tetra ammonium cerium (IV) sulphate dihydrate (6 g) was dissolved in 1.75 mol/l sulphuric acid and adjusted to a final volume of 500 ml with the same acid solution.

***Iodine calibrators*** - In a 100 ml volumetric flask, 168.6 mg of potassium iodate was dissolved in water to make a 7.88 mmol/l stock solution (1000 mg/l iodine). The stock solution was diluted 100- and 10 000-fold, and working solutions of 0.039 – 4.73 mmol/l (5– 600 mg/l iodine) were prepared.

**Steps**

1. In microplate pipette out 50 µl of standards known values samples and urine samples in the wall as shown below.
2. A1 (blank) - nothing was added.
3. In the first 2 columns standards were added (as shown in figure) and in H2 a known value sample was added.
4. From column 3 to 12, urine samples to be analyzed were added.
5. Add 100 µl of Ammonium Per Sulphate (freshly prepared) in each well (using multi-channel pipette).
6. Put the urine plate in heating cassette. Seal the cassette and put it in oven at 95°C, for 90 minutes.
7. Take out the cassette from oven and allow to cool to room temperature.
8. Take out the digested microplate and take another microplate and transfer 50 µl of digested standards and urine samples in corresponding wells (using multi-channel pipette).
9. Add 100 µl of Arsenious Acid solution (using multichannel pipette). Wait for 15 minutes and then add 50 µl of Ceric Ammonium Sulphate in each well (using multi-channel pipette).
10. Measure optical density (OD) using Microplate reader at 405 nm. Plot the graph using log of absorbance on Y-axis vs iodine

concentration (µg/l) on X-axis. Extrapolate the values of urine samples from the graph.

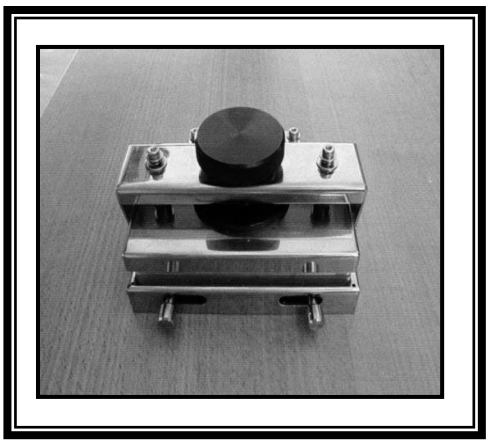


**Figure 3.2: Sealing cassette** (The cassette is used for digestion after a micro plate is placed inside) The sealing cassette includes a Teflon (fluorinated ethylene propylene)-laminated silicon rubber gasket and a handle to seal the wells of a micro plate

**Table 3.3: Epidemiological criteria from the WHO for assessment of iodine nutrition in a population based on median or range of UI**

| UI (µg/L) | Iodine Intake |
|---|---|
| Pregnant women | |
| <150 | Insufficient |
| 150-249 | Adequate |
| 250-499 | More than adequate |
| ≥500[a] | Excessive |
| Lactating women[b] | |
| <100 | Insufficient |
| ≥100 | Sufficient |
| Children <2 years of age | |
| <100 | Insufficient |
| ≥100 | Sufficient |

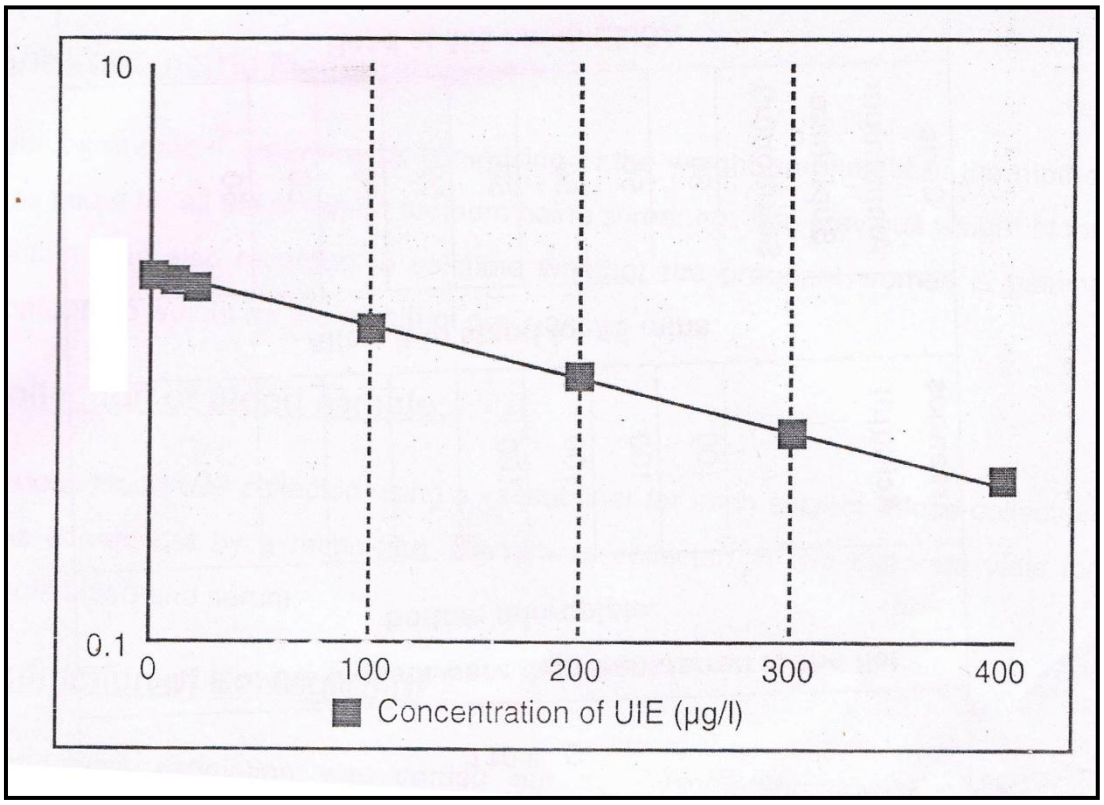
*A* The term excessive means in excess of the amount needed to prevent and control iodine deficiency.
*b* In lactating women, the numbers for median UI are lower than the iodine requirements because of the iodine excreted in breast milk.

**Source: WHO/UNICEF/ICCIDD, 2007**

**Figure 3.3: Sample of microplate for urinary iodine analysis**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | Blank | 100 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample |
| **B** | 0 | 200 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample |
| **C** | 0 | 200 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |
| **D** | 10 | 300 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |
| **E** | 10 | 300 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |
| **F** | 20 | 400 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |
| **G** | 20 | 400 | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |
| **H** | 100 | Known value sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine sample | Urine sample | Urine Sample | Urine sample | Urine sample | Urine Sample |

**Standard curve for Urinary iodine**




| Standard | Concentration (µg/l) | Log of absorbance |
|---|---|---|
| $S_1$ | 0 | 1.8484 |
| $S_2$ | 10 | 1.7459 |
| $S_3$ | 20 | 1.2474 |
| $S_4$ | 100 | 1.0597 |
| $S_5$ | 200 | 0.7859 |
| $S_6$ | 300 | 0.5062 |
| $S_7$ | 400 | 0.3282 |

## 3.8 THYROID HORMONE ANALYSIS

Thyroid hormone analysis was performed at Radiation Medicine Centre, Bhabha Atomic Research Centre, Tata Memorial Cancer Hospital, Parel, Mumbai. Prior to this, six day training was availed from the same place under the guidance of Dr. M. G. R. Rajan and Mrs. Chandrakala S. Gholve.

**Method: Radio Immuno Assays (RIA)**

**Principle:** To perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma radioactive isotopes of iodine attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two chemically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radio labeled variant, and reducing the ratio of antibody-bound radio labeled antigen to free radio labeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.

**Precautions**

**General remarks**

- Bring all reagents to room temperature before pipetting

- Do not mix the reagents from kits of different lots.
- A standard curve must be included with each assay.
- The correct setting of the shaker is very important for the reproducibility of the assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used once only.

**Basic rules of radiation safety**

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipetting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subjected to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.

- Radioactive waste should be handled according to the rules established in the country of use.

**Equipments and apparatus required**

Precision micro pipettes
Semi-automated pipettes
Vortex mixer
Horizontal or orbital shaker
Aspiration system
Aspiration system
Gamma counter set for $^{125}I$

**Figure 3.4: Stratec SR300 Automated Radio Immunoassay Analyzer**


STRATEC SR 300 is a fully automated radioimmunoassay analyzer containing the following modules:

1. Pipetting station
2. Washing station
3. Incubating station
4. Gamma counter

**Determination of Thyroid Stimulating Hormone (TSH)**

**Principle**

The Immuno Radio Metric Assay (IRMA) of thyroid stimulating hormone is a 'sandwich' type assay. Mouse monoclonal antibodies directed against two different epitopes of TSH (antigen) and hence not competing 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 added, 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 samples or calibrators are incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labelled with $^{125}I$. After incubation the content of tubes is aspirated and the tubes are rinsed so as to remove unbound $^{125}I$ labelled antibody. The bound radioactivity is then determined in a gamma counter. The TSH concentrations in the samples are obtained by interpolation from the standard curve. *(The concentration of TSH in the samples in directly proportional to the radioactivity)*

**Kit:** IMMUNOTECH, Beckman Coulter, Prague, Czech Republic

**Performance characteristics of the kit**

| | |
|---|---|
| Analytical sensitivity: | 0.025 mIU/l |
| Functional sensitivity: | 0.141 mIU/l |
| Measurement range : | 0.025-50 mIU/l |

**Reagents provided in kit:**

**Anti-TSH antibody-coated tubes:** 100 tubes (ready to use)

**$^{125}I$-labelled monoclonal anti-TSH antibody:** 11 ml vial (ready to use)

**Calibrators:** seven 1 ml vials (ready to use), the calibrator vials contain from 0 to 50 mIU/l of TSH in bovine serum with sodium azide (0.1 %). The exact concentration in indicated on each vial label. The

calibrators were calibrated against the international standard, WHO 2nd IRP 80/558.

**Control serums:** two vials (lyophilized), the vials contain TSH lyophilized in bovine serum. The expected values are in the concentration range indicated on the vial label.

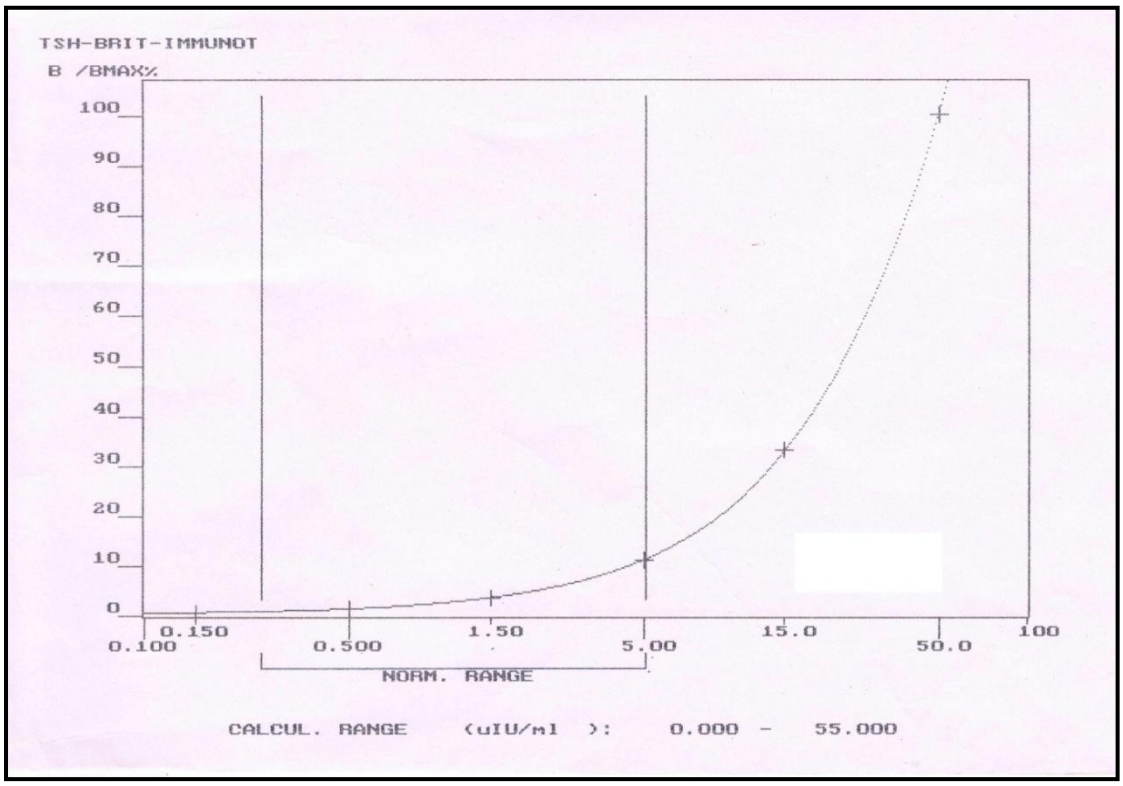
**Wash solution (20x):** one 50 ml vial, concentrated solution has to be diluted before use.

**Reagent Preparation –** let all the reagents come to room temperature.

**Reconstitution of control samples –** the content of the vials is reconstituted with the volume of distilled water indicated on the label. Wait for 30 minutes following reconstitution and mix gently to avoid foaming before dispensing. Store the reconstituted solutions at 2-8°C for one day or aliquoted at <-18 °C for a longer time, until the expiry date of the kit.

**Preparation of the wash solution** -pour the content of the vial into 950 ml of distilled water and homogenize. The diluted solution may be stored at <-18 °C for a longer time, until the expiry date of the kit.

**Standard curve for TSH**




**Normal value: 0.25-5.10 µIU/ml**

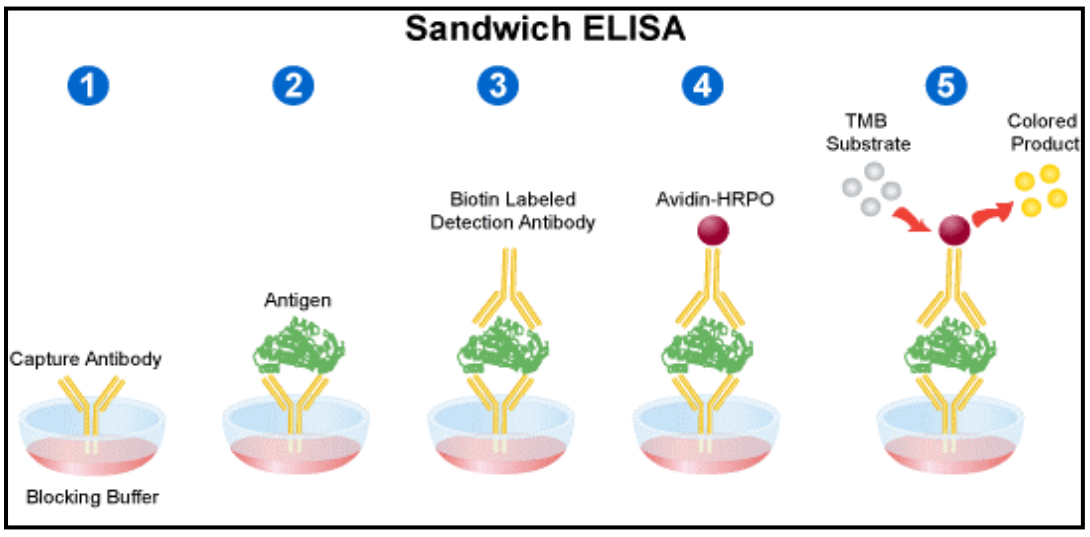
| Serial no. | Tube no. | Expected value (µIU/ml) | Actual value (µIU/ml) | Difference % | Percentage bound (% B) |
|---|---|---|---|---|---|
| 1 | $S_1$ | 0.000 | 0.000 | - | 0.1 |
| 2 | $S_2$ | 0.150 | 0.150 | - | 0.4 |
| 3 | $S_3$ | 0.500 | 0.500 | - | 1.2 |
| 4 | $S_4$ | 1.500 | 1.500 | - | 3.5 |
| 5 | $S_5$ | 5.000 | 5.000 | - | 11.0 |
| 6 | $S_6$ | 15.000 | 15.000 | - | 32.9 |
| 7 | $S_7$ | 50.000 | 50.000 | - | 100.0 |

**Assay procedure**

| Step 1Additions* | Step 2Incubation | Step 3Counting |
|---|---|---|
| To coated tubes, add successively-<br><br>100 µl of calibrator, control or sample<br>100 µl of tracer<br><br>Mix | Incubate 1 hour at 18-25°C with shaking (>280 rpm) | Aspirate carefully the contents of tubes (except the 2 tubes <<total cpm>>)<br><br>Wash twice with 2 ml of wash solution<br><br>Count bound cpm (B) and total cpm (T) for 1 minute |

*add 100 µl of tracer to 2 additional tubes to obtain total cpm

**Figure 3.5: Sandwich ELIZA method**



**Determination of Free thyroxine ($FT_4$)**

**Principle**

The Radio Immuno Assay of free thyroxine ($FT_4$) is a competition assay based on the principle of labelled antibody. Samples and calibrators are incubated with 125I labelled monoclonal antibody specific for $T_4$, as tracer, in the presence of a biotinylated analog of thyroxine (ligand) in avidin-coated tubes. There is competition between the free thyroxine of the sample and the ligand for the binding to the labelled antibody. The fraction of antibody complexes with the biotinylated ligand binds to avidin-coated tubes. After incubation, the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve.

**Kit:** IMMUNOTECH, Beckman Coulter, Prague, Czech Republic

**Performance characteristics of the kit**

| | |
|---|---|
| Analytical sensitivity: | 0. 5 pM |
| Functional sensitivity: | 2.4 pM |
| Measurement range : | 0.5-75 pM |

**Reagents provided in kit:**

**Coated tubes for the binding of ligand:** 1000 tubes (ready to use)

**$^{125}$I monoclonal antibody:** one 45 ml vial (ready to use)**,** the vial contains 310 kBq, at the date of manufacture, of $^{125}$I-labelled immunoglobulins in liquid form with bovine serum albumin, sodium azide (<0.1%) and a dye.

**Calibrators:** five 0.5 ml vials (ready to use), the calibrator vials contain from 0 to 75 pM of free thyroxine in human serum and sodium azide (<0.1 %). The exact concentration is indicated on each vial label. The vials must be tightly capped immediately after pipetting and stored at 2-8$^{o}$C. The evaporation of the calibrator solutions from

open vials may influence results of the further determination. Calibrators are verified to an internal reference standard.

**Ligand:** one 12 ml vial (ready to use), the vial contains a ligand solution which includes also bovine proteins and sodium azide (< 0.1 %).

**Control serum:** one vial (lyophilized), the vial contains $T_4$ in human serum. The expected values are in the concentration range indicated on the supplement. The vial must be tightly capped immediately after pipetting and stored according to #. The evaporation of the solution from open vial may influence results of the further determination.

**Attention:** all liquid reagents should be examined for the absence of precipitates; the antibody solution should be clear and blue-green, the calibrators may be opalescent and the ligand should be clear and colourless.

**Reagent preparation –** let all the reagents come to room temperature.

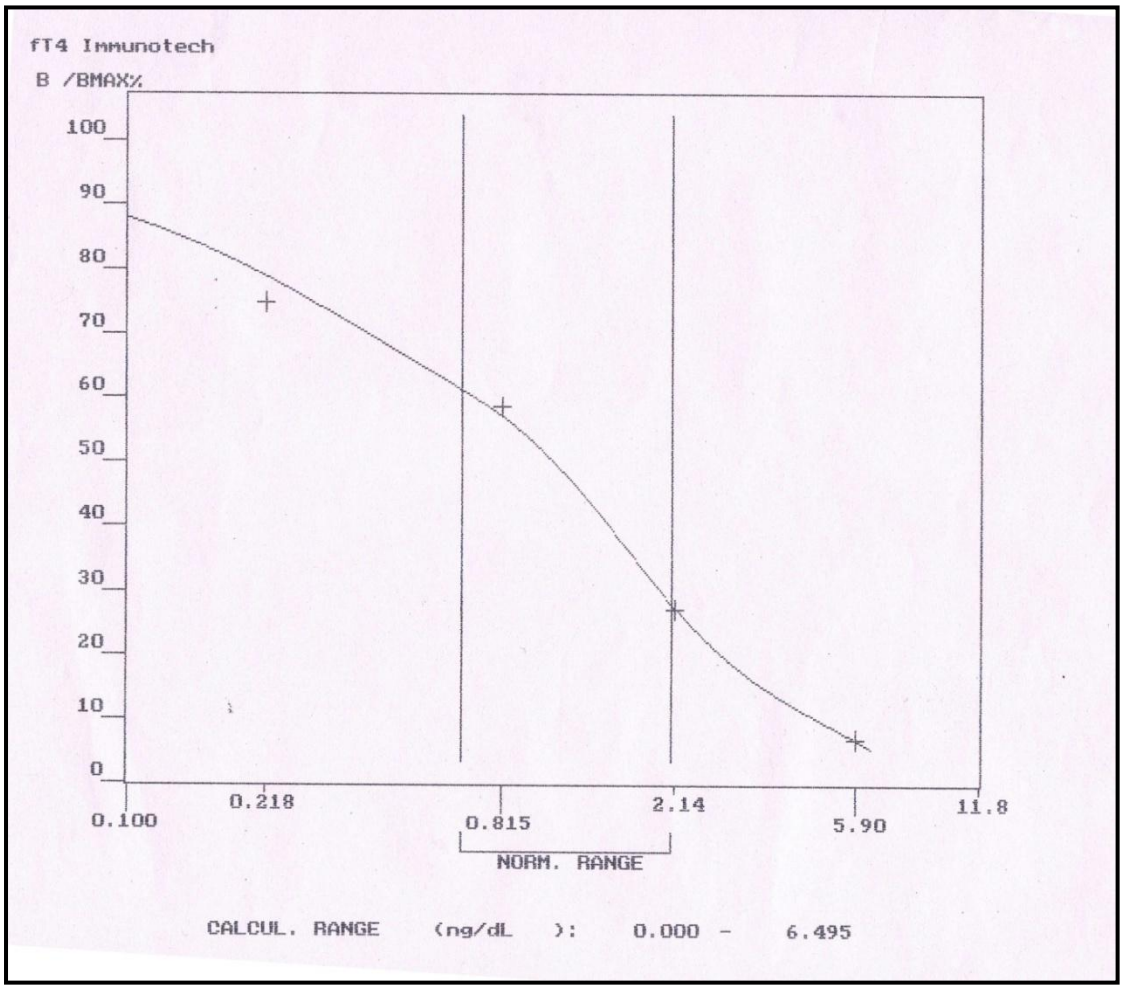
**Reconstitution of control samples –** the content of the vials is reconstituted with the volume of distilled water indicated on the label. Wait for 10 minutes following reconstitution and mix gently to avoid foaming before dispensing. Store the reconstituted solutions at 2-8°C for one week or aliquoted at <-18 °C for a longer time, until the expiry date of the kit.

**Assay procedure**

| **Step 1 Additions*** | **Step 2 Incubation** | **Step 3Counting** |
|---|---|---|
| To coated tubes, add successively-<br><br>25 µl of calibrators, control or sample<br>and<br>400 µl of tracer<br>100 µl of ligand<br>Mix | Incubate 1 hour at 18-25°C with shaking (>350 rpm) | Aspirate carefully the contents of tubes (except the 2 tubes <<total cpm>>)<br><br>Count bound cpm (B) and total cpm (T) for 1 minute |

*add 400 µl of tracer to 2 additional tubes to obtain total cpm

**Standard Curve for $FT_4$**




**Normal Value: 0.65-2.10 ng/dl**

| Serial no. | Tube no. | Expected value (ng/dl) | Actual value (ng/dl) | Difference % | Percentage bound (% B) |
|---|---|---|---|---|---|
| 1 | $S_1$ | 0.000 | 0.001 | - | 100.0 |
| 2 | $S_2$ | 0.218 | 0.281 | -29.36 | 74.7 |
| 3 | $S_3$ | 0.815 | 0.760 | 6.81 | 58.1 |
| 4 | $S_4$ | 2.144 | 2.154 | -0.48 | 27.0 |
| 5 | $S_5$ | 5.905 | 5.897 | 0.14 | 6.6 |

**Determination of Thyroglobulin (Tg)**

**Principle**

The Radio Immuno Assay of thyroglobulin (Tg) is a competition assay based on the principle of labelled antibody. Samples and calibrators are incubated with 125I labelled monoclonal antibody specific for Tg, as tracer, in the presence of a biotinylated analog of thyroxine (ligand) in avidin-coated tubes. There is competition between the thyroglobulin of the sample and the ligand for the binding to the labelled antibody. The fraction of antibody complexes with the biotinylated ligand binds to avidin-coated tubes. After incubation, the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve.

**Kit:** BARC (In-house)

**Reagents provided in kit:**

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

**Reagent preparation**

**PBS-EDTA:** dissolve 35.49 g $Na_2HPO_4$ (0.25M); 8.76 g NaCl (0.15M); 3.72 g EDTA (0.01M) and 1 g sodium azide in 800 ml distilled water. Adjust pH 7 and raise the volume to 1000 ml.

**Standard Tg:** Thyroid tissue is collected and connective tissue is removed. It is then chopped into small pieces and extracted in normal saline. It is separated by salting-out method with ammonium sulphate. This is further purified by separation of Sephadex G-200 column chromatography followed by another column chromatography (Sepharose-6B). Purified native Tg is obtained. Tg obtained in this way is from a single specimen and is used in preparation of standards, labelled Tg and to produce anti-Tg antibodies in rabbits.

**r-antiTg antibody:** Normal rabbit is subcutaneously injected with 400 to 500 µg of purified native Tg in complete Freund's adjuvant. Following the initial dose, booster doses with the same amount of Tg was administered after 2-4 weeks respectively. The rabbits were than bled at fortnightly intervals after the final booster and the sera obtained were tested for its antibody activity in an Ouchterlony double diffusion system against purified Tg, thyroid extract and normal serum. The anti-sera gave a single intense precipitin line with purified Tg and thyroid extract. However, no precipitin line was visible in reaction with normal human serum. The antibodies were stored at -20$^{o}$C till further use. Before use, diluted (1:75000) in PB-EDTA.

**DAB-magnetic particles:** Rabbit serum is separated by fractional precipitation using ammonium sulphate, followed by DEAE-Sephadez A-50 ion exchange chromatography and were used for immunizing a goat. The rabbit used here is a different one (not the one in which Tg was injected).

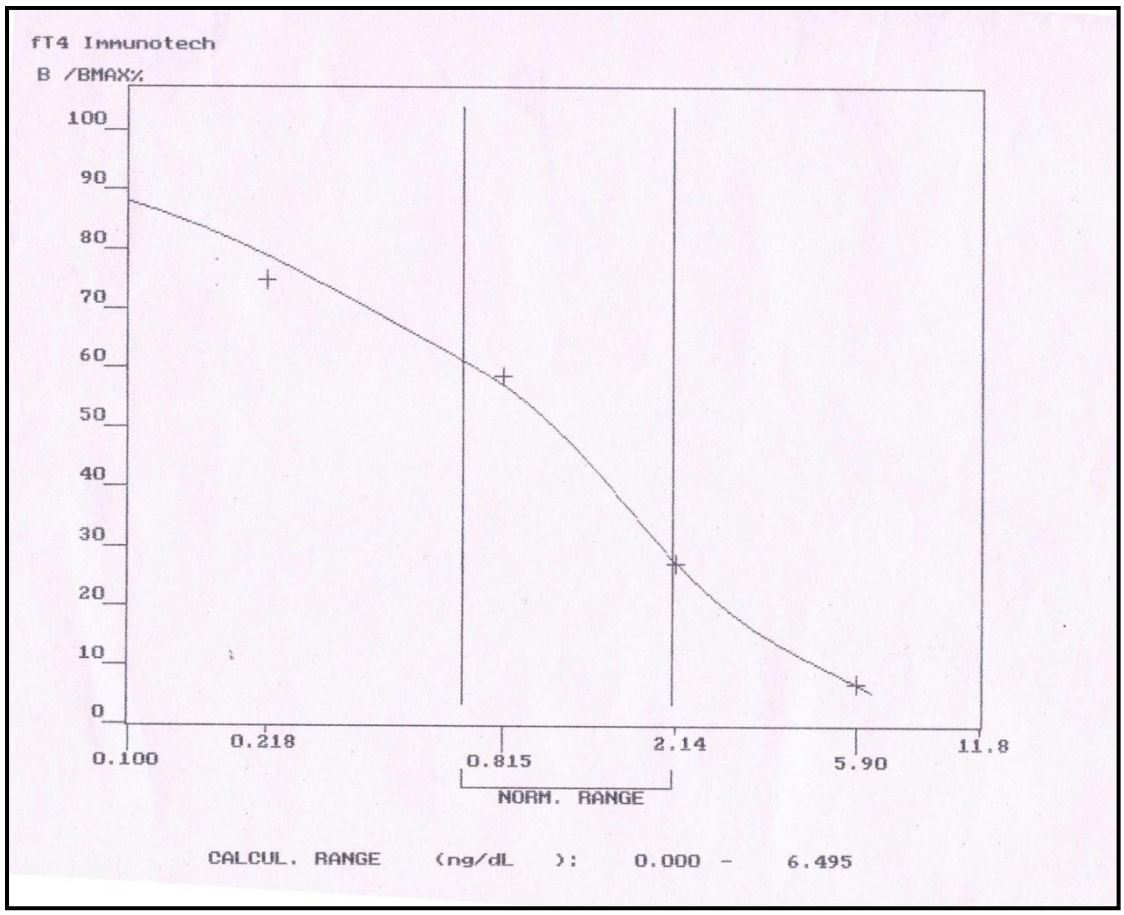
**Assay procedure for Tg**

| **Tube** | **Buffer (µl)** | **Free serum (µl)** | **Tracer 125I (µl)** | **Standard / Serum/ Control (µl)** | **Anti-serum (µl)** | Incubate at 40oC 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 | - | - | | - | |
| $S_1$ | 100 | 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 | |
| $S_8$ | - | 100 | 100 | 100 | 100 | | 100 | |
| Control | 100 | - | 100 | 100 | 100 | | 100 | |
| Sample | 100 | - | 100 | 100 | 100 | | 100 | |

**Procedure**

1. Following components were added to polystyrene tubes in sequence. The tubes were made in duplicates.
   a) Standard Tg (100 µl, range: 0-800 ng/ml)
   b) Tg free serum (100 µl) tubes containing standard Tg and 100 µl of PBS-EDTA buffer in the serum sample tubes.
   c) r-anti-Tg antibodies (100 µl, final dilution, 1:75000) in PBS-EDTA buffer.
   d) Labelled Tg (100 µl).
2. The tubes were incubated for 3 overnights at 4°C.
3. After incubation, 50 µl of DBA- magnetic particles were added to each tube and after incubated for 2 hours on shaker.
4. After 2 hours, 1 ml of PBS-EDTA buffer was added and left for 20 minutes on magnetic rack.
5. Decanted and dried, the tubes were counted for bound radioactivity.

**Standard curve for Tg**



**Normal value: 0.0-50.0 ng/dl**

| Serial no. | Tube no. | Expected Value (ng/ml) | Actual value (ng/ml) | Difference % | Percentage bound (% B) |
|---|---|---|---|---|---|
| 1 | $S_1$ | 0.15849 | 0.15849 | - | 47.84 |
| 2 | $S_2$ | 12.50000 | 10.84695 | -13.22 | 40.39 |
| 3 | $S_3$ | 25.00000 | 25.34220 | 1.37 | 34.79 |
| 4 | $S_4$ | 50.00000 | 52.40718 | 4.81 | 28.62 |
| 5 | $S_5$ | 100.00000 | 109.94882 | 9.95 | 21.99 |
| 6 | $S_6$ | 200.00000 | 202.87504 | 1.44 | 17.11 |
| 7 | $S_7$ | 400.00000 | 368.38590 | -7.90 | 13.32 |
| 8 | $S_8$ | 800.0 | 736.12403 | -7.98 | 10.19 |

**Determination of Total Thyroxine ($TT_4$)**

**Principle**

The Radio Immuno Assay of thyroxine ($T_4$) is a competition assay based on the principle of labelled antibody. Samples and calibrators are incubated with $^{125}I$ labelled monoclonal antibody specific for Thyroxine, as tracer, in the presence of a biotinylated analog of thyroxine (ligand) in avidin-coated tubes. There is competition between the thyroxine of the sample and the ligand for the binding to the labelled antibody. The fraction of antibody complexes with the biotinylated ligand binds to avidin-coated tubes. After incubation, the content of tubes is aspirated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve.

**Kit:** BARC (In-house)

**Reagents provided in kit:**

**Standard $T_4$-** 5 vials containing 10 ml each (conc. 0, 2.5, 5, 10, 20, µg/dl), ready to use

**Tracer:** 1 vial (10 ml), ready to use

**Antibody-magnetic particles:** 1 vial (20 ml), ready to use

**Control:** 2 vials (10 ml), ready to use

**Procedure**

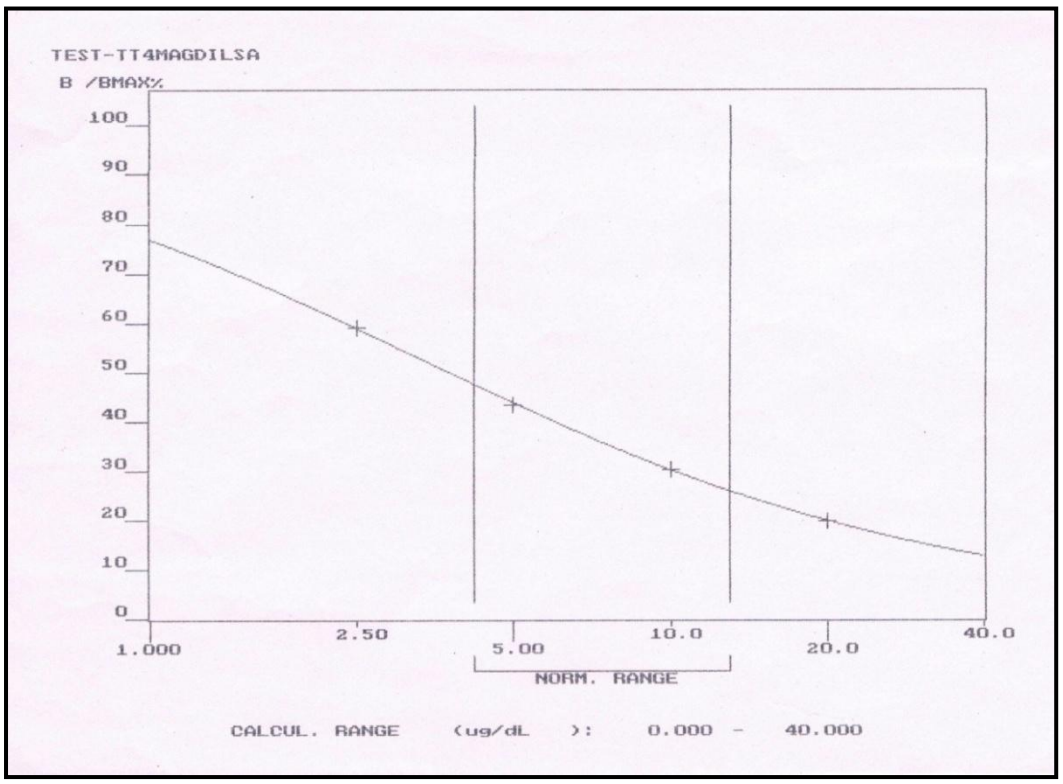
1. Bring all the reagents to room temperature.
2. Pipette 100 µl of standard, control or sample in the respective tubes.
3. Add 100 µl of tracer ($^{125}I$ labelled $T_4$) to it followed by 1000 µl of antibody-magnetic particles.
4. Vortex all the tubes in vortex mixture.
5. Cover the tubes with aluminium foil and incubate the tubes in a 37$^{o}$C water bath for 2 hours.
6. Keep the racks containing assay tubes over a magnetic rack for 15 minutes.
7. Decant the tubes into sink (used for liquid-radioactive discard) and leave the tubes inverted over absorbent sheet for 10 minutes.
8. Wipe the mouth of the tubes with a tissue paper wick while the rack is inverted and still in the magnetic field.
9. Count the tubes in a Gamma Counter for 1 minute; plot the standard graph of % bound and T4 concentration. Extrapolate % bound values of unknown samples to determine T4 concentration.

**TSH IRMA kit** **FT4 RIA kit**


**Assay procedure for $TT_4$**

| Tube | Standard/control/ Sample (µl) | Tracer (µl) | Antibody Magnetic Particles (µl) |
|---|---|---|---|
| Standard 1 | 100 | 100 | 100 |
| Standard 2 | 100 | 100 | 100 |
| Standard 3 | 100 | 100 | 100 |
| Standard 4 | 100 | 100 | 100 |
| Standard 5 | 100 | 100 | 100 |
| Control 1 | 100 | 100 | 100 |
| Control 2 | 100 | 100 | 100 |
| Control 3 | 100 | 100 | 100 |
| Test Sample | 100 | 100 | 100 |
| Vortex all the tubes, Incubate all the tubes in 37oC water bath for 2 hours. Keep them on magnetic racks | | | |
| Decant the tubes and dry them | | | |
| Count the tubes on Gamma Counter for 1 minute | | | |

**Standard curve for $TT_4$**



**Normal value: 4.20-13.00 µg/dl**

| Serial no. | Tube no. | Expected value (µg/dl) | Actual value (µg/dl) | Difference % | Percentage bound (% B) |
|---|---|---|---|---|---|
| 1 | $S_1$ | 0.002 | 0.002 | - | 100.0 |
| 2 | $S_2$ | 2.500 | 2.463 | 1.48 | 58.9 |
| 3 | $S_3$ | 5.000 | 5.078 | -1.56 | 43.2 |
| 4 | $S_4$ | 10.000 | 9.957 | 0.43 | 30.0 |
| 5 | $S_5$ | 20.000 | 19.997 | 0.02 | 19.5 |

## 3.9 DETERMINATION OF HEMOGLOBIN (HB) CONCENTRATION

**Method: Sahli (Acid Hematin) method**

**Principle**

When blood is added to 0.1 N Hydrochloric Acid hemoglobin is converted to brown coloured acid hematin. The resulting colour after dilution is compared with standard brown glass reference blocks of a sahli hemoglobinometer.

**Specimen:** Capillary blood or thoroughly mixed anti-coagulated (EDTA), venous blood (0.02 ml).

**Requirements**

Sahil hemoglobinometer: it consists of-

a. A standard brown glass mounted on a comparator
b. A graduated tube
c. Hb pipette (0.02 ml)

0.1 N HCL, distilled water and Pasteur pipettes

**Method**

1. By using a Pasteur pipette, add 0.1 N HCl acid in the tube upto the lowest mark (20 % mark).

2. Draw blood upto 20 µl mark in the Hb-pipette, adjust the blood column, carefully without bubbles. Wipe excess of the blood on the sides of the pipette by using a dry piece of cotton.
3. Transfer blood to the acid in the graduated tube, rinse the pipette well, mix the reaction mixture and allow the tube to stand for atleast 10 minutes.
4. Dilute the solution with distilled water by adding few drops at a time carefully add by mixing the reaction mixture, until the colour matches with the glass plate in the comparator.
5. The matching should be done up against natural light, the level of the fluid is noted at its lower meniscus and the reading corresponding to this level on the state is recorded in g/dl.

**Remarks**

This method is useful for places where a photometer is not available.

It can give an error of even 1 g/dl

Immediately after use rinse the Hb pipette by using tab water in beaker. This prevents blocking of the pipette.

**Method: Cyanmethemoglobin method (Winkleman, 1974)**

**Principle**

Hemoglobin is oxidized to methemoglobin. Met hemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which is measured photometrically. The concentration of hemoglobin in the sample is directly proportional to the intensity of the coloured complex which is measured at 540 nm (520-560 nm or with green filter).

Hemoglobin + potassium ferricyanide $\xrightarrow{Oxidation}$ Methemoglobin

Methemoglobin $\xrightarrow{Potassium\ Cyanide}$ Cyanmethemoglobin

**Kit:** Reckon Diagnostics P. Ltd., Vadodara

**Characteristics of the kit- linear upto 25 gm/dl**

**Regents provided**

1. Drabkin's reagent (1 bottle/1000ml)
2. Hemoglobin standard (5ml, strength 60 mg/dl)

**Reagent storage and stability**

The reagent provided is stable at room temperature until the expiry date printed on the label. Hemoglobin standard is stable at 2 to 8 $^0$C until the expiry date printed on the label. Hemoglobin reagent contains potassium cyanide, hence use automated pipettes. Avoid ingestion.

**Requirements**

Test tubes, Pipettes (5 ml and 0.2 ml) and Spectrophotometer

**Specimen:** Capillary blood or thoroughly mixed anti-coagulated (EDTA), venous blood (0.02 ml).

**Procedure**

| **Pipette into test tubes** | **Test** |
|---|---|
| Working reagent (ml) | 5.00 |
| Sample (ml) | 0.02 |

Mix by inversion and read absorbance against distilled water after 3 minutes at 540 nm. The colour stability of the reaction mixture is 24 hours. Read the standard directly without dilution.

For a spectrophotometer or standard instrument (with regular calibrations) use of standard is not required. Use factor directly or read in the chart.

Details of calculation are given below-

For colorimeter-

**Calculation Hb**

**(g/dl)**= $\frac{Absorbance\ of\ Test}{AbsorbanceofStandard} \times \frac{DilutionofSample}{1} \times \frac{StrengthofStandard}{1000}$

**Calculation Hb (g/dl)** = $\frac{OpticalDensityofTest}{OpticalDensityofStandard} \times \quad 15$

For spectrophotometer-

**Calculation Hb (g/dl)**= $Absorbance\ of\ test\ \times F\ (F = 36.77)$

Where F = $\frac{Mol.\ weightofHb}{mmol.\ Ext.\ Coeff.} \times \frac{DilutionFactor}{1000} \times \frac{1}{10}$

Mol wt. of Hb= 64458

Mmolar Extinction Coefficient of Hb= 44.0

Dilution Factor= 251

**Table 3.4: Hemoglobin cut-offs to define iron deficiency anemia during pregnancy**

| HB (g/dl) | Degree of IDA |
|---|---|
| <7 | Severe |
| 7-9.9 | Moderate |
| 10-10.99 | Mild |
| ≥11 | Normal |

**Source: UNICEF, 2001**

### 3.10 INFANT DEVELOPMENT TESTING

**Method: Baroda Development Screening Tests for Infants (BDSTI)**

BDSTI contains 54 items (32 mental and 22 motor) for evaluating child's development from 2-30 months, these items are selected from Bayley Scales of Infant development (BSID). BSID has a total of 230 items (163 mental and 67 motor) for testing development of a child from 2-30 months. Many items of the BSID use standardized equipment (cubes, pegboard, form-board etc.) and standardized techniques (certain performances timed by stop-watch). The BSID is a detailed test and has many items for testing the development of one skill-some of them very close in the development sequence. For instance, there are ten items related to the skill of sitting. Items like-sits with support, effort to sit, sits alone momentarily, sits alone 30 seconds or more and sits down require some experience and good judgment on the part of the health worker. Only those items which were simple and easy to administer and to asses and not requiring any special training, experience and equipment were selected. Since in present study, infants were followed till 12 months only. Hence, a total of 35 (20 mental and 15 motor) items out of 54 items from full scale of BDSTI (Baroda norms) have been used for present study (Appendix-iii).

**Table 3.5: Phatak's Screening Test items and age-grouping**

| Months | Sr. No. | Items |
|---|---|---|
| 1 | 1 | Arms and legs thirst in play * |
| | 2 | Momentary regard |
| | 3 | Lateral head movement (prone) * |
| 2 | 4 | Responds to sound |
| | 5 | Follows moving person |
| | 6 | Free inspection of surroundings |
| 3 | 7 | Social smile/vocalizes |
| | 8 | Eye co-ordination |
| | 9 | Head erect and steady* |
| 4 | 10 | Holds head steady* |
| | 11 | Recognizes mother |
| | 12 | Elevates on arms* |
| 5 | 13 | Play with rattle/hand play |
| | 14 | Reaches for dangling ring |
| | 15 | Sits with slight support* |
| 6 | 16 | Turns head to sound |
| | 17 | Turns from back to side* |
| | 18 | Exploitive paper play |
| 7 | 19 | Discriminates strangers |
| | 20 | Pulls to sit* |
| 8 | 21 | Bangs to play |
| | 22 | Sits alone steadily* |
| 9 | 23 | Retails two things in two hands |
| | 24 | Pulls to stand* |
| | 25 | Playful response to mirror images |
| | 26 | Sits with good coordination* |
| 10 | 27 | Pulls string-secures toy |
| | 28 | Co-operates in play |
| | 29 | Crawling (pre-walking) * |
| 11 | 30 | Rings bell purposefully |
| | 31 | Fine prehension* |
| | 32 | Raises to sit* |
| | 33 | Stands by furniture* |
| 12 | 34 | Adjusts to words |
| | 35 | Says da-da |

***motor development items***

**Source: Phatak and Khurana, 1991**

**Developmental curve**

The 50 and 97 % level age placements of each item is placed against its serial number and then joined to have 2 smooth curves, the upper curve representing the 50 % pass level and the lower representing the 97 % pass level (Appendix-ix).

**Table 3.6: Screening efficiency of BDSTI**

| Parameter | BDSTI vs BSID full scale |
|---|---|
| Sensitivity | 65.62 to 93.33 |
| Specificity | 77.37 to 94.44 |
| Over-referral | 6.67 to 34.37 |
| Under-referral | 5.56 to 22.63 |
| Screening validity | 76.00 to 94.05 |

The screening validity, sensitivity and specificity of BDSTI are above 65 %, they can be used as valid for reliable screening tests for early detection of infants with delayed motor and mental development.

**Ethical Approval**

Permission for the study was obtained from concerned health authorities of the state and ethical approval was obtained from Baroda Medical College, Vadodara, Gujarat, India [ethical approval no: FCScFN ME67].

**Statistical Analysis**

Data was entered and saved into Microsoft excel (2007). For analyzing data, SPSS (Statistical Package for Social Sciences, version 14.0) and MedCalc (version 12.2.1.0) software were used.

Quantitative (parametric) data is represented as mean ± sd or mean (sd) and non parametric data is represented as median (95 % CI). Categorical data is represented as percentages. Before applying any test, data was tested for normality using Kolmogorov-Smirnov test,

histogram and Q-Q plot. Where indicated data was normalized using log transformations to facilitate the use of normal-theory analytic methods. Data was then back transformed and reported as geometric mean. Such transformation was performed for TSH.

In case of urinary iodine, where data showed skewness, non parametric tests were used.

For determining effect of 3 trimesters, repeated measures of ANOVA was used (paired samples) for parametric data and for non-parametric data Friedman test was used. Similarly for determining effect of pregnancy and postpartum period, one way ANOVA was used (unpaired samples) for parametric data and for non-parametric data Kruskal-Wallis test was used. After performing ANOVA, wherever required poct-hoc test (Bonferoni) was used for checking with-in group and between group differences.

To determine association between variables Pearson's correlation (parametric data) or Spearman's rank (non parametric data) correlation were calculated. Where data was found to be significantly associated further regression analysis (95% prediction level) was performed.

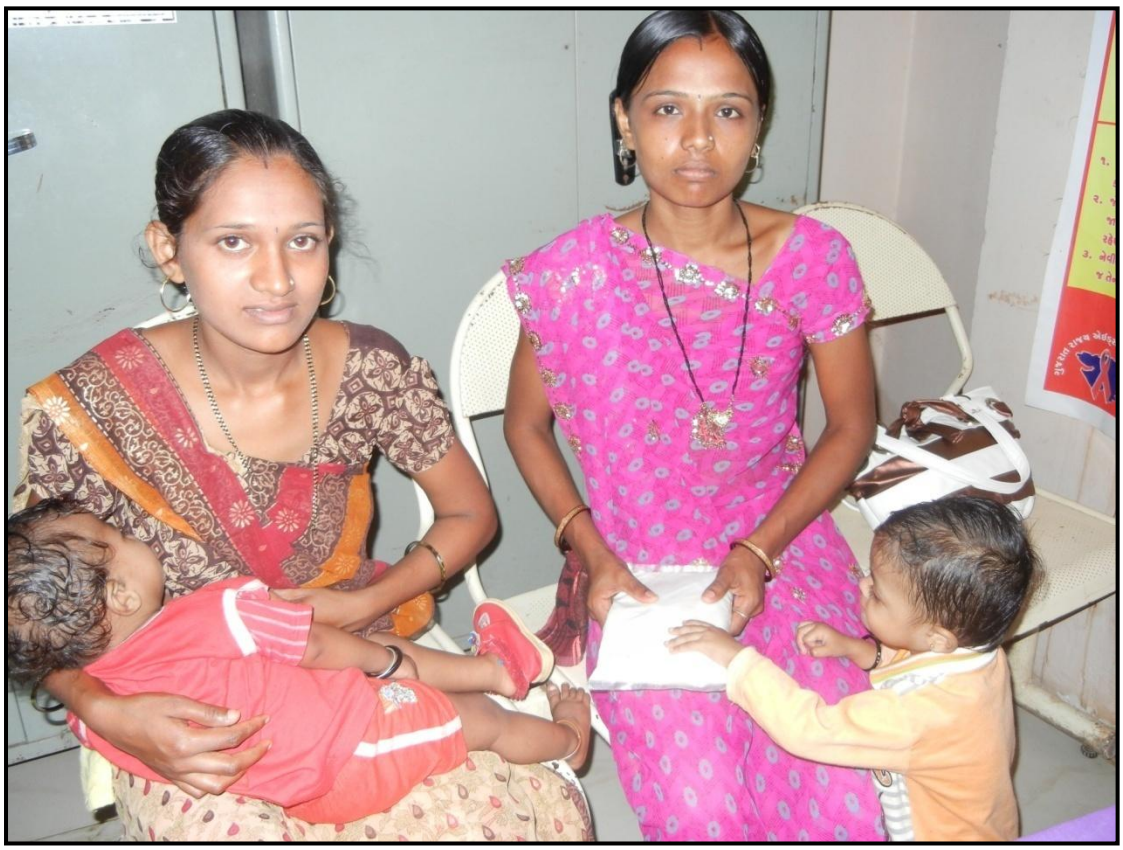
For paired samples paired t-test (parametric data) and Wilcoxon test (non parametric data) were used. Non independent samples, independent t test (parametric data) and Mann Whitney test (non parametric data) were used.

For comparing two diagnosis tests, diagnostic test was used as sensitivity, specificity, positive predictive value and negative values were calculated.

A two-tailed p value $<0.05$ was considered statistically significant, which was denoted as * [$p<0.01$ high significance denoted as **, $p<0.001$ very high significance denoted as *** and $p<0.0001$ very very high significance denoted as ****].

**Picture 3.1: Enrolment of pregnant women and counselling**


**Picture 3.2: Blood collection and DFS supplementation**

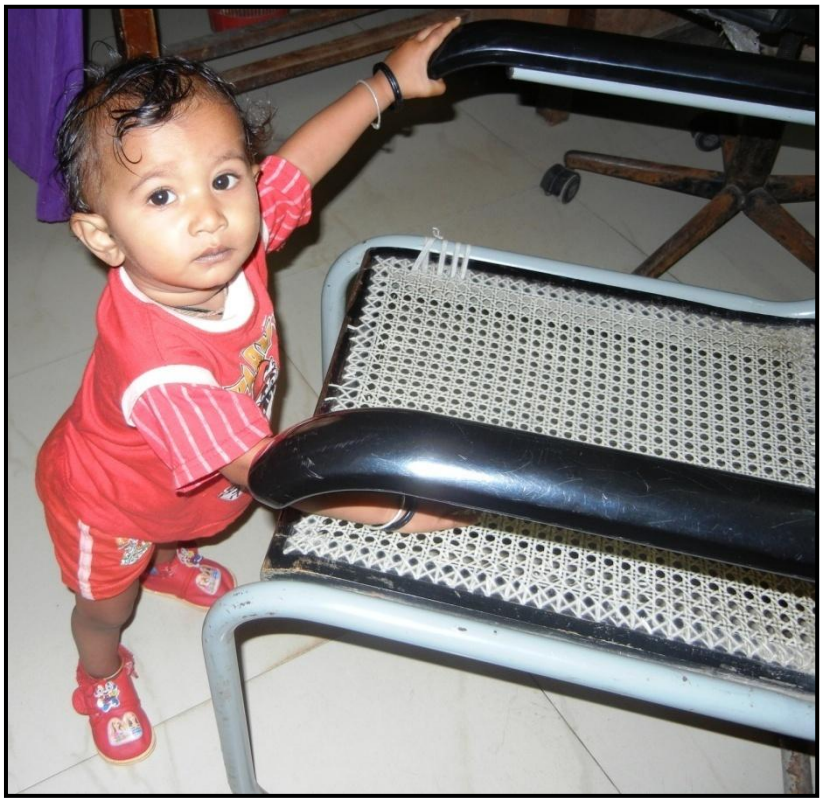


**Picture 3.3: Child sitting with good co-ordination and holding cubes [retain two things in two hands]**

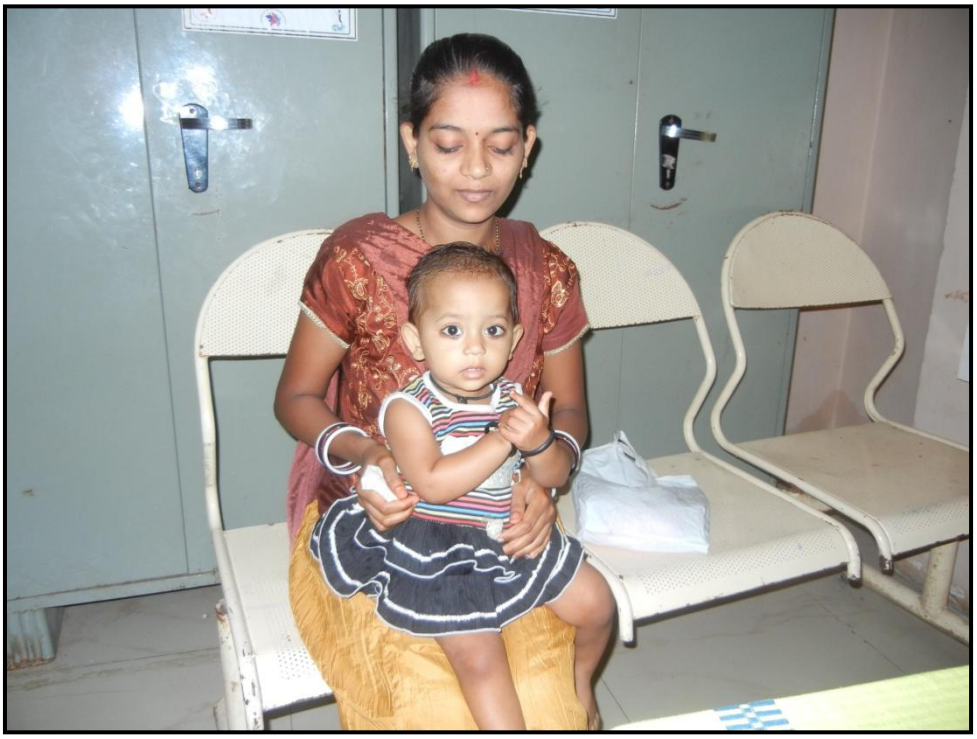
**Picture 3.4: Child trying to stand up**

**Picture 3.5: Child using furniture for walking**

**Picture 3.6: Child stands up by furniture**



**Picture 3.7: Parents with their infants**



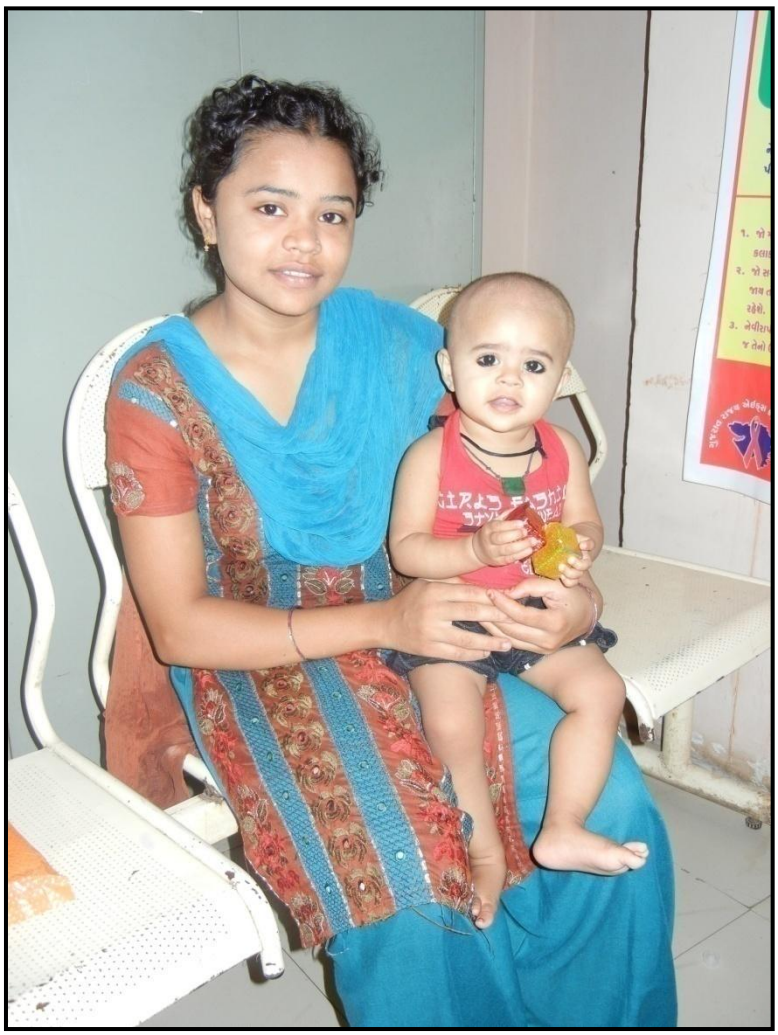


**Figure 3.8: Mother with her newborn baby**

# RESULTS AND DISCUSSIONS

# RESULTS AND DISCUSSION

4.2.9 KAP

4.2.10 Food Frequency

4.2.11 Maternal health and child care

4.3 Phase III: Screening of neonates

4.3.1 Characteristics of neonates

4.3.2 Thyroid profile of neonates

4.3.3 Newborn screening

4.4a Phase IV: Effect of thyroid dysfunction during early gestation on infant development

4.4b Phase IV: Effect of DFS supplementation on iron and iodine status of lactating women

4.4.1 Characteristics of women during postpartum period

4.4.2 Comparison of dietary intake during pregnancy and postpartum

4.4.3 Micronutrient deficiency during pregnancy and postpartum

4.4.4 Prevalence of thyroid dysfunction during postpartum period

4.4.5 Comparison of diagnostic test

4.4.6 Comparison of biochemical parameters of subjects during pregnancy (I, II and III trimester) and postpartum

4.4.7 Characteristics of infants

4.4.8 Nutritional status of infants

4.4.9 DFS supplementation to combat anemia

4.4.10 Effect of early gestation thyroid dysfunction on infant development

Present study is a longitudinal study [18 months follow up of pregnant women (from 4th month of pregnancy till one year postpartum along with one year follow up of infant)], which is divided into 4 phases. Because of long follow up we encountered many difficulties/obstacles during each phase of this study which we did not anticipate at the onset of the study. Hence we had to modify the study design with respect to sample size as and when required. However, all possible measures were taken to minimize any errors.

**Phase 1-Screening of pregnant women**

During Phase [1] 225 pregnant women were enrolled for the study. After data entry and cleaning 25 pregnant women were excluded (applying exclusion criteria). Hence results of phase [1] are based on observations of 200 (parent sample size) pregnant women.

**Phase 2-Follow up till delivery**

During phase [2] out of these 200 women, 100 women were purposively selected (50%) for follow up till delivery. Follow up of all 200 women in the given time period was not feasible due to time taken for enrolling 200 women (which took 3 months). If one wants to carry out the follow up for all 200 women then, by the time one takes the second trimester sample for 199th women, the 1st woman would be due for delivery and hence we considered and reached a conclusion that, dealing with different situations altogether was not the appropriate approach.

Out of these 100 women, 5 women migrated and 15 women refused to further participate in the study. Hence we had 80 women for follow up. After completing data collection, data entry and analysis, 7 women [2 premature babies, 1 twin pregnancy, 2 low birth weight babies, 2 Intra Uterine Fetal Death (IUFD)] were excluded. Hence results of phase [2] are based on observations of 73 (100-20=80, 80-7=73) pregnant women.

**Phase 3-Screening of neonates**

During phase [3] out of 73 pregnant women, we could collect cord blood from 32 subjects. However, we could follow 49 women within 24 hours of delivery. Results of this phase are based on 32 samples for cord blood thyroid hormones, 49 samples for birth length and head circumference and 73 samples for birth weight.

**Phase 4a) Effect of thyroid dysfunction during early gestation on infant development and 4b) Effect of DFS supplementation on iron and iodine status of lactating women**

During phase [4] out of 73 women, 23 women refused to further participate in the study. Hence, we approached remaining 100 women from our parent sample size. Out of these 100 women 31 agreed to participate in the study. Hence, for this phase, we had a total of 81 pregnant women [50 from follow up group + 31 from parent group].

Phase [4] is further divided into 2 parts, part 4a is effect of thyroid dysfunction during early gestation on infant development and part 4b is effect of DFS supplementation on iron and iodine status of lactating women. During phase 4a, on the basis of thyroid status of women (81) during early gestation we had categorized them into 2 groups, namely with normal thyroid function and with thyroid dysfunction during early gestation. Hence after categorization 42 women fell into group with normal thyroid function and 39 women fell into group with thyroid dysfunction. During phase 4b, out of 81 lactating women, 48 women (voluntary basis) were supplemented with DFS and the remaining 33 lactating women (consuming adequately iodized salt) were considered as control.

# *RESULTS*

# Phase I

Screening of pregnant women during early gestation

### 4.1.1 General Characteristics

Mean age of pregnant women was 23.3(3.6) years and they all were housewives. No pregnant women were consuming alcohol and cigratte or *bidi*. Information about general characteristic of pregnant women reveals that most (69%) of them were Hindu and were living in joint family (Table 4.1.1).

**Table 4.1.1: General characteristics of pregnant women**

| S.No. | Determinants | Variable | Percentage |
|---|---|---|---|
| 1 | Food Habits: | Religion | |
| | | Hindu | 69 |
| | | Muslim | 31 |
| 2 | Maternal Education: | Studies | |
| | | Illiterate | 7 |
| | | Primary | 66 |
| | | High school | 18 |
| | | Intermediate | 9 |
| 3 | Obstetric History: | Parity | |
| | | Primpara | 48 |
| | | Multipara | 52 |
| | | Abortions | |
| | | No abortions | 82 |
| | | 1 or more abortions | 18 |
| 4 | Care takers: | Type of Family | |
| | | Nuclear | 27 |
| | | Joint | 73 |
| | | Total family member | |
| | | 2 | 8 |
| | | 3-4 | 34 |
| | | 5-6 | 33 |
| | | >6 | 25 |
| 5 | Socio economic status: | Per capita income | |
| | | <500 | 3 |
| | | 500-1,000 | 75 |
| | | 1,001-5,000 | 21 |
| | | >5,000 | 1 |

Maternal education revealed that most (66%) of them were educated till primary level. Mean parity was found to be 0.7(0.1) and women with history of abortions were few (6%). Mean per capita income was 967(637) rupees.

**Nutritional status of pregnant women in India**

Nutrition plays a major role in maternal and child health. Poor maternal nutritional status has been related to adverse birth outcomes; however, the association between maternal nutrition and birth outcome is complex and is influenced by many biologic, socioeconomic, and demographic factors, which vary widely in different population (Villar et al, 2003).

Pregnancy is dynamic, anabolic, characterized by a series of small adjustments whose purpose is to allow growth and development of the fetus while maintaining maternal homeostasis and preparing for breast feeding. These adjustments relate to changes in maternal behavior and affect the metabolism of all nutrients. They depend primarily on the nutritional status of the mother before conception and explain its ability to adapt to various nutritional situations (Basdevant et al, 2007). Assessing the nutritional status during the reproductive period, especially during pregnancy, is a widely used method that requires few resources and is likely to provide much useful information. Weight gain during pregnancy is an essential element of fetal growth and fate of pregnancy. The expectant mother must be well nourished to meet the needs of her fetus, her own needs and to prepare your body for breast feeding. The deleterious effects of severe deficiency, especially in the peri-conceptional period, are established for many nutrients.

Numerous studies in India and elsewhere have shown that, in chronically undernourished women subsisting on unchanged dietary intake, pregnancy and lactation have an adverse effect on maternal nutritional status. Maternal under nutrition is associated with low birth weight and all its attendant adverse consequences.

Epidemiological studies from India documented the magnitude and adverse consequences of chronic energy deficiency (CED) on the mother child dyad and paved way for effective intervention programmes to address under nutrition during pregnancy and lactation. Over 75% of pregnant women in India are anemic and anemia remains to be a major factor responsible for maternal morbidity, mortality and low birth weight. Too early, too close, too many and too late pregnancies adversely affect nutrition and health status of the mother child dyad; timely contraceptive care has become an indirect effective intervention to prevent deterioration in maternal and child nutrition (Ramachandran, 2002). Yet another important indirect cause of under nutrition continues to be infections; under nutrition increases the susceptibility for infections; infections aggravate under nutrition. While under nutrition continues to be major problem as in the earlier decades, the current decade has witnessed the progressive rise of over nutrition in women during reproductive age especially among the affluent segments of population both in urban and in rural areas. It has become imperative to assess the nutritional status of pregnant women and give them appropriate advice and care.

**Time trends in dietary intake in pregnant women**

Data from NNMB surveys (using 24 hour dietary recall method) show that between 1975 and 1995 there has been some increase in dietary intake. By the mid-nineties average intake of cereals almost met the RDA. Since then there has been a reduction in cereal intake inspite of the fact that food is available, accessible and affordable. There has been a progressive reduction in the pulse intake, which might be related to the rise in the cost of pulses. Intake of vegetables and fruits continue to be low (Table 4.1.2). Dietary intake of pregnant and lactating women is not different from that of the non-pregnant and non-lactating women.

**Table 4.1.2.: Time trends in dietary intake (g/day) in pregnant and lactating women**

| Groups | Year | Cereals & Millets | Pulses & Legumes | Milk & Milk Products | GLV's | Roots & tubers | Other vegetables | Fruits | Fats & Oil | Sugar & Jaggery |
|---|---|---|---|---|---|---|---|---|---|---|
| **NPNL Women** | 1975-79 | 386 | 31 | 56 | 11 | 51 | 47 | 11 | 9 | 16 |
| | 2000-01 | 389 | 26 | 67 | 18 | 69 | 50 | 20 | 12 | 16 |
| | 2005-06 | 365 | 27 | 80 | 18 | 63 | 52 | 26 | 13 | 14 |
| **Pregnant Women** | 1975-79 | 359 | 34 | 75 | 12 | 58 | 44 | 11 | 12 | 19 |
| | 2000-01 | 408 | 28 | 77 | 15 | 69 | 44 | 21 | 12 | 17 |
| | 2005-06 | 362 | 27 | 87 | 16 | 55 | 49 | 25 | 14 | 14 |
| **Lactating Women** | 1975-79 | 436 | 30 | 58 | 15 | 48 | 45 | 13 | 10 | 16 |
| | 2000-01 | 442 | 28 | 65 | 18 | 69 | 54 | 24 | 13 | 13 |
| | 2005-06 | 406 | 30 | 80 | 17 | 63 | 56 | 24 | 14 | 13 |

NPNL-non pregnant non lactating, GLV's-green leafy vegetables

**Source: NNMB report, 1979-2002**

**Table 4.1.3: Time trends in nutrient intake in pregnant and lactating women**

| Groups | Years | Protein (g) | Total Fat (g) | Energy (kcal) | Calcium (mg) | Iron (mg) | Vitamin A (µg) | Thiamin (mg) | Riboflavin (mg) | Niacin (mg) | Vitamin C (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **NPNL Women** | 1975-79 | 45.4 | 17.1 | 1,698 | 330 | 21.0 | 118.0 | 1.00 | 0.70 | 11.0 | 24 |
| | 2000-01 | 48.2 | 27.6 | 1,878 | 445 | 14.1 | 219.8 | 1.20 | 0.60 | 14.9 | 45 |
| | 2005-06 | 46.5 | 21.8 | 1,738 | 443 | 13.8 | 254.0 | 1.10 | 0.60 | 14.2 | 47 |
| **Pregnant Women** | 1975-79 | 40.8 | 18.8 | 1,597 | 390 | 20.0 | 160.0 | 1.00 | 0.60 | 10.0 | 21 |
| | 2000-01 | 49.7 | 25.9 | 1,933 | 463 | 14.0 | 227.0 | 1.20 | 0.70 | 15.1 | 45 |
| | 2005-06 | 46.8 | 22.5 | 1,726 | 456 | 14.0 | 261.0 | 1.10 | 0.60 | 13.7 | 42 |
| **Lactating Women** | 1975-79 | 47.6 | 18.3 | 1,797 | 358 | 23.0 | 133.0 | 1.10 | 0.70 | 12.0 | 23 |
| | 2000-01 | 50.3 | 25.9 | 2,028 | 408 | 14.6 | 212.0 | 1.30 | 0.60 | 16.3 | 48 |
| | 2005-06 | 49.6 | 22.1 | 1878 | 447 | 14.7 | 249.0 | 1.20 | 0.60 | 15.5 | 46 |

NPNL-non pregnant non lactating, GLV's-green leafy vegetables

**Source: NNMB report, 1979-2002**

Nutrient intake in pregnant and lactating women over the last three decades is given in Table 4.1.3. Between 1975 and 1996 there was an increase in the total energy, protein and fat intake. However over the last decade there has been a reduction in the energy and fat intake. In all periods of time there is no difference in nutrient intake of pregnant and lactating women and NPNL women. All these data clearly indicate that in India women do not consume more food during pregnancy and lactation.

Studies carried out by National Institute of Nutrition (NIN) during the seventies and early eighties confirmed that among urban and rural low income group population in Hyderabad there was no increase in dietary intake during pregnancy and lactation. Dietary intake ranged from 1,200-1,800 kcal per day. These women weighed an average 43 kg prior to pregnancy and gained 6 kg during pregnancy (Table 4.1.4.).

**Table 4.1.4: Change in weight during pregnancy**

| Category | Weight (KG) |
|---|---|
| NPNL | 42.3 |
| First trimester | 41.5 |
| Second trimester | 44.6 |
| Third trimester | 46 |

**Source: NNMB report, 1979-2002** NPNL-non pregnant non lactating

**Table 4.1.5: Birth weight and socio economic status**

| Income Group | No. | Age (years) | Parity | Weight (kg) | Height (cm) | Hb (g/dl) | BWt (kg) |
|---|---|---|---|---|---|---|---|
| Low | 1468 | 24.1 | 2.4 | 45.7 | 151.5 | 10.9 | 2.7 |
| Middle | 108 | 24.3 | 1.6 | 49.9 | 156.3 | 11.1 | 2.9 |
| High | 63 | 27.8 | 1.6 | 56.2 | 156.3 | 12.4 | 3.1 |

**Source: NNMB report, 1979-2002** NPNL-non pregnant non lactating, BWt-birth weight

Studies carried out at NIN Hyderabad in late seventies showed that there was a socioeconomic gradient in dietary intake but in majority of women in all the three groups' dietary intake was not higher in pregnant women as compared to non-pregnant women from same income group. The low income group women weigh ten kg less than high income group of women and birth weight of the offspring was only 2.7 kg (Table 4.1.5). Women from the upper income group consumed 2,000 to 2,500 kcal per day during pregnancy. In middle and high income groups, pregnant women do not perform hard physical labor during pregnancy and there is a reduction in physical activity during pregnancy. The pre-pregnancy weight in this population group ranges between 45-55 kg and pregnancy weight gain was 11 kg. The mean birth weight of infants is 3.1 kg (Table 4.1.5). These data suggest that among habitually well-nourished women who eat to appetite, there is no increase in dietary intake during pregnancy; unchanged dietary intake did not have any adverse effect either on their own nutritional status or on the course and outcome of pregnancy.

The Tenth Plan envisaged that, efforts will be made to weigh all women as early in pregnancy as possible and to monitor their weight gain. Well-nourished women will be advised not to increase their dietary intake to prevent over nutrition and obesity. Women who weigh less than 40 kg will be identified and:

- given food supplements consistently throughout pregnancy
- given adequate antenatal care
- monitored for weight gain during pregnancy
- if weight gain is sub-optimal, efforts are to be made to identify the causes and attempt remedial measures

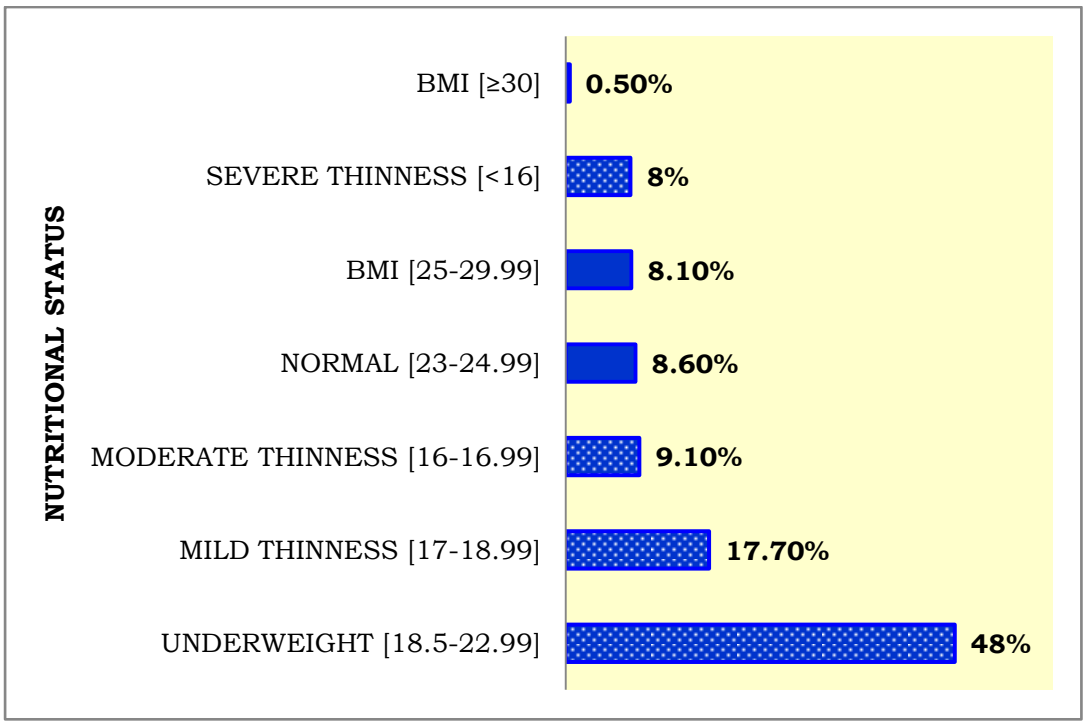
The National Rural Health Mission (NRHM) envisages that, there will be village health and nutrition days where in the ANM and AWW will

work together and provide the needed health and nutrition care. As a part of this, weighing of pregnant women is to be carried out, those with body weight less than 45 kg can be identified and given food supplementation on priority and monitored for weight gain during pregnancy.

### 4.1.2 Anthropometric measurements and BMI of pregnant women

Mean weight, height and BMI was 45.7(8.0) kg, 150.8(8.9) cm and 20.7(11.9) kg/m$^2$ respectively. Only 8.6% pregnant women had normal BMI. Mild, moderate and severe thinness was found in 17.7%, 9.10% and 8% pregnant women respectively. Almost half (48%) of pregnant women were falling under underweight category during early gestation indicating that these women were at risk of delivering low birth weight babies. Percentage of women with BMI between 25 to 29.99 and >30 was observed to be 8.10% and 0.5% respectively (Figure 4.1.1).

**Figure 4.1.1: Nutritional status of pregnant women according to BMI**



**Nutritional Anemia during pregnancy**

The World Health Organization estimates that 58% of pregnant women in developing countries are anemic (ACC/SCN, 1997). For women, the consequences of anemia include reduced energy and capacity for work (Levin, 1986), poor pregnancy and birth outcomes including premature delivery, low birth weight, and increased perinatal mortality (Murphy et al, 1986; Scholl and Hediger, 1994), and increased risk of death during delivery and postpartum (Llewellyn-Jones, 1965; Ojo and Savage, 1974; Zucker et al, 1994; Sarin, 1995). It is estimated that as many as 20% of maternal deaths are caused by anemia and that anemia may be an associated cause in as many as 50% of maternal deaths worldwide (Gillespie et al, 1991).

Most Ministries of Health in developing countries have policies to supplement pregnant women either iron by itself or combined with folate in tablet form or in prenatal vitamins. For example, national protocols in India require the provision of 100 tablets containing 60 mg elemental iron and 0.5 mg folic acid for daily consumption to all women during pregnancy and lactation. The Government of Indonesia provides 50–60% of the recommended number of iron supplements (60 mg elemental iron each with folate) for women (90 tablets during pregnancy and 40 tablets during the postpartum period). Despite these policies, anemia prevalence has not declined significantly (Gillespie et al, 1991). Many nutrition experts believe that one of the main reasons why national iron supplementation programs have failed is women's "non-compliance/non-adherence" with taking iron supplements daily because of gastrointestinal upset and other side effects that sometimes occur when taking iron (deMaeyer, 1989).

Recent reviews on the topic suggest that, there are a number of reasons for ineffective programs including sporadic or inadequate supplies, poor quality tablets, problems with delivery and distribution systems, poorly trained and uncommitted health providers, ineffective communication materials to promote behavior change, lack of access

to or use of prenatal care, and poor monitoring of the problem. (Gillespie et al, 1991; Galloway and McGuire, 1994; Yip, 1996).

Women in developing countries are always in a state of precarious iron balance during their reproductive years. Their iron stores are not well developed because of poor nutritional intake, recurrent infections, menstrual blood loss, and repeated pregnancies. Gender discrimination in a country like India results in girls lacking access to a balanced diet, adequate healthcare, and proper education. Thus the average Indian woman enters her reproductive years, and particularly pregnancy, with iron and folate deficiency (Mukherji, 2002).

During the first 2 trimesters of pregnancy, iron-deficiency anemia increases the risk for preterm labor, low-birth-weight babies, and infant mortality and predicts iron deficiency in infants after 4 months of age (Brabin et al, 2001). It is estimated that anemia accounts for 3.7% and 12.8% of maternal deaths during pregnancy and childbirth in Africa and Asia, respectively (Khan et al, 2006). Therefore it is important to diagnose and treat anemia to ensure the optimal health of the mother and the newborn (Khan et al, 2006).

**Table 4.1.6: Prevalence of anemia among pregnant women in India and other Asian countries**

| Country | Prevalence of anemia in pregnant women |
|---|---|
| India | 87% |
| Bangladesh | 74% |
| Bhutan | 68% |
| Nepal | 63% |

**Source: Kalaivani, 2009**

The high prevalence of iron deficiency in the developing world has substantial health and economic costs (Gautam et al, 2008). Dieticians should educate pregnant mothers about careful selection of food and meal planning and preparation during their routine

antenatal checkups. Even simple alterations in food habits like separating tea drinking from meal time can increase iron absorption.

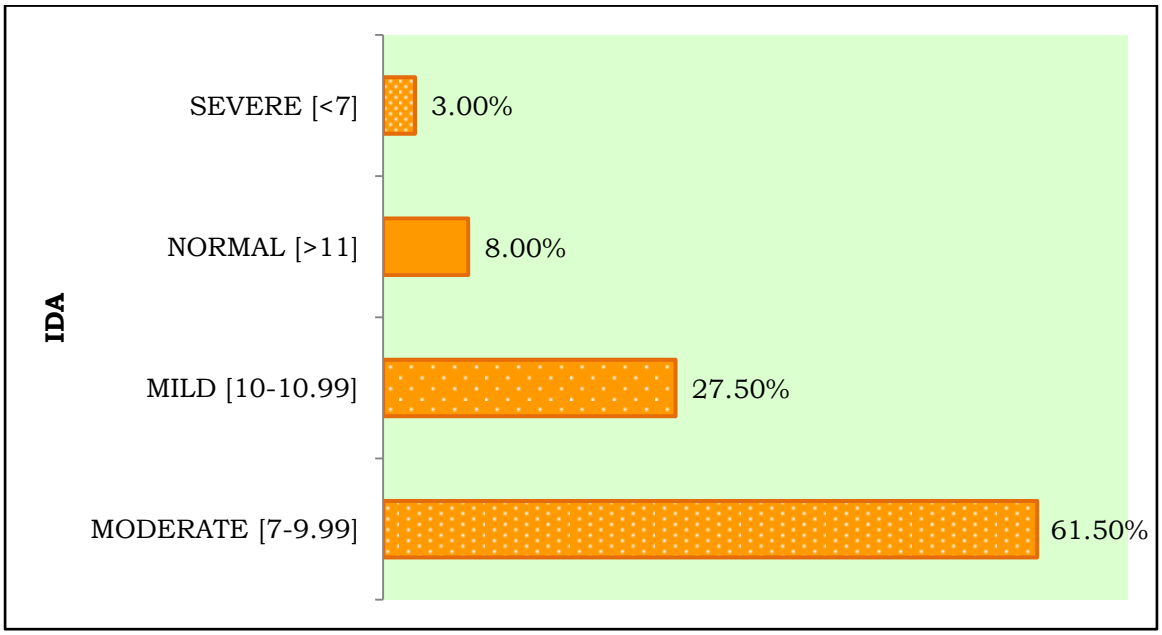
### 4.1.3 Iron Deficiency Anemia

Mean hemoglobin was 9.3 g/dl (Table 4.1.7) reflecting moderate anemia during early pregnancy. Iron deficiency anemia was found in 92% pregnant women of which 3% were severe anemic. Moderate anemia was found in 61.5% pregnant women and remaining 27.5% had mild anemia (Figure 4.1.2). These results reflect that almost all women had low iron stores from the start of pregnancy. When we compared mean hemoglobin of Hindu pregnant women (9.2 g/dl) with Muslim pregnant women (9.5 g/dl) we found a significant difference of 0.3 g/dl. This difference could be due to high consumption of non vegetarian food items by Muslims (79%) compared to Hindus (38%).

**Table 4.1.7: Hemoglobin level of pregnant women**

| Indicator | Mean | 95 % CI | Status |
|---|---|---|---|
| HB (g/dl) | 9.30 | 9.15-9.46 | Moderate IDA |

**Figure 4.1.2: Iron Deficiency Anemia using hemoglobin**



Low BMI (82.8%) along with low iron stores (92%) is an alarming state for both maternal and fetal health. Anemia in pregnancy is associated with adverse consequences both for the mother and the fetus. Studies have shown that the adverse consequences of maternal anemia may affect not only the neonate and infant but also increase the risk of low birth weight in the next generation (Kalaivani, 2009).

**Iodine Deficiency Disorders (IDD) during pregnancy**

Over the past 20 years, a worldwide effort has been under way to reduce the number of people at risk of iodine deficiency disorders (UNICEF, 2008). These disorders result from a diet low in iodine, which is particularly damaging during early pregnancy because it retards fetal development, especially brain development, causing a range of intellectual, motor and hearing defects.

Pregnancy is associated with profound changes in thyroid function and consequentially requirements of iodine are increased (Delange, 2004). The factors responsible for increased iodine requirement during pregnancy are (1) an increase in the production of thyroxine (T4) by the mother to maintain her euthyroid state and (2) the transfer of iodine in form of thyroid hormone to the fetus and (3) increased loss of iodine through the kidney due to an increase renal clearance of iodide. Taking account of these factors, the recommended dietary intake of iodine during pregnancy and the cut off values for Urinary Iodine (UI) concentration were revised by the Technical Consultation convened by WHO Secretariat in 2007 (WHO/ICCIDD/UNICEF, 2007). The recommended iodine intake during pregnancy was increased from 200 to 250 µg/day and median UI concentration cut off was increased from 100 µg/L to 150 µg/L. This upward revision means that current level of iodine supplementation in salt (at 15 parts per million level of iodine, daily average salt consumption of 10 gm will provide only 150 µg/day of iodine) may not be sufficient to meet the increased requirement during pregnancy. Also, the increase in median UI

concentrations cut offs will lead to greater proportion of pregnant women being classified as iodine deficient.

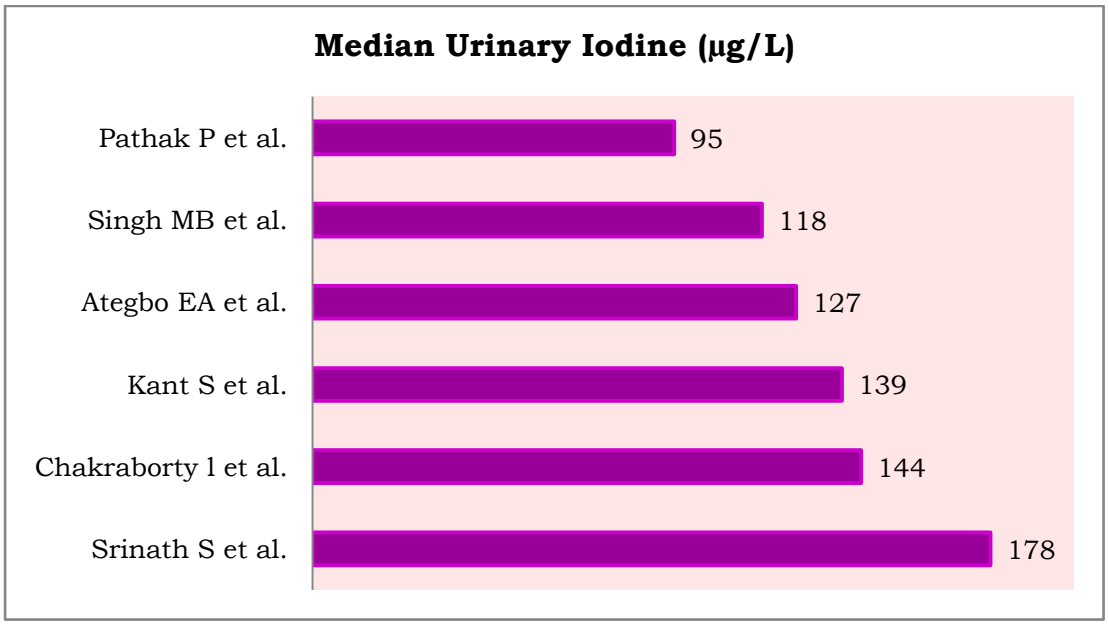
**Table 4.1.8: Summary of studies on iodine nutrition status of pregnant women in India**

| S.N. | Studies | Sample size | Study setting | Study area | Adequate IS consumption |
|---|---|---|---|---|---|
| 1 | Singh MB | 384 | Community based | Rajasthan | 77.3% |
| 2 | Ategbo E-A | 349 | Community based | Rajasthan | 59.5% |
| 3 | Chakraborty I | 267 | Hospital based | West Bengal | - |
| 4 | Srinath S | 400 | Hospital based | Haryana | 64% |
| 5 | Pathak P | 151 | Community based | Uttaranchal | - |
| 6 | Kant S | 149 | Community based | Delhi | 95% |
| 7 | Kapil U | 768 | Hospital based | Delhi | 89% |
| 8 | Kapil U | 137 | Hospital based | Himachal Pradesh | - |
| 9 | Dodd NS | 429 | Community based | Mumbai | 81% |

Recently a systematic literature review was performed by Yadav et al (2012) to identify studies that evaluated iodine nutrition status of the pregnant women in India. Their study reviewed nine studies, which were cross sectional studies conducted in different parts of India [Rajasthan (Singh et al, 2009; Ategbo et al, 2008), West Bengal (Chakraborty et al, 2006), Delhi (Kapil et al, 1999; Kant S et al, 2003), Haryana (Srinath, 2004), Uttaranchal (Pathak et al 2003), Himachal Pradesh (Kapil et al, 1997) and Maharashtra (Dodd and Madan, 1993)] from 1993 to 2009. Five out of nine studies were community based studies while four studies were hospital based. Three out of nine studies were carried out in rural areas and the remaining six were in urban/urban slum areas (Table 4.1.8).

Only five out of nine studies reported percentage of pregnant women consuming adequately iodized salt. The percentage of pregnant women consuming adequately iodized salt ranged from 59.5% to 95%.

**Figure 4.1.3: Median Urinary Iodine Excretion (µg/L) of the population of the studies reviewed**




Median UI concentration was reported by 6 out of 9 studies and the value ranged from 95 µg/L to 178 µg/L (Figure 4.1.3). Only in one study [Haryana], pregnant women had median UI concentration greater than the cut off level of 150 µg/L. Based on median UI concentration pregnant women were iodine deficient in five out of six studies. One study [Rajasthan] reported percentage of pregnant women with UI concentration less than 150 µg/L. All remaining eight studies reported percentage of women less than 100 µg/L (as per the old cut off levels). As per the imputation, the percentage of pregnant women with UI concentration less than 150 µg/L ranged from 30.4% to 95.3%. In six out of nine studies the percentage of pregnant women with UI concentration more than 150 µg/L was greater than 50%.

The current available data in India shows that, pregnant women in India are iodine deficient as per the WHO/UNICEF/ICCIDD criterion. No national representative study exists on iodine nutrition status of

pregnant women in India. Only handfuls of sub-national/regional studies are available. In few studies available on iodine nutrition of pregnant women, the data are reported as per the old cut off values.

The UI concentration represents the recent iodine intake and is widely accepted as the best indicator for iodine nutrition status (WHO/UNICEF/ICCIDD, 2007). The cut-off values of adequate iodine nutrition in pregnancy are higher (150 µg/L) as compared to normal population (100 µg/L). This review showed that in most (eight out of nine) of the studies the median UI concentration was less than the cut off value of 150 µg/L. The exact percentage of pregnant women with UI concentration less than 150 µg/L was reported by only one study. Author's imputation showed that 6 out of total 9 studies reviewed had greater than 50 % of pregnant women with UI concentration less than 150 µg/L. As of date, no cut off values are defined to grade the iodine deficiency status of pregnant women into mild, moderate and severe as is available for general population.

The presence of iodine deficiency amongst pregnant women in India as documented by this review warrants that immediate efforts need to be undertaken to increase adequately iodized salt consumption at household level to USI target of 90% from current level of 71% (UNICEF CES, 2009).

### 4.1.4 Iodine Deficiency

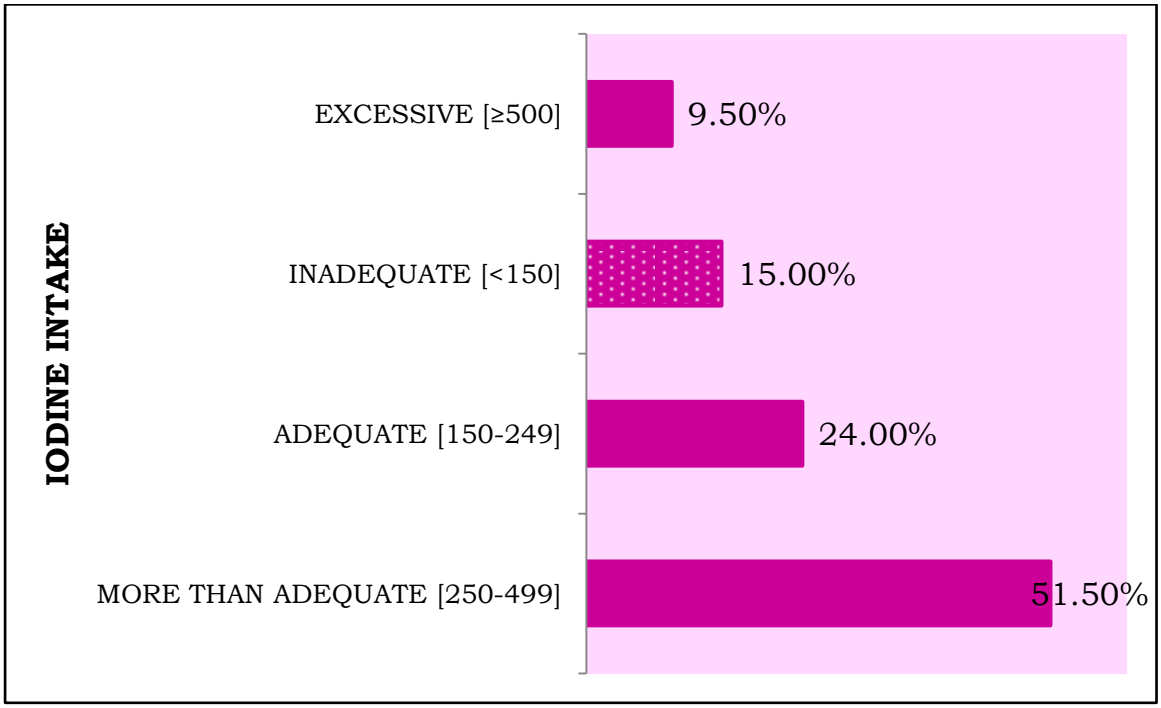
Median urinary iodine was 283.8 µg/L (Table 4.1.9) indicating adequate iodine intakes among these women. All these women were receiving iodized salt from their respective *Angawadi Centres*. Percentage of women having inadequate, adequate, more than adequate and excessive iodine intake was 15%, 24%, 51.5% and 9.5% respectively (Figure 4.1.4). As demonstrated in a recent study by Moleti et al (2008), iodine deficiency plays a pivotal role in favoring

thyroid impairment during gestation. In the present study, 15 % of pregnant women had low urinary iodine levels.

**Table 4.1.9: Urinary iodine level of pregnant women**

| Indicator | Median | 95 % CI | Status |
|---|---|---|---|
| UI (µg/L) | 283.8 | 262.8-313.1 | Adequate iodine intake |

**Figure 4.1.4: Iodine intake of pregnant women using UI**




### 4.1.5 Thyroid dysfunction

Over the past several years it has been proved that, maternal thyroid disorder influence the outcome of mother and fetus, during pregnancy and also in postpartum period. The most frequent thyroid disorder in pregnancy is maternal hypothyroidism. It is associated with fetal loss, placental abruptions, pre-eclampsia, preterm delivery and reduced intellectual function in the offspring (Abalovich et al, 2007).

Incidence of subclinical hypothyroidism is 2.5% and these women have no clinical features and are often asymptomatic. Overt hypothyroidism occurs only in about 5% of all women who have a

high TSH (Klein et al, 1991). During the last decade, it has become apparent that untreated maternal hypothyroidism and subclinical hypothyroidism in pregnancy is associated with adverse fetal and obstetric outcomes, which can be ameliorated by adequate levo-thyroxine therapy [Casey el al, 2005; Negro, 2010 and Agarwal et al, 2011 (unpublished)].

There is a greater prevalence of subclinical hypothyroidism in women with delivery before 32 weeks and there is even an association between thyroid autoimmunity and adverse obstetric outcome, which is independent of thyroid function (Lao, 2005). Higher maternal TSH levels even within the normal reference range are associated with an increased risk of miscarriages, fetal and neonatal distress as well as preterm delivery (Benhadi et al, 2009; Stagnaro-Green et al, 2005).

The availability of thyroxine to the developing fetal neurons is vital for their maturation and proper function (Williams, 2008). Either due to iodine deficiency or autoimmune thyroid disease reduction of circulating maternal thyroxine has been shown to result in lower IQ in infants and young children in retrospective (Haddow et al, 1999) and prospective studies (Pop et al, 2003). Isolated hypothyroxinemia has been found to be associated with reduced motor and intelligence performance in neonates (Kooistra et al, 2006). The strength of evidence relating maternal hypothyroidism to low IQ in children suggests the need for screening pregnant women for thyroid dysfunction during early gestation.

Pregnant women were screened using two TSH cut-off values as 2.5 μIU/ml (reduced upper limit) and 5.0 μIU/ml. Mean TSH, FT4, TT4 and TG were found to be falling under normal range (Table 4.1.10). Screening with TSH reveals that, 28% women were at low risk and 5.5% women were at high risk of developing hypothyroidism, while 66.5% were under normal range (Figure 4.1.5). Thyroid function in

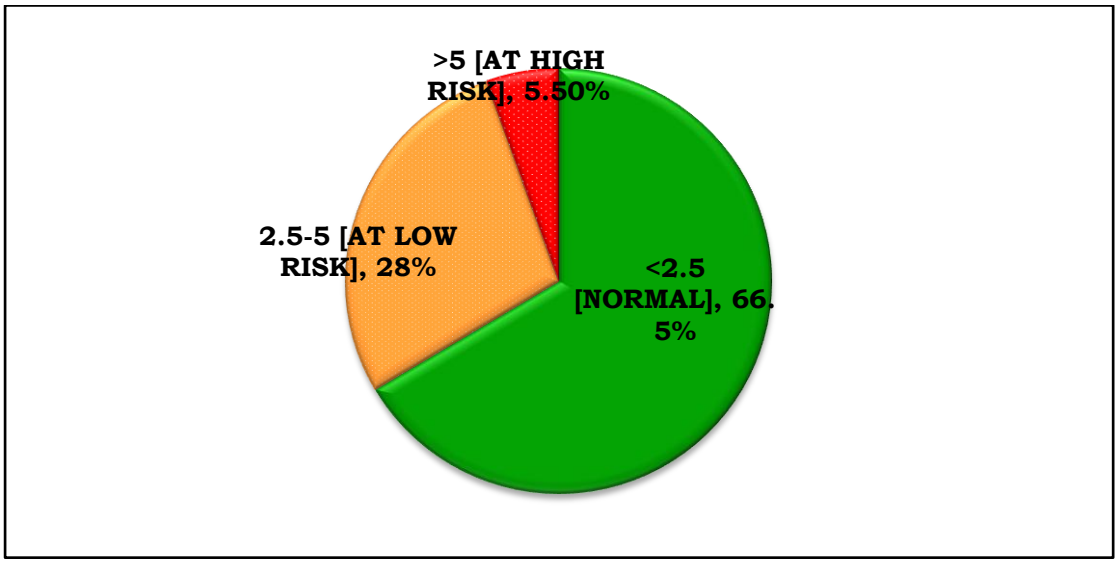
these women may be associated with increased risk of adverse pregnancy and perinatal outcomes.

**Table 4.1.10: Thyroid Hormone level of pregnant women**

| Thyroid Hormones | Mean | 95 % CI | Median | 95 % CI | Range* |
|---|---|---|---|---|---|
| TSH (µIU/ml) | 1.77 | 1.59-1.98 | 1.89 | 1.70-2.16 | 0.25-5.10 |
| FT4 (ng/dl) | 0.80 | 0.77-0.83 | 0.82 | 0.78-0.85 | 0.65-2.10 |
| TT4 (µg/dl) | 10.46 | 10.09-10.82 | 10.32 | 10.05-10.77 | 4.20-13.0 |
| TG (ng/ml) | 6.27 | 5.10-7.44 | 4.00 | 3.33-5.06 | 0.0-50.0 |

*Normal range for non-pregnant adults

**Figure 4.1.5: Percentage of at risk women for developing hypothyroidism**




### 4.1.6 Screening of pregnant women for thyroid dysfunction during early gestation

Globally, screening for thyroid dysfunction during pregnancy is being debated contextually. Currently there are no recommendations for universal screening for thyroid dysfunction in women before or during pregnancy. As the overall benefits of screening for thyroid dysfunction have not yet been justified by current evidence based medicine, recent

international guidelines (Abalovich et al, 2007) have recommended 'aggressive' case finding among the following groups of women who are at risk, preferably already prior to pregnancy or in early gestation.

Abalovich et al 2007 suggested that, since maternal thyroid function, especially hypothyroidism, is associated with adverse outcomes, recognizing those at risk of thyroid dysfunction might be beneficial. According to Vaidya et al 2007, this kind of targeted high risk case finding would fail to identify one third of pregnant women with overt/subclinical hypothyroidism. Negro et al (2010) compared targeted high risk case findings with universal screening. Authors found that up to 16% of elevated TSH level would have been missed by high risk case findings. This study also showed that, treatment of subclinical hypothyroidism during pregnancy with levothyroxine is beneficial considering adverse pregnancy and perinatal outcomes. It is suggested that universal screening of women with mildly elevated TSH levels should be recommended [Alexander, 2010 and Agarwal et al, 2011 (unpublished)].

Although treatment of thyroid dysfunction has been found to be beneficial considering adverse outcomes of pregnancy, there has not yet been any cost-effectiveness analysis concerning prevention of adverse outcomes with levothyroxine (Negro et al, 2010). According to Thung et al 2009 universal screening for hypothyroidism during pregnancy is considered cost-effective if treatment can prevent the possible neuropsychological damage that untreated hypothyroidism can impose on the child.

Recommendation on universal screening for thyroid dysfunction-

American Association of Clinical Endocrinologists (AACE) in the year 1999 believed that-

- Routine serum TSH testing early during pregnancy is reasonable but should be left to the discretion of the physician, in consultant with the patient.

- Serum TSH testing should be done in all women considering pregnancy so that hypothyroidism can be diagnosed early and treated before pregnancy.

Mitchell and Klein from USA (2004) suggested that-

- It should be the responsibility of the medical community to outline a course of action that will bring relief to pregnant hypothyroid women and their unborn children.
- Maternal screening programme is an effective tool in early diagnosis and treatment of subclinical hypothyroidism. Unfortunately, pregnant women with subclinical hypothyroidism seem to escape early clinical detection.
- In case of infant, major malformations and loss of IQ could be prevented by early diagnosis and treatment of mother.

They further opined that, if screening of all pregnant women be implemented, the mother, the infant and society all will be benefited.

Aziz et al from India (2006) stated that their data regarding hypothyroidism supports all the criteria needed to justify routine screening during pregnancy. They proposed inclusion of TSH as a screening test for hypothyroidism during the antepartum period, at the time of booking visit. After 5 years, Banerjee from India (2011) supported Aziz et al recommendation that TSH should be used for screening. Author also stated that if necessary FT4 and FT3 may also be tested.

Recently in 2012, Sahasrabuddhe and Pitale have found 59% pregnant women having TSH >2 μIU/ml during early gestation. After looking at high percentage of abnormal TSH in pregnancy, authors have suggested that universal screening for thyroid dysfunction during early gestation should be considered.

Over the past decade the normal upper limit of TSH levels during pregnancy has been an area of rising concern. Panesar et al in 2001 (11 wk, China) reported TSH upper limit of 2.3 μIU/ml, Stricter et al

in 2007 (7-12 wk, Switzerland) reported upper limit of as 2.8 μIU/ml and Gilbert et al in 2008 (9-13 wk, Australia) reported upper limit of 2.2 μIU/ml. These recent studies confirm that a redefinition of TSH concentration during first trimester is required, resulting in a shift to an upper limit to approximately 2.5 μIU/ml.

Hence for present study, pregnant women were screened during early gestation (first trimester) using two TSH cut-off values as 2.5 μIU/ml and 5.0 μIU/ml, while during second and third trimester TSH cut-off of 3 μIU/ml was followed. During postpartum period (when thyroid function again becomes normal as pregnancy induced changes are gone), we compared these two TSH cut-offs.

# *RESULTS*

# Phase II

Follow-up of pregnant women

&

Intervention

### 4.2.1 General characteristics

General characteristics of pregnant women in this group were similar as compared to parent group [as a sub sample (n=73) of parent group (n=200) was selected for follow-up].

### 4.2.2 Dietary information

Adequate maternal nutrition is important for the health and reproductive outcome of women, child survival and development. Pregnancy is physiologically and nutritionally a highly demanding period. Nutrient-dense food is required to meet the requirements of the fetus. In India, it is observed that diets of women from the low socioeconomic groups are essentially similar during pre-pregnant, pregnant and lactating periods. Consequently, there is widespread maternal malnutrition leading to high prevalence of low birth weight infants and very high maternal mortality. Additional nutrient-dense foods are required to improve pregnancy weight gain and birth weight of infants.

Pregnant women consuming vegetarian diet were 68% and 32% were consuming non vegetarian diet. Mean calorie, protein and visible fat intake were 1,617(367) kcal, 46.5(13.3) g and 52.3(14.7) g respectively. When compared with RDA (NIN, 2010) for Indian pregnant sedentary women differences in calorie intake, protein and visible fat were -633 kcal, -35.7 g and +22.3 g respectively.

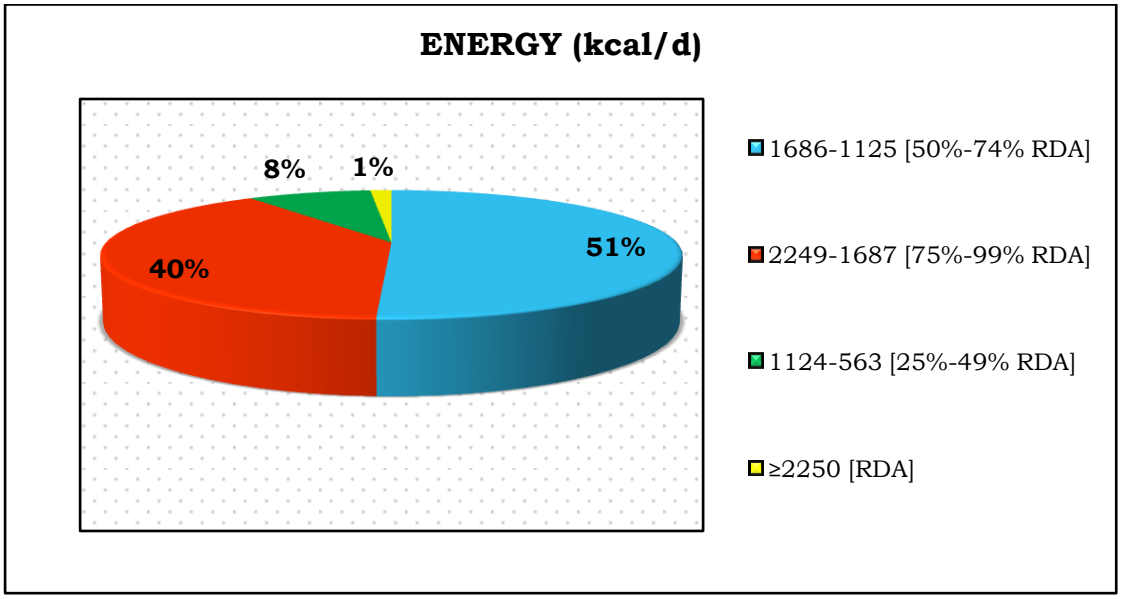
Parikh and Nair (2012) carried out dietary survey for urban pregnant women (Vadodara) and found that their mean calorie, protein and fat intake was 1022 kcal, 31 g and 45 g respectively.

**Energy intake**

The daily diet of a pregnant woman should contain an additional 350 calories. Mean calorie intake was equivalent to 71.9% of RDA. Only

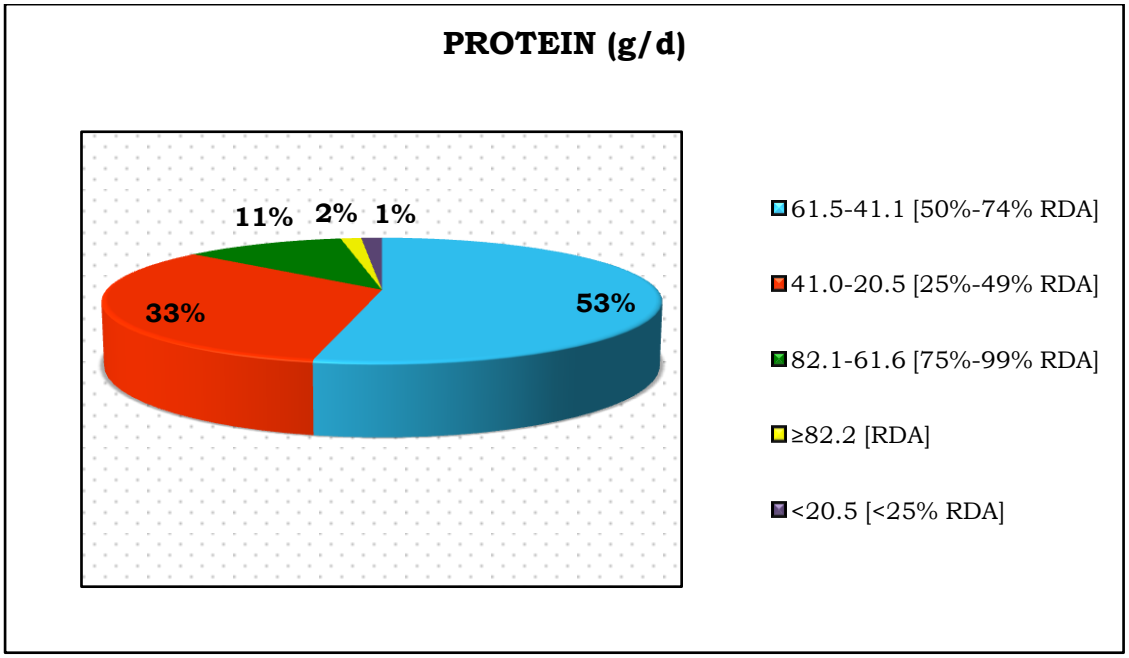
1.4% pregnant women met the requirements for daily calorie needs (Figure 4.2.1). Half (50.74%) of the population had calorie intake between 50-74% RDA.

**Figure 4.2.1: Energy intake of pregnant women according to RDA**




[RDA for energy-2250 kcal]

**Figure 4.2.2: Protein intake of pregnant women according to RDA**




[RDA for protein-82.2 g]

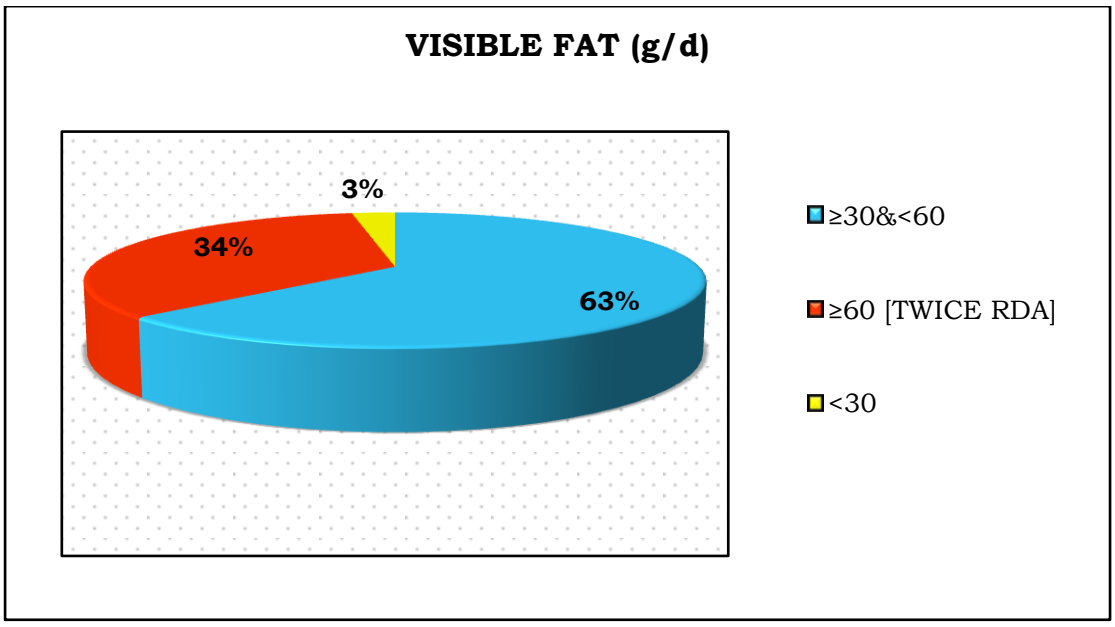
**Protein Intake**

The daily diet of a pregnant woman should contain 82.2 g protein. Mean protein intake was equivalent to 56.6% of RDA. Protein intake between 50- 74% of RDA was found in 53.4% of the population (Figure 4.2.2).

**Visible Fat**

The daily diet of a pregnant woman should contain 30 g of fat. Mean visible fat was well in excess of RDA. Percentage of population having fat intake <30 g was 2.7% (Figure 4.2.3). Women having visible fat consumption more than twice the RDA was 34.2%.

**Figure 4.2.3: Visible fat intake of pregnant women according to RDA**




[RDA for fat inatke-30 g]

Calorie intake of these pregnant women was not satisfying. Only one subject had energy intake close to RDA. Protein intake of these pregnant women was just above 50% of RDA. One subject had protein intake below 25% of RDA. Fat intake of the population was well in excess of what is recommended during pregnancy. This is due to the

eating habits of Gujarati families; consumption of oil and oily snack items *(farsan)* is high in this part of India. Only one subject was consuming fat below RDA.

### 4.2.3 Anthropometric measurements

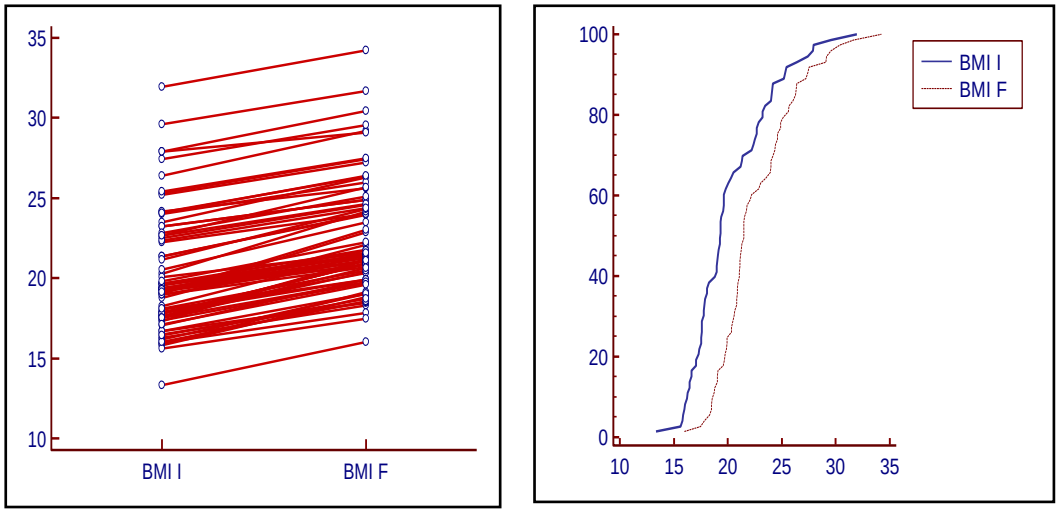
Low pre-pregnancy BMI is a risk factor for poor birth outcome and delivery complaints. Mean height and mean weight gain of pregnant women was found to be 151.4(5.3) cm and 5.4(1.3) kg respectively (Table 4.2.1).

**Table 4.2.1: Anthropometric measurements of pregnant women**

| Variable | Mean ± SD |
|---|---|
| Height (cm) | 151.4 ± 5.3 |
| Weight at 3 M [initial weight] (kg) | 46.3 ± 9.2 |
| Weight at 9 M [final weight] (kg) | 51.8 ± 8.9 |
| Weight gain (kg) | 5.4 ± 1.3 |

M-month

**Figure 4.2.4: Subjects-wise increment in BMI of pregnant women from 3rd month to 9th month and cumulative frequency**




BMI I-BMI initial (3rd month), BMI F-BMI final (9th month)

Weight gain was just half (5.4 kg) as compared to standard weight gain of 10-12 kg. A significant increase of 2.38 kg/m$^2$ in BMI from initial stages of pregnancy to final stage of pregnancy was found (Table 4.2.2 and Figure 4.2.4). This increase in BMI due to weight gain resulted in improvement in percentage of normal pregnant women from 9.59% to 16.44%. Severity of thinness disappeared and there was a shift from mild and moderate thinness to underweight category. Percentage of overweight and obese also increased by a few percent (Table 4.2.3). However during pregnancy it can be considered as normal because it is desirable.

**Table 4.2.2: Mean difference in BMI (kg/m$^2$) from 3$^{rd}$ to 9$^{th}$ month**

| BMI | Mean | 95 % CI | Difference | t value | DF | P |
|---|---|---|---|---|---|---|
| Initial 3$^{rd}$ M | 20.21 | 19.34-21.07 | 2.3875 | 32.28 | 72 | P <.0001 |
| Final 9$^{th}$ M | 22.59 | 21.76-23.43 | | | | |

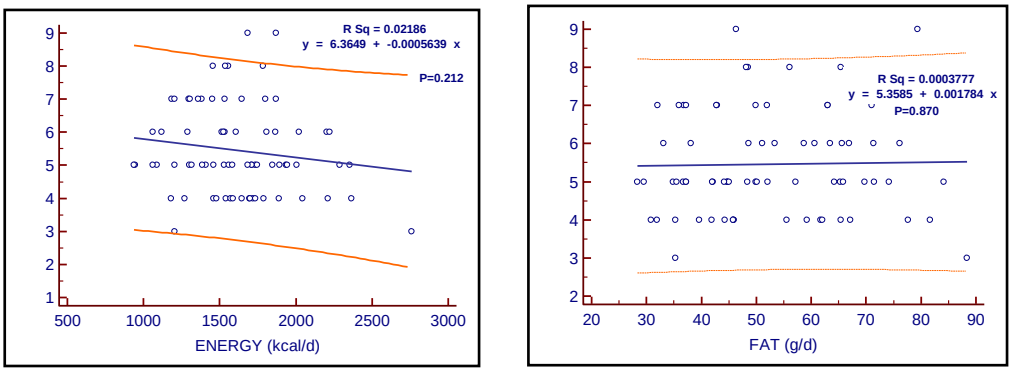
M-month

**Table 4.2.3: Nutritional Status during initial and final stages of pregnancy**

| Nutritional status | Cut-off | BMI (3 months) | BMI (9 months) |
|---|---|---|---|
| Severe thinness | <16 | 6.85% (5) | - |
| Moderate thinness | 16-16.99 | 9.59% (7) | 1.37% (1) |
| Mild thinness | 17-18.49 | 21.92% (16) | 5.48% (4) |
| Underweight | 18.5-22.99 | 39.73% (29) | 54.79% (40) |
| Normal | 23-24.99 | 9.59% (7) | 16.44% (12) |
| Overweight | 25-29.99 | 10.96% (8) | 17.81% (13) |
| Obese | ≥30 | 1.37% (1) | 4.11% (3) |

**Figure 4.2.5: Correlation and regression**

[Orange line denotes 95% prediction interval]




**(a) weight gain and energy intake (b) weight gain and fat intake**

Adequate intake of a nutrient-dense diet is reflected in optimal weight gain during pregnancy (10-12kg) by the expectant woman. Low calorie and protein intake along with high fat intake in these women resulted in low weight gain (Table 4.2.1) during entire pregnancy. This indicates that the consumption of carbohydrate rich foods was low among the population. When we tried to correlate weight gain with energy intake (Figure 4.2.5a), we found negative association (r=-0.1478, NS p=0.2119) while between fat intake and weight gain (Figure 4.2.5b) we found a week positive correlation (r=0.01943, NS p=0.8704). Hence, we can conclude that high fat intake had contributed more towards weight gain of 5.4 kg along with other natural phenomenon (weight gain due to fetus growth).

**DFS supplementation during pregnancy**

A pilot study with DFS supplementation of six months to urban pregnant women (Vadodara) was carried out by Joshi and Nair in 2010 (unpublished). Authors have randomly selected 50 pregnant women as control (receiving iodized salt and IFA for 100 days) and 50 as experimental (receiving DFS and IFA for 100 days). After supplementation, when comparison was made between initial and final hemoglobin in control and experimental group, authors have

found a decline of 0.20 g/dl ($p<0.05$) in mean hemoglobin of control group while an increase of 0.42 g/dl ($p<0.05$) was observed in experimental group. There was 1.5% increase in proportion of pregnant women with normal hemoglobin (changed from mild category to normal category) in experimental group, whereas in control group 11.1% reduction in normal category was observed. Since both groups were consuming iodized salt, when compared a non-significant difference was observed in median urinary iodine levels. In both groups median urinary iodine was >150 µg/L throughout gestation.

Hence, considering the above discussed beneficial effect of DFS, all pregnant women in present study were given double fortified salt in order to improve their iodine and iron status and its outcome to be observed in neonates.

### 4.2.4 Iron Deficiency Anemia

Iron is needed for hemoglobin synthesis, mental function and body defense. In India iron deficiency is common particularly in women of reproductive age. Iron deficiency during pregnancy increases maternal mortality and low birth weight in infants. Iron intake from diets is around 18 mg as against 35 mg RDA (NIN, 2010). An iron supplement (60 mg elemental iron, 500 µg folic acid) is recommended for 100 days during pregnancy from 16 week onwards to meet the demand of pregnancy.

#### 4.2.4.1 Iron status

Mean hemoglobin (g/dl) during first, second and third trimester was found to be 9.11(0.9), 9.23(0.9) and 9.46(1.0) respectively (Table 4.2.4 and Figure 4.2.6). These values of mean hemoglobin indicated presence of moderate IDA in all three trimesters. We observed an increase in mean hemoglobin with advancing gestation and the 10$^{th}$

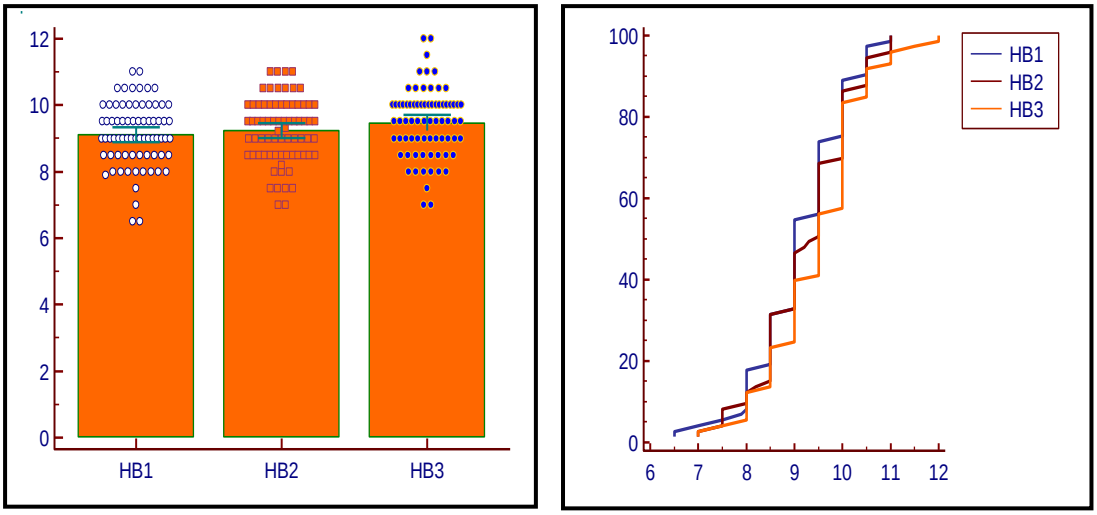
and 90th percentile value did not change with advancing gestation, suggesting an overall improvement in iron status of these pregnant women.

**Table 4.2.4: Mean and 95 % CI for hemoglobin (g/dl) during each trimester**

| Parameter | Trimester | Mean | 95% CI | 10th P | 90th P |
|---|---|---|---|---|---|
| HB | First | 9.11 | 8.89-9.3376 | 8.00 | 10.50 |
| | Second | 9.23 | 9.01 to- 9.45 | 8.00 | 10.50 |
| | Third | 9.46 | 9.22-9.70 | 8.00 | 10.50 |

P-Percentile

**Figure 4.2.6: Box plot of hemoglobin and cumulative frequency**




HB1, HB2 and HB3 denote HB during trimester

After performing repeated measures of ANOVA, we found a significant difference in mean hemoglobin in all three trimesters (Table 4.2.5 and Figure 4.2.7). Trends were analyzed and showed significant linear relations. After applying post hoc test, we have found mean difference of 0.12 from first to second trimester (non significant), mean difference of 0.23 from second to third trimester (non significant) and mean difference of 0.35 g/dl from first to third trimester (significant).

**Figure 4.2.7: Trends in mean hemoglobin (g/dl)**

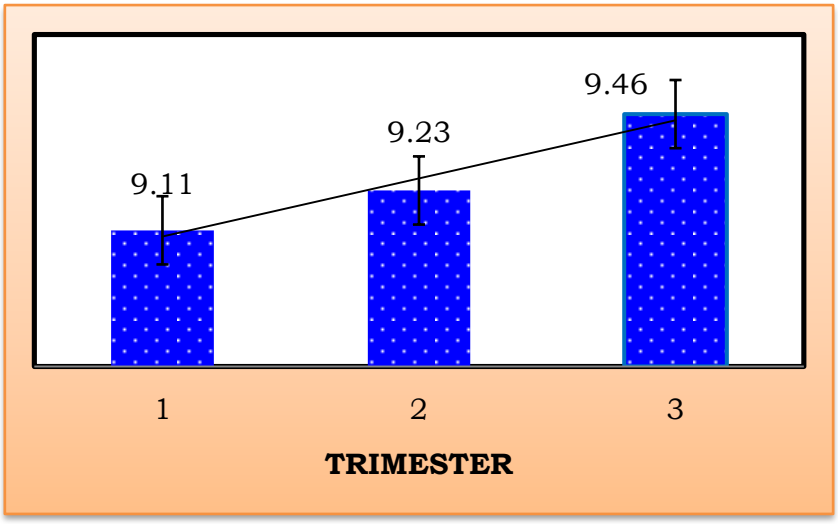



**Table 4.2.5: Repeated measures of ANOVA, trend analysis and post hoc test for hemoglobin**

| HB | | | |
|---|---|---|---|
| **Groups** | **F value** | **DF** | **P** |
| Trimester (1,2 & 3) | 4.75 | 2 | 0.010* |
| **Trend analysis** | | | |
| **Trend** | **t value** | **DF** | **P** |
| Linear | 2.7893 | 72 | 0.0068** |
| **Within-subjects factors** | | | |
| **Factor** | **Mean** | **Std. Error** | **95% CI** |
| HB_1 | 9.11 | 0.11 | 8.89-9.33 |
| HB_2 | 9.23 | 0.11 | 9.01-9.45 |
| HB_3 | 9.46 | 0.11 | 9.22-9.70 |
| **Pair wise comparisons** | | | |
| **Factors** | **Mean Difference** | **Std. Error** | **P** |
| HB_1 HB_2 | -0.12 | 0.11 | 0.8936$^{ns}$ |
| HB_2 HB_3 | -0.23 | 0.10 | 0.0962$^{ns}$ |
| HB_3 HB_1 | 0.35 | 0.12 | 0.0203* |

**4.2.4.2 IDA prevalence during each trimester**

Prevalence of IDA in first, second and third trimester was found to be 97.26%, 94.52% and 91.78% respectively (Table 4.2.6). Figure 4.2.8 and Table 4.2.6 shows that there was an improvement in hemoglobin level of pregnant women as pregnancy progressed (severity disappeared, percentage of normal increased and there was a shift from severe and moderate category to moderate and mild category).

**Table 4.2.6: Prevalence (%) of IDA during each trimester**

| Iron Deficiency Anemia | Cut-off (g/dl) | Trimester | | |
|---|---|---|---|---|
| | | I | II | III |
| Severe | <7 | 2.74% (2) | - | - |
| Moderate | 7-9.9 | 71.23% (52) | 69.86% (51) | 57.53% (42) |
| Mild | 10-10.99 | 23.29% (17) | 24.66% (18) | 34.25% (25) |
| Normal | ≥11 | 2.74% (2) | 5.48%(4) | 8.22% (6) |

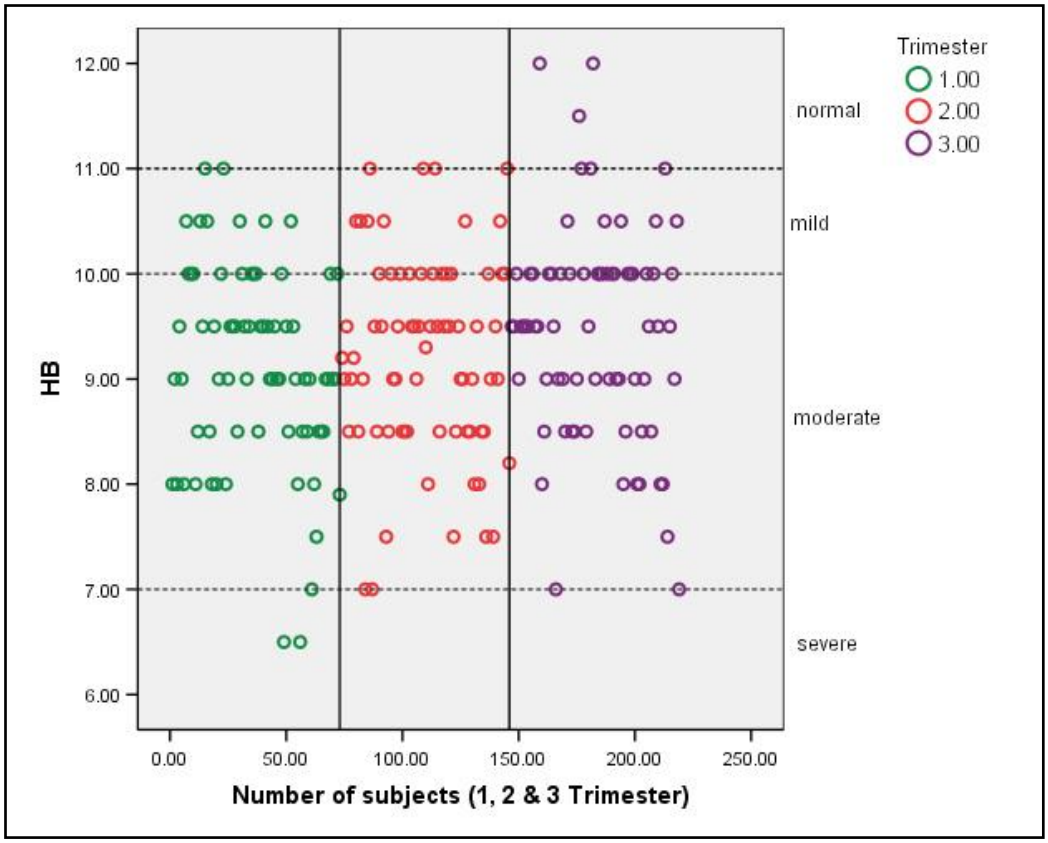
Figure in parenthesis denote number of subjects

A major problem in maintaining iron balance in pregnancy is that iron requirements are not equally distributed over its duration. Although reduced during the first trimester, iron requirements rise to between 4 and 6 mg in the second and third trimesters, respectively (FAO, 1988).

In our study we have found a significant increase of 0.35 g/dl of hemoglobin from first to third trimester. Pregnant women with cessation of menstruation during first trimester had mean hemoglobin of 9.11 g/dl; at this stage the absorption of iron also reduces (Bothwell, 2000). After first trimester or fourth month onwards pregnant women were consuming IFA [60 mg of elemental iron along with 500 µg folic acid] and DFS [1ppm or 10mg/10g salt] daily. This 70 mg of iron [60 mg IFA + 10 mg DFS] could bring an increase of 0.12 g/dl in hemoglobin levels from first to second trimester and an

increase of 0.23 g/dl from second to third trimester. Mean increase in hemoglobin was doubled during second to third trimester when compared between first and second trimester. This indicates that, with advancing gestation the absorption of iron from IFA and DFS also increased.

**Figure 4.2.8: Subject-wise prevalence of IDA**




From our results we can conclude that, 60 mg of elemental iron which was given to all pregnant women (government initiative) to control anemia did not result in complete success. This could be due to poor compliance (pregnant women often forget to take IFA or discontinuation due to side effects) or low absorption of IFA. However, it was proven beneficial in reducing severity cases and it also brought about a shift from severity and moderate category to mild and normal category. Hence we recommend that apart from IFA, other strategies

should also be introduced during pregnancy to help pregnant women to combat anemia.

### 4.2.5 Iodine Deficiency during pregnancy

Iodine deficiency during pregnancy is the commonest worldwide cause of preventable intellectual impairment.

#### 4.2.5.1 Iodine status

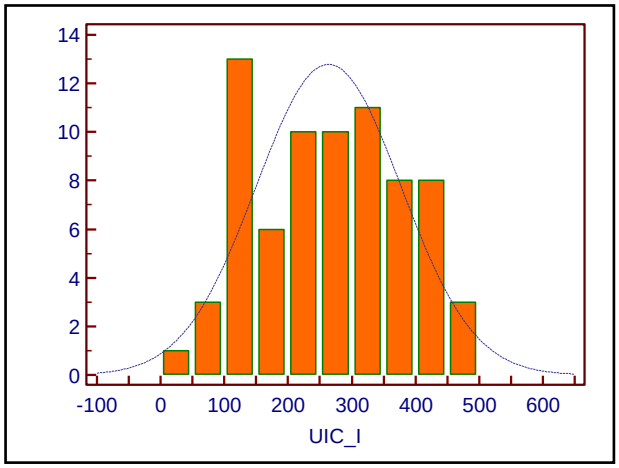
Urinary iodine excretion is an appropriate indicator of dietary iodine intake since 90 per cent of ingested iodine is excreted in the urine. Median urinary iodine (µg/L) during first, second and third trimester was found to be 270.1, 292.7 and 284.7 respectively (Table 4.2.7 and Figure 4.2.9). These figures are indicative of adequate iodine intake among the population in all three trimesters. During second trimester, minimum 20$^{th}$ percentile and maximum 80$^{th}$ percentile value was observed with highest median urinary iodine value, this could be due to skewness.

**Table 4.2.7: Median Urinary Iodine (µg/L) during pregnancy**

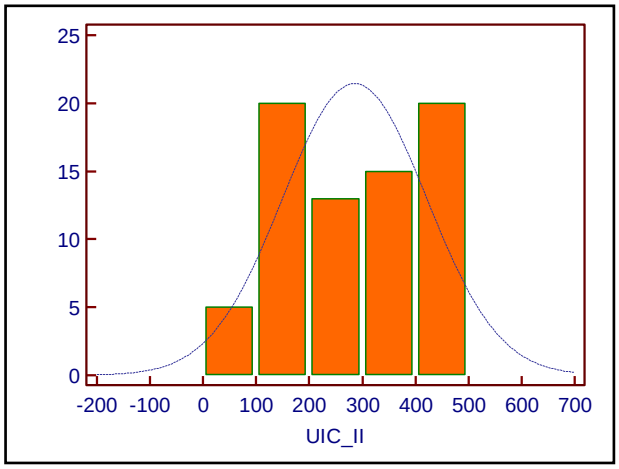
| Parameter | Trimester | Median | 95% CI of Median | 20$^{th}$ Percentile | 80$^{th}$ Percentile |
|---|---|---|---|---|---|
| UI | First | 270.1 | 224.2-306.5 | 140.1 | 375.6 |
| | Second | 292.7 | 243.6-360.0 | 123.5 | 423.0 |
| | Third | 284.7 | 232.0-307.4 | 155.4 | 393.6 |

Median urinary iodine levels increased by 22.6 µg/L form first to second trimester and then decreased by 8 µg/L from second to third trimester (Figure 4.2.10). After applying non parametric test we found that differences in urinary iodine levels among 3 trimesters were non significant (Table 4.2.8).

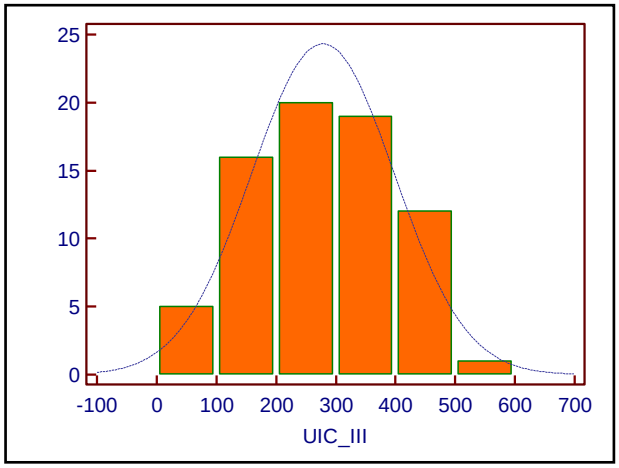
**Figure 4.2.9: Frequency distribution of median urinary iodine during pregnancy**




**First trimester**




**Second trimester**



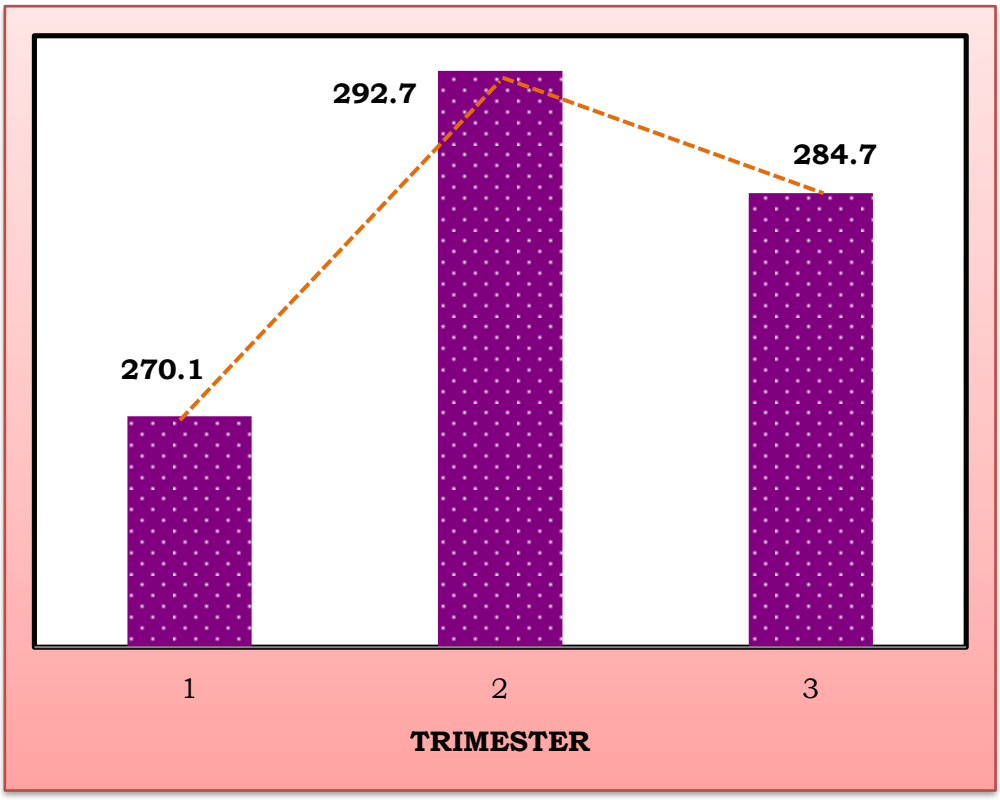

**Third trimester**

**Table 4.2.8: Non parametric test (Friedman) for urinary iodine during all 3 trimesters**

| | Minimum | 25th Percentile | Median | 75th Percentile | Maximum |
|---|---|---|---|---|---|
| **UI_I** | 49.6400 | 155.303 | 270.190 | 363.078 | 491.380 |
| **UI_II** | 81.6000 | 141.620 | 292.780 | 414.917 | 498.010 |
| **UI_III** | 74.1500 | 167.175 | 284.730 | 363.637 | 543.640 |

| F value | DF1 | P |
|---|---|---|
| 2.1856 | 2 | 0.116ns |

**Figure 4.2.10: Trends in urinary iodine (µg/L)**




### 4.2.5.2 ID prevalence during each trimester

Percentage of population having inadequate iodine intake during first, second and third trimester was 23.29%, 30.14% and 19.18% respectively (Table 4.2.9).

**Table 4.2.9: Iodine intake of pregnant women**

| Iodine Intake | Cut-off (µcg/L) | Trimester | | |
|---|---|---|---|---|
| | | I | II | III |
| Inadequate | <150 | 23.29% (17) | 30.14% (22) | 19.18% (14) |
| Adequate | 150-249 | 20.55% (15) | 10.96% (8) | 21.92% (16) |
| More than adequate | 250-499 | 56.16% (41) | 58.9% (43) | 57.53% (42) |
| Excessive | ≥500 | - | - | 1.37% (1) |

Figure in parenthesis denotes actual number

Median urinary iodine is the most commonly used indicator for measuring iodine status in a population. There are limited studies from India on iodine deficiency during pregnancy. A few authors have studied urinary iodine levels of pregnant women in West Bengal (Chakraborty et al 2006), Himachal Pradesh (Kapil et al, 1997), Delhi (Kant et al, 2003), Rajasthan (Singh et al, 2009) and Uttarakhand (Pathak et al, 2003). These studies are cross-sectional and have reported median urinary iodine levels in pregnant women. None of these authors have studied the relationship between gestation age and urinary iodine. Only one recent study by Chakraborty et al (2010) has reported median urinary iodine values of pregnant women during each trimester. Hence due to less number of databases from Indian population we have compared our data from well documented data with other countries along with Chakraborty et al (2010) study.

Stiwell et al (2008) studied the influence of gestational age on urinary iodine in Tasmania (an Island state of the Commonwealth of Australia with mild iodine deficiency). These Authors have studied 686 pregnancy samples (232 single sample, 143 two samples, 56 three samples) and found that median urinary iodine declined during pregnancy at an average rate of change of -0.44 µg/ per week of

gestation. The relationship with gestation was nonlinear, however this was found to be statistically significant.

Other similar studies on mild iodine deficient population from Switzerland (Brander et al, 2003) and United Kingdom (Smyth, 1999) have shown that urinary iodine values decrease with advancing gestation. Median urinary iodine levels in Switzerland and United Kingdom study was found to be 267 μg/L (first trimester), 206 μg/L (Second trimester), 172 μg/L (third trimester) and 135 μg/L (first trimester), 124 μg/L (second trimester), 122 μg/L (third trimester) respectively. In an Asian study from Dhaka (Mehdi et al, 2009) authors have reported that women progressively become more iodine deficient as pregnancy advances. Median urinary iodine levels during first, second and third trimester was found to be 143 μg/L, 132 μg/L and 120 μg/L respectively.

Unlike from above results, studies from Hong Kong (Kung et al, 2000) and Spain (Alvarez-Pedrerol et al, 2009) have shown an increase in median urinary iodine values with advancing gestation. Median urinary iodine values during first, second and third trimester in Hong Kong and Spain study were 106 μg/L (first trimester), 115 μg/L (Second trimester), 124 μg/L (third trimester) and 95 μg/L (first trimester) and 104 μg/L (third trimester) respectively.

Recent studies from Portugal (Costeria et al, 2009) and India (Chakraborty et al, 2010) have shown different results from above discussed studies. In these two studies authors have found that urinary iodine levels decreased from first to second trimester (Portugal study 65-57 μg/L and Indian study 137-135 μg/L) and then increased from second to third trimester (Portugal study 57-70 μg/L and Indian study 135-160 μg/L). In both studies maximum urinary iodine levels were found in third trimester.

In our study we have found a different pattern in the results. Median urinary iodine values increased from first to second trimester and then decreased form second trimester onwards. The explanation for

these differences could be physiological adjustments due to thyroid hormone fluctions. However, difference in dietary iodine intake among all these countries and degree of iodine deficiency might have played a role.

Stiwell et al (2008) suggested that studies from populations that are both iodine sufficient as well as mildly iodine deficient, it should be expected that:

1. Median urinary iodine should be high in pregnancy as in non-pregnant women of reproductive age.
2. The proportion of pregnant women with urinary iodine less than 50 mcg/L should be less than 10 %.
3. Median urinary iodine should not decline with advancing gestation.

In our study proportion of population with urinary iodine <50 µg/L was very less. During second and third trimester none of the pregnant women had urinary iodine levels <50 µg/L. Only one subject had urinary iodine level just <50 µg/L during first trimester. Median urinary iodine in our study declined only after second trimester; however the decline was very minor. Hence, in general we can conclude that iodine intake of our population was adequate.

### 4.2.6 Thyroid function during pregnancy

Pregnancy may affect the course of thyroid disorders and, conversely, thyroid diseases may affect the course of pregnancy. Moreover, thyroid disorders may affect both the pregnant woman and the developing fetus.

#### 4.2.6.1 Thyroid status

Mean TSH (µIU/ml) and FT4 (ng/dl) during first, second and third trimester were found to be 1.63, 1.82, 2.20 and 0.85, 0.75, 0.91 respectively. Mean TT4 (µg/dl) and TG (ng/ml) during first, second and third trimester were 10.87, 11.20, 12.12 and 7.04, 12.27, 23.37

respectively. Median values of thyroid hormones are presented in Table 4.2.10.

**Table 4.2.10: Mean and median thyroid hormone of pregnant women**

| Thyroid hormones | Trimester | Mean | 95% CI of Mean | Median | 95% CI of Median |
|---|---|---|---|---|---|
| TSH | First | 1.63* | 1.34-1.98 | 1.80 | 1.53-1.95 |
| (μIU/ml) | Second | 1.82* | 1.56-2.13 | 1.98 | 1.79-2.19 |
| N=73 | Third | 2.20* | 1.89-2.57 | 2.38 | 2.12-2.63 |
| FT4 | First | 0.85 | 0.81-0.89 | 0.85 | 0.80-0.89 |
| (ng/dl) | Second | 0.75 | 0.70-0.79 | 0.72 | 0.68-0.80 |
| N=73 | Third | 0.91 | 0.87-0.95 | 0.91 | 0.89-0.95 |
| TT4 | First | 10.87 | 10.05-11.68 | 10.74 | 9.80-11.70 |
| (μg/dl) | Second | 11.20 | 10.68-11.71 | 11.11 | 10.58-11.90 |
| N=73 | Third | 12.13 | 11.59-12.66 | 11.71 | 11.29-12.28 |
| TG | First | 7.04 | 4.26-9.82 | 3.80 | 2.59-6.98 |
| (ng/ml) | Second | 12.27 | 8.85-15.70 | 8.15 | 5.20-12.65 |
| N=73 | Third | 23.37 | 18.91-27.83 | 21.55 | 11.76-29.16 |

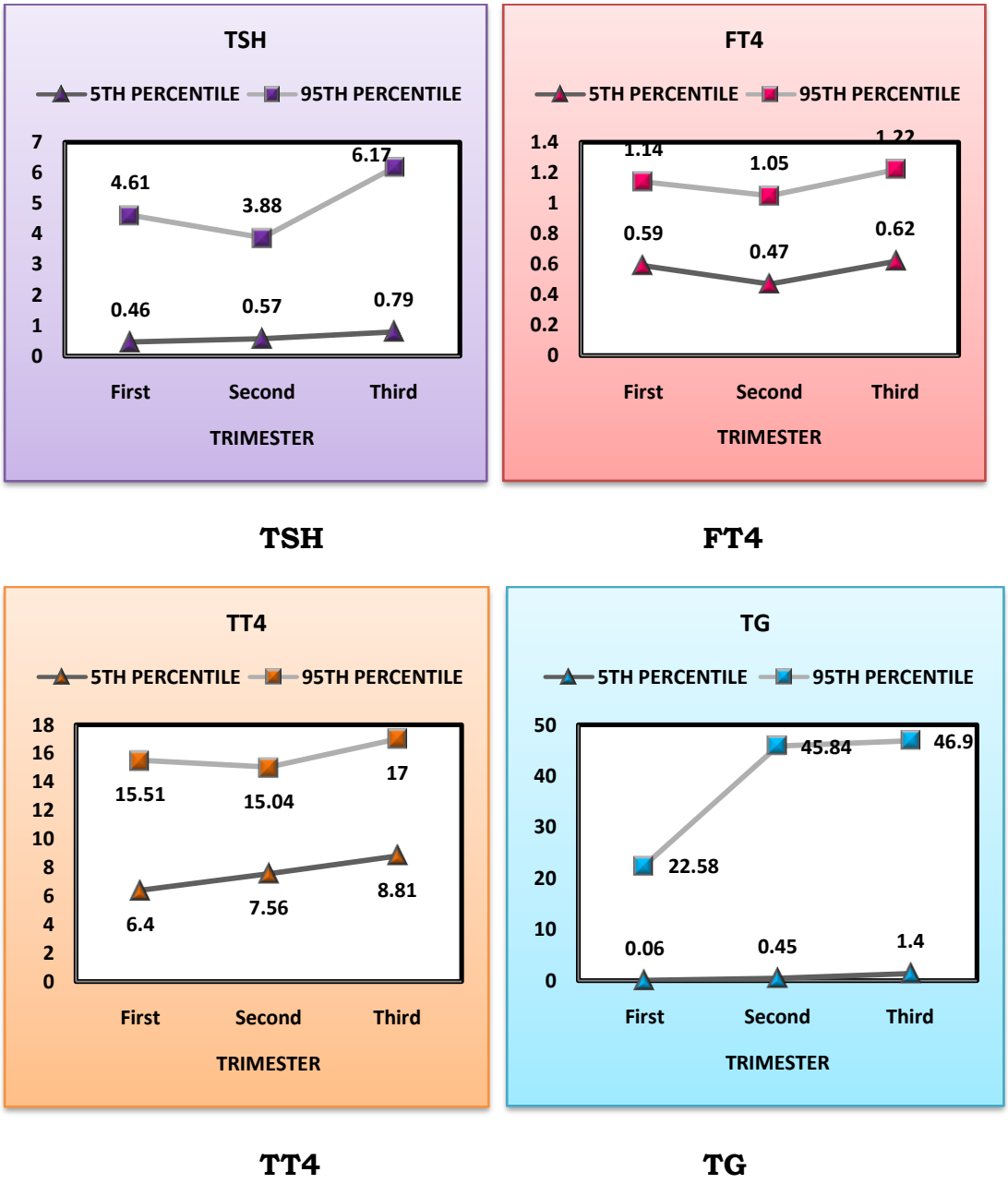
*geometric mean

Mean and median values for TSH, FT4, TT4 and TG were in normal range according to non pregnant adult reference range. The 5$^{th}$ and 95$^{th}$ percentile values of TSH, FT4, TT4 and TG are given in Figure 4.2.11. Maximum variation was found in TG followed by TSH and TT4. Much variation among FT4 values was not observed.

When compared, 5$^{th}$ percentile values of TSH, TT4 and TG, a similar trend was observed. We have observed a linear relationship between

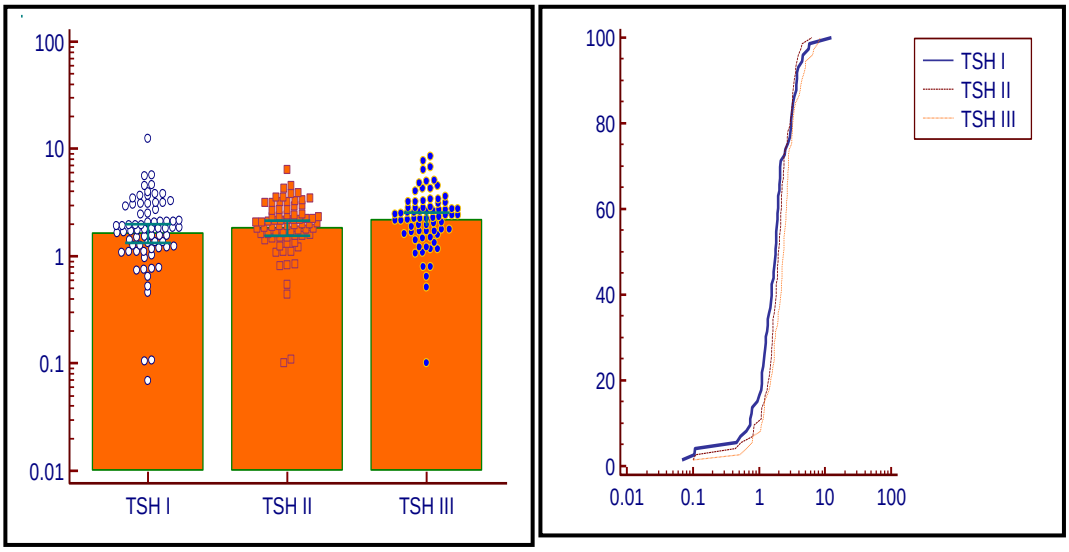
5th percentile values with advancing gestation (Figure 4.2.11). However, the 95th percentile value for these three parameters did not follow the same trend. In case of TSH, TT4 and FT4, firstly it decreased from first to second trimester and then increased from second to third trimester. Unlike TSH, TT4 and FT4, in case of TG the value increased form first to second trimester and then it remained more or less similar.

**Figure 4.2.11: 5th and 95th percentile values of thyroid hormones**



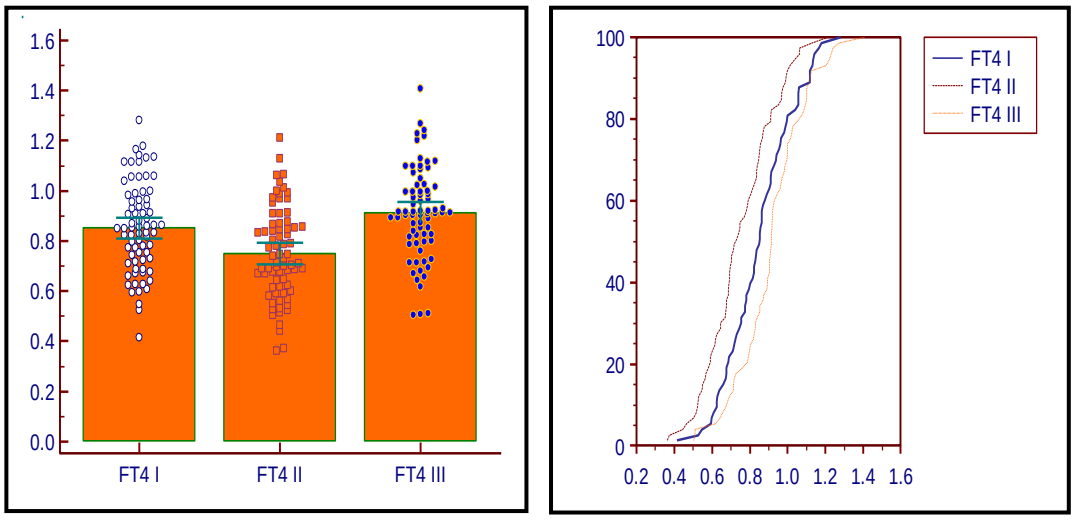
TSH and FT4 values of all pregnant women during first, second and third trimester can be seen in Figure 4.2.12 and 4.2.13 respectively. These figures are also helpful for outlier detection for trimester-specific reference interval generation. Cumulative frequency figure (right side) gives us the idea of trend in TSH and FT4 increase during each trimester.

**Figure 4.2.12: Box-plot and cumulative frequency of TSH**




TSH I, TSH II, TSH III denotes TSH during trimester

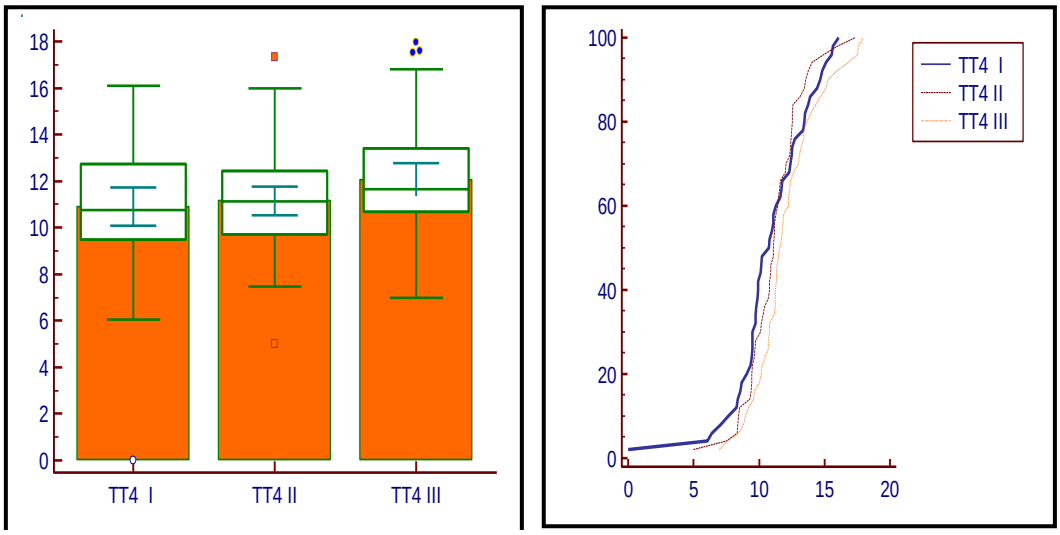
**Figure 4.2.13: Box-plot and cumulative frequency of FT4**




FT4 I, FT4 II, FT4 III denotes FT4 during trimester

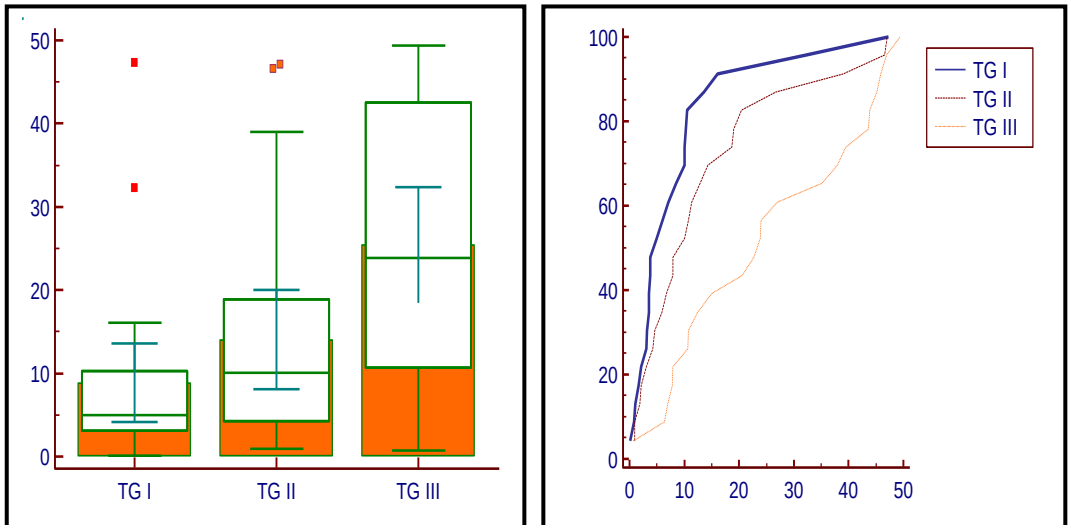
TT4 and TG values of all pregnant women during first, second and third trimester can be seen in Figure 4.2.14 and 4.2.15 respectively. Cumulative frequency figure (right side) gives us the idea of trend in TT4 and TG increase during each trimester. Variations were observed in TT4 and TG increase during each trimester.

**Figure 4.2.14: Box-plot and cumulative frequency of TT4**




TT4 I, TT4 II, TT4 III denotes TT4 during trimester

**Figure 4.2.15: Box-plot and cumulative frequency of TG**




TG I, TG II, TG III denote TG during each trimester

### 4.2.6.2 Thyroid dysfunction during each trimester

In present study we have defined overt hypothyroidism as pregnant women having TSH value >2.5 μIU/ml during first trimester and >3.0

μIU/ml during second and third trimester and FT4 value <0.65 ng/dl. Subclinical hypothyroidism was defined as pregnant women having TSH value >2.5 μIU/ml during first trimester and >3.0 μIU/ml during second and third trimester and FT4 value >0.65 ng/dl. Pregnant women with TSH value <2.5 μIU/ml during first trimester and <3.0 μIU/ml during second and third trimester and FT4 value <0.65 ng/dl were defined as hyothyroxinemic.

**Table 4.2.11: Thyroid dysfunction among pregnant women**

| Thyroid Status | Trimester | | |
|---|---|---|---|
| | I | II | III |
| Overt hypothyroidism | 10.96 (8) | 5.48 (4) | 1.37 (1) |
| Subclinical hypothyroidism | 17.81 (13) | 13.7 (10) | 24.66 (18) |
| Hypothyroxinemia | 4.11 (3) | 24.66 (18) | 5.48 (4) |
| Normal | 67.12 (49) | 56.16 (41) | 68.49 (50) |

Figure in parenthesis actual number

Prevalence of overt hypothyroidism, subclinical hypothyroidism and hypothyroxinemia during each trimester is given in Table 4.2.11 Figure 4.2.16 and 4.2.17 present distribution of TSH and FT4 during each trimester.

Thyroid dysfunction was found in 32.88%, 43.84% and 31.51% pregnant women during first, second and third trimester respectively. Prevalence of overt hypothyroidism, hypothyroxinemia and subclinical hypothyroidism was maximum during first, second and third trimester respectively. Prevalence of overt hypothyroidism decreased with advancing gestation. Subclinical hypothyroidism was minimum during second trimester and prevalence of hypothyroxinemia increased sharply from first to second trimester and then decreased sharply from second to third trimester.

**Figure 4.2.16: Distribution of TSH during each trimester**

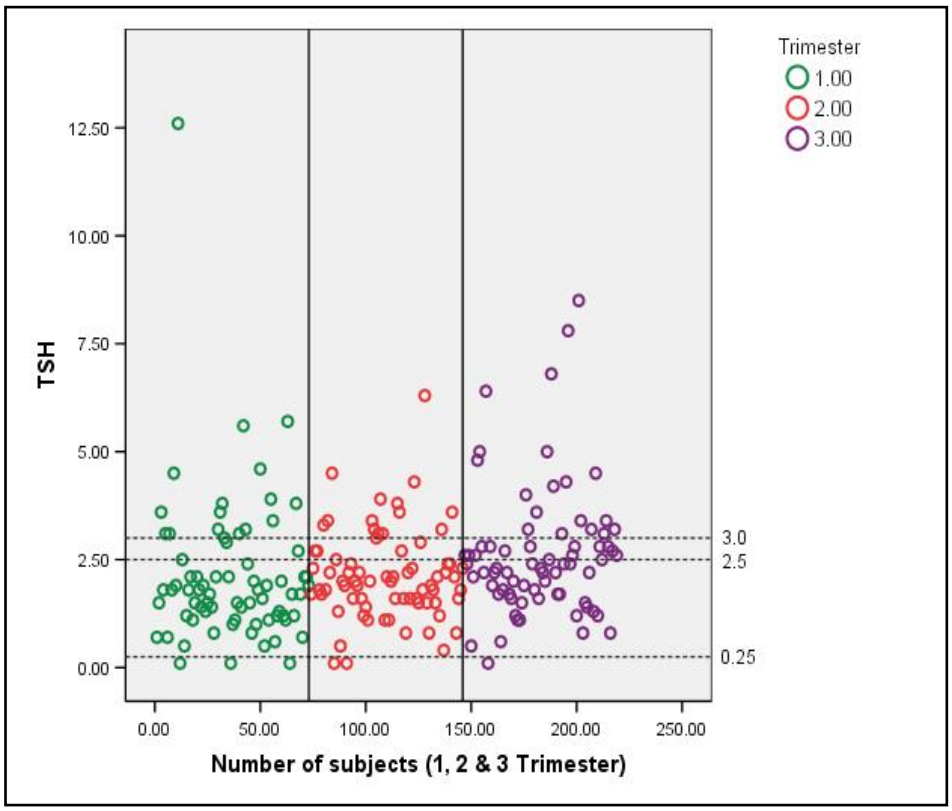
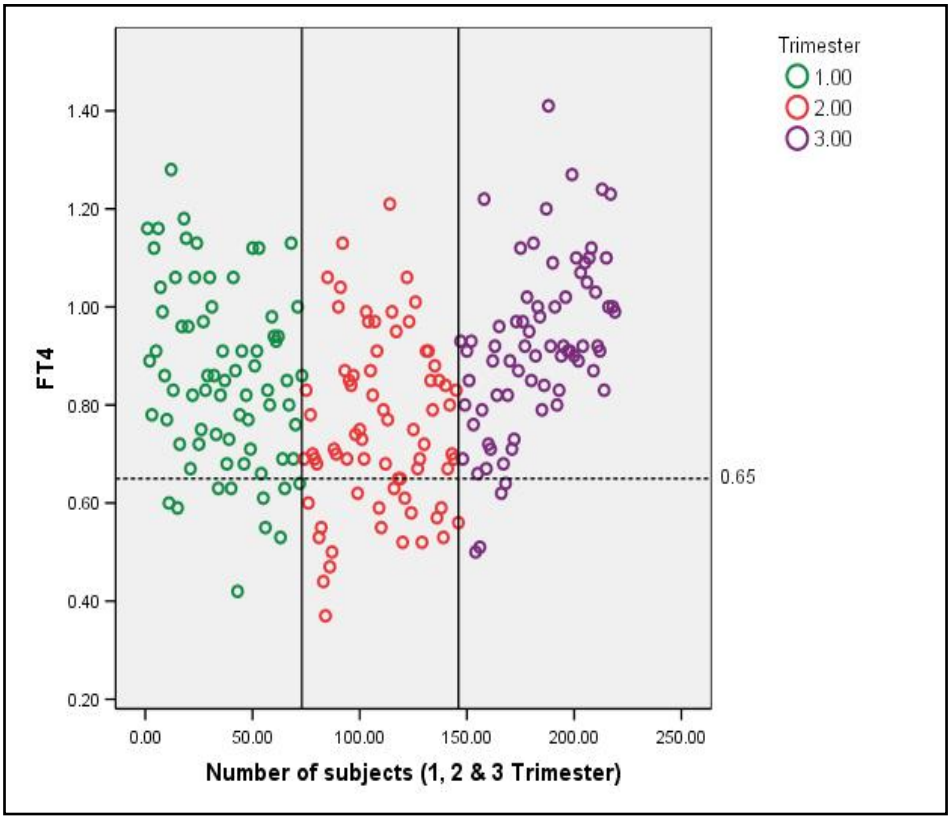



**Figure 4.2.17: Distribution of FT4 during each trimester**



#### 4.2.6.3 TSH AND FT4 DUIRNG PREGNANCY

Mean TSH was 1.63 μIU/ml during first trimester. The values were observed to rise through second trimester (11.6%) to a mean level of 1.82 μIU/ml, the levels then further increased in the third trimester (20.9%) to 2.2 μIU/ml. Analysis of TSH during each trimester showed significant differences (Table 4.2.12). Differences were primarily observed between second and third trimester and first and third trimester but not between first and second trimester (p=0.324). Trend analysis showed linear trend in TSH increase between each trimester (Figure 4.2.18).

Mean FT4 was found to be 0.85 ng/dl during first trimester. These seemed to decline through second trimester (11.8%) to a mean level of 0.75 ng/dl, the levels then increased in the third trimester (21.3%) to 0.91 ng/dl. Analysis of FT4 during each trimester also showed significant differences (Table 4.2.13). Differences were primarily seen between first and second trimester and second and third trimester but not between first and third trimester (p=0.08). Unlike TSH, in case of FT4, we have found quadratic trend (Figure 4.2.19).

Price et al (2001) studied thyroid function in pregnant and non pregnant Asian and Western Caucasian women. They have found TSH to increase from first to second trimester and FT4 decreased with advancing gestation. Kumar et al (2003) studied thyroid function in 124 pregnant women from India. Authors have reported an increase in TSH value with advancing gestation.

Soldin et al (2004) studied trimester-specific changes in thyroid hormones (Sweden). In their study, mean TSH increased with advancing gestation from first to second trimester, while it remained stable from second to third trimester. However, mean value of FT4

decreased by 15% from first to second trimester, while it remained stable from second to third trimester.

In 2008 Marwah et al have studied thyroid function of normal pregnant women from India. Analysis of TSH between each trimester did not show any significant difference in TSH values. However, FT4 was decreased significantly with advancing gestational age. In 2009, Mehdi et al studied maternal iodine status and thyroid function during pregnancy in Dhaka. They had studied thyroid hormones during first and third trimester only. Mean TSH was significantly increased (first trimester-2.0 and third trimester-3.10) and mean FT4 was significantly decreased (first trimester-12.3 and third trimester 9.6) with advancing gestation.

In 2010, Chakraborty et al studied iodine status of pregnant women in Kolkata. Unlike our results and results from other studies discussed before, TSH values (μIU/ml) in this study were found to be maximum during first trimester (4.94) and it decreased to 2.82 during second trimester ($p<0.05$). TSH value further declined to 1.72 from second to third trimester ($p<0.05$). FT4 values increased from first to second trimester ($p<0.05$) and then decreased from second to third trimester ($p<0.05$). The author's had mentioned that an initial increase in TSH and a decrease in FT4 may have resulted from a transient increase in TBG and a consequently lower FT4.

As discussed above, increase in TSH with advancing gestation as found in our study was observed in other studies also. Majority of authors have found a decline in FT4 with advancing gestation, however in our study we found dissimilar results. FT4 firstly decreased and then increased from second to third trimester. Mean FT4 was found to be maximum during third trimester.

Changes in FT4 concentrations during pregnancy have been controversial. Some authors had reported a decrease in FT4 (Boss and Kingstone 1979; Kurtz et al, 1979), whereas others had reported no change (Guillaume et al, 1985) or increase (Harada et al, 1979;Malkasian et al, 1970; Osathanondh et al, 1975) in FT4 concentration. Fantz et al (1999) mentioned that discrepancies in FT4 changes during pregnancy may have been attributable to the techniques used for free hormone measurement.

Another team (Roti et al, 1991) reported variability in serum free thyroid hormones in pregnant women at term among 10 commercially available methods. Albumin dependent methods gave 50% of subnormal values towards term, suggesting that such methods are unsuitable for use during pregnancy because of marked negative bias. Conversely, because of an increase in the pool of protein bound T4 during pregnancy, methods that require a high degree of sample dilution could be expected to show positive bias in relation to standard that contain a normal concentration of TBG.

Methods that are based on dialysis of free tracer to determine free fraction tend to overestimate free T4 in the presence of TBG excess, thus obscuring the normal decline in FT4 as pregnancy progresses. Regardless of the method, however, pregnant women, on an average had lower free thyroid hormone concentrations at term than non pregnant women.

Thus, thyroid function during pregnancy should be assessed using FT4 reference values that are both trimester-specific and method-specific. However, unlike TSH no recommendations on international basis have been given by endocrine societies on FT4 reference intervals that are both method and trimester specific.

**Figure 4.2.18: Mean difference in TSH during first, second and third trimester**

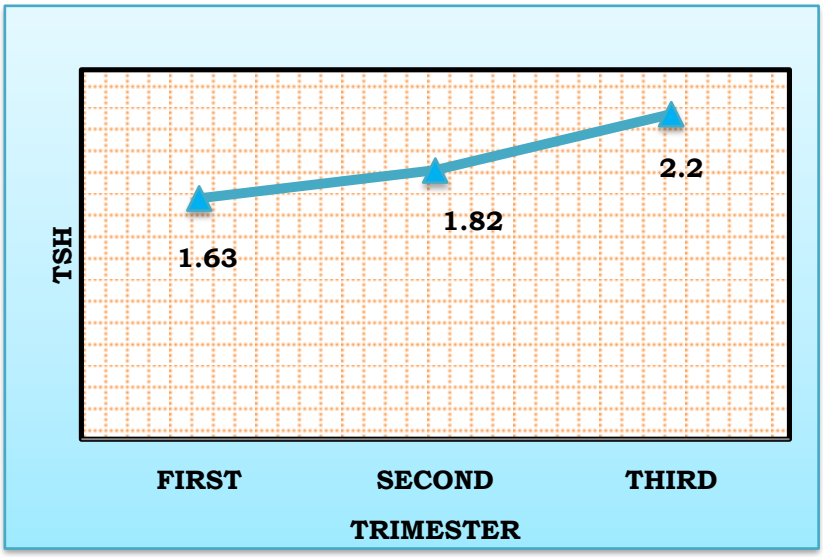



**Table 4.2.12: Repeated measures of ANOVA for logTSH**

| **Groups** | **F value** | **DF** | **P** |
|---|---|---|---|
| Trimester (1,2 & 3) | 10.41 | 2 | <0.001*** |
| | **Trend analysis** | | |
| **Trend** | **t value** | **DF** | **P** |
| Linear | 3.97 | 72 | 0.0002*** |
| **Factor** | **Geometric Mean** | **95% CI** | |
| TSH_I | 1.63 | 1.34-1.98 | |
| TSH_II | 1.82 | 1.56-2.13 | |
| TSH_III | 2.20 | 1.89-2.57 | |
| **Pair wise comparisons** | | | |
| **Factors** | **Geometric Mean** | **95% CI** | **P** |
| TSH_I TSH_II | 0.89 | 0.75-1.05 | 0.3242ns |
| TSH_II TSH_III | 0.82 | 0.72-0.94 | 0.0024** |
| TSH_III TSH_I | 1.35 | 1.12-1.63 | 0.0005*** |

**Figure 4.2.19: Mean difference in FT4 during first, second and third trimester**

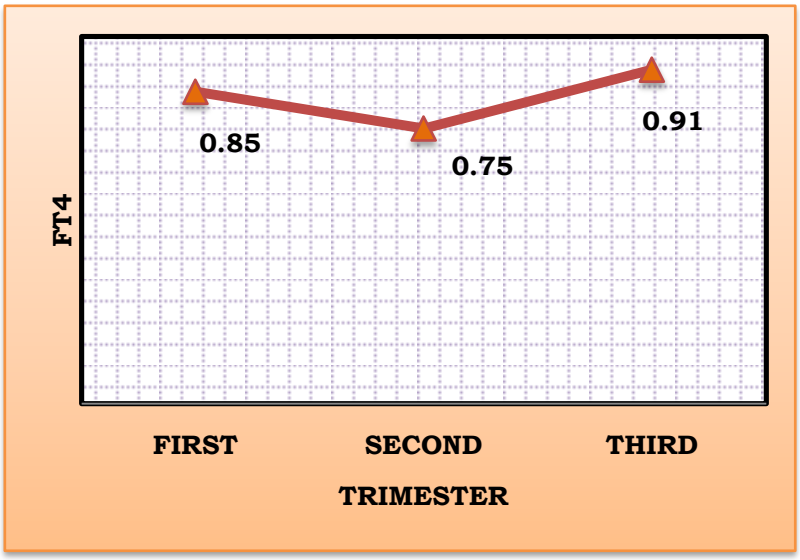



**Table 4.2.13: Repeated measures of ANOVA for FT4**

| **Groups** | | **F value** | **DF** | **P** |
|---|---|---|---|---|
| Trimester (1,2 & 3) | | 23.79 | 2 | <0.001*** |
| **Trend analysis** | | | | |
| **Trend** | | **t value** | **DF** | **P** |
| Quadratic | | 7.64 | 72 | < 0.0001**** |
| **Factor** | | **Mean** | **Std. Error** | **95% CI** |
| FT4_I | | 0.85 | 0.02 | 0.81-0.89 |
| FT4_II | | 0.75 | 0.02 | 0.70- 0.79 |
| FT4_III | | 0.91 | 0.021 | 0.87- 0.95 |
| **Pair wise comparisons** | | | | |
| **Factors** | | **Mean Difference** | **Std. Error** | **P** |
| FT4_I | FT4_II | 0.10 | 0.02 | 0.0001*** |
| FT4_II | FT4_III | -0.16 | 0.02 | <0.0001**** |
| FT4_III | FT4_I | 0.06 | 0.02 | $0.0820^{ns}$ |

### 4.2.6.4 TT4 AND TG DURING PREGNANCY

Mean TT4 was found to be 10.89 µg/dl during first trimester. These were seen to rise through second trimester (3%) to a mean level of 11.15 µg/dl. The levels then further increased in the third trimester (8.3%) to 12.05 µg/dl. Levels of TT4 increase markedly during the first trimester of pregnancy, it reaches a peak at 20 weeks and then high levels of TT4 are maintained through the second and third trimesters.

Analysis of TT4 between each trimester showed significant differences (Table 4.2.14). Differences were primarily seen between second and third trimester and first and third trimester but not between first and second trimester (p=1.000). Trend analysis showed linear trend in TT4 increase between each trimester (Figure 4.2.20). A similar finding is echoed in study of Erem et al (2001), who investigated maternal thyroid function in 51 pregnant women without goiter in Turkey.

Mean TG was 8.8 ng/ml during first trimester. These were seen to rise through second trimester (74.3%) to a mean level of 14.0 ng/ml and then further increased in the third trimester (90.5%) to 25.3 ng/dl. Although thyroglobulin lacks specific hormonal activity, it can indicate the activity status of injury to the thyroid gland (Spencer and Wang, 1995). TG is frequently increased during pregnancy, reflecting the increased activity of the gland during pregnancy (Glinoer, 1997).

Analysis of TG between each trimester showed significant differences in mean values (Table 4.2.15). Differences were primarily seen between first and second trimester and first and third trimester but not between second and third trimester (p=0.0698). Trend analysis showed linear trend in TG increase between each trimester (Figure 4.2.21). Similar to our study Glinoer (1997) reported that increase in TG can be seen as early as the first trimester, but it is more pronounced during latter part of pregnancy.

**Figure 4.2.20: Mean difference in TT4 during first, second and third trimester**

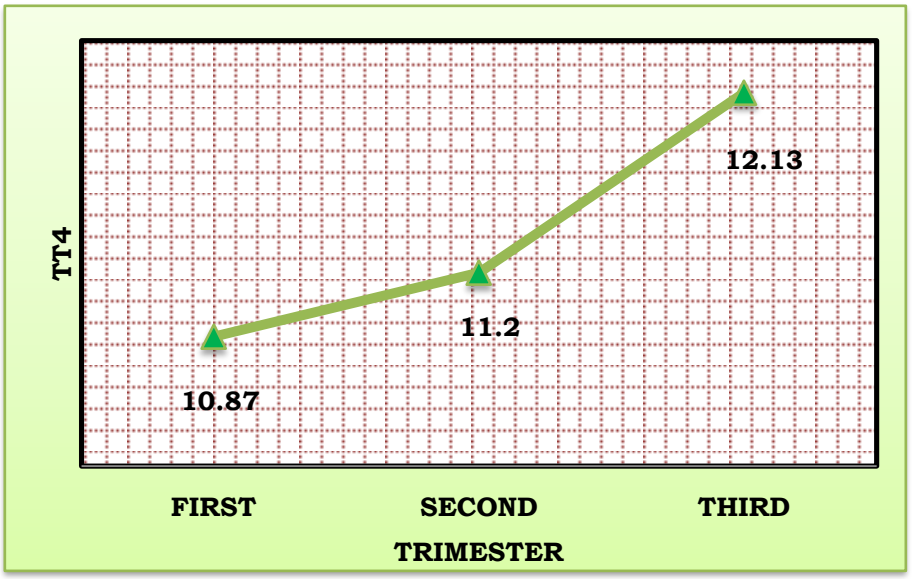



**Table 4.2.14: Repeated measures of ANOVA for TT4**

| **Groups** | **F value** | **DF** | **P** |
|---|---|---|---|
| Trimester (1,2 & 3) | 5.99 | 2 | 0.004** |
| | **Trend analysis** | | |
| **Trend** | **t value** | **DF** | **P** |
| Linear | 2.7775 | 72 | 0.0077** |
| **Factor** | **Mean** | **Std. Error** | **95% CI** |
| TT4__I | 10.87 | 0.41 | 10.06-11.72 |
| TT4_II | 11.20 | 0.30 | 10.54-11.77 |
| TT4_III | 12.13 | 0.35 | 11.35-12.76 |
| **Pair wise comparisons** | | | |
| **Factors** | **Mean Difference** | **Std. Error** | **P** |
| TT4__I TT4_II | -0.33 | 0.34 | 1.0000$^{ns}$ |
| TT4_II TT4_III | -0.93 | 0.27 | 0.0059** |
| TT4_III TT4__I | 1.26 | 0.41 | 0.0232* |

**Figure 4.2.21: Mean difference in TG during first, second and third trimester**

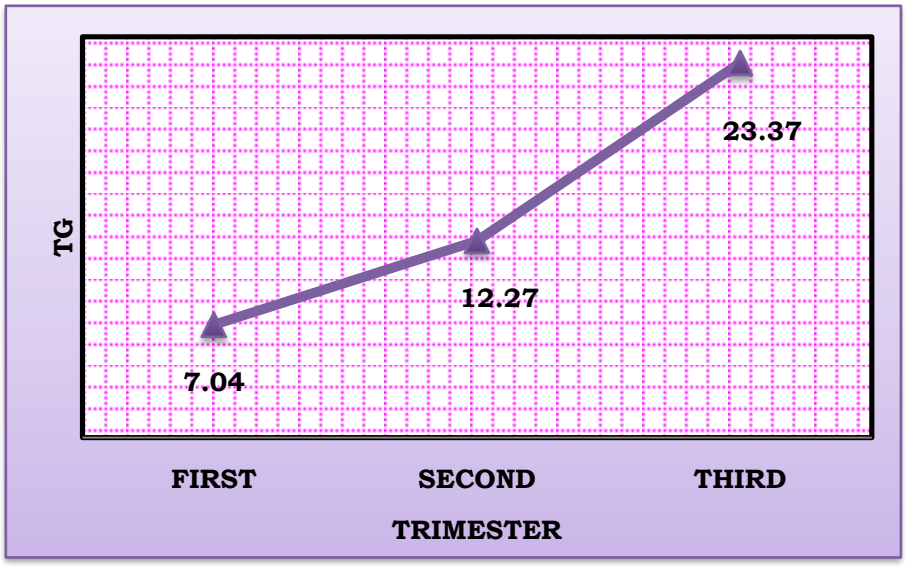



**Table 4.2.15: Repeated measures of ANOVA for TG**

| **Groups** | **F value** | **DF** | **P** |
|---|---|---|---|
| Trimester (1,2 & 3) | 10.11 | 2 | 0.002** |
| | **Trend analysis** | | |
| **Trend** | **t value** | **DF** | **P** |
| Linear | 2.10 | 72 | 0.0006*** |
| **Factor** | **Mean** | **Std. Error** | **95% CI** |
| TG_I | 7.04 | 2.27 | 4.13-13.55 |
| TG_II | 12.27 | 2.87 | 8.07-20.01 |
| TG_III | 23.37 | 3.33 | 18.46-32.32 |
| **Pair wise comparisons** | | | |
| **Factors** | **Mean Difference** | **Std. Error** | **P** |
| TG_I TG_II | -5.23 | 1.95 | 0.0423* |
| TG_II TG_III | -11.10 | 4.65 | $0.0698^{ns}$ |
| TG_III TG_I | 16.33 | 4.13 | 0.0018** |

### 4.2.7 Reference interval for TSH and FT4

Trimester specific reference intervals for TSH and FT4 were developed using Robust method (CLSI C28-A3 Medcal software), 95 % CI of mean, upper limit and lower limit. Before developing reference intervals, outliers were tested and outside and far out values were excluded.

**Table 4.2.16: Trimester specific reference intervals for TSH (μIU/ml) and FT4 (ng/dl)**

| Thyroid hormone | Trimester | N | Mean | Median | Lower limit | Upper limit |
|---|---|---|---|---|---|---|
| **TSH** | First | 67 | 1.79 | 1.81 | 0.59 | 5.48 |
| | Second | 68 | 2.02 | 2.04 | 0.93 | 4.52 |
| | Third | 68 | 2.31 | 2.40 | 0.92 | 5.82 |
| **FT4** | First | 73 | 0.85 | 0.85 | 0.49 | 1.20 |
| | Second | 73 | 0.75 | 0.72 | 0.37 | 1.11 |
| | Third | 71 | 0.91 | 0.91 | 0.58 | 1.25 |

Table 4.2.16 shows mean and reference intervals for TSH (μIU/ml) during first, second and third trimester were 1.79 (0.59-5.48), 2.02 (0.93-4.52) and 2.31 (0.92-5.82) respectively. For FT4 (ng/dl) mean and reference interval were 0.85(0.49-1.20, first trimester), 0.75 (0.37-1.11, second trimester) and 0.91 (0.58-1.25, third trimester). Our trimester specific reference intervals were different from normal range for non-pregnant adults.

In 2007 International Guidelines for management of hypothyroidism during pregnancy and postpartum were published (Albanovich et al,

2007), according to these guidelines TSH (μIU/ml) upper limit of 2.5 during first trimester and 3.0 during second and third trimester should be considered for diagnosing hypothyroidism. However, till now no consensus has been reached for FT4 (ng/dl) lower limit.

**Table 4.2.17: Comparison of trimester specific reference intervals**

| **Thyroid hormone** | **Trimester** | **Reference interval (present study)** | | **Reference interval (Marwah et al)** | | **Manufacturer's range** | |
|---|---|---|---|---|---|---|---|
| | | **Lower limit** | **Upper limit** | **Lower limit** | **Upper limit** | **Lower limit** | **Upper limit** |
| **TSH** | First | 0.59 | **5.48** | 0.6 | **5.0** | 0.25 | **5.10** |
| **(μIU/ml)** | Second | 0.93 | **4.52** | 044 | **5.78** | | |
| | Third | 0.92 | **5.82** | 0.74 | **5.7** | | |
| **FT4** | First | **0.49** | 1.20 | **0.93** | 1.51 | **0.65** | 2.10 |
| **(ng/dl)** | Second | **0.37** | 1.11 | **0.73** | 1.52 | | |
| | Third | **0.58** | 1.25 | **0.87** | 1.37 | | |

Marwah et al were the first group who came up with trimester specific reference intervals for Indian pregnant women in 2008. They had studied 331 pregnant women (107 in first trimester, 137 in second trimester and 87 in third trimester). Their trimester specific upper limit for TSH was close to our range; however their lower limit for FT4 was far from our range (Table 4.2.17). The reason for these differences could be the study type, method used for thyroid hormone analysis and method used for developing reference ranges. Marwah et al study was a cross-sectional study and our study was a longitudinal study.

ECLIA method was used in Marwah et al study and we have used RIA method.

Trimester specific reference intervals can be developed using 3 methods, 1) normal distribution method 2) percentile method and 3) robust method for small sample size. In present study, since sample size was small we have used robust method. In contrast, Marwah et al have developed their reference ranges using percentile method [5$^{th}$ (lower limit) and 95$^{th}$ Percentile (upper limit)].

Looking into the variation caused by these 3 different factors, we recommend that each laboratory dealing with samples of pregnant women must develop their own trimester specific reference intervals using large sample size and with same subjects. Since more and more researchers are aware of the importance of evaluating maternal thyroid function during pregnancy by gestation-specific reference intervals manufacturer's reference range should not be used for pregnant women. If a non pregnant reference interval is used, a number of pregnant women with thyroid dysfunction could be potentially misclassified.

In 2007 Stricker et al have reported that 5.6-18.3% of misdiagnoses and missed diagnoses likely occur in clinical practice due to the use of non-pregnant reference values as basis for diagnosis. In China (Shan et al, 2009), Malaysia (Thevarajah et al, 2009) and Australia (Gilbert et al, 2008), the percentage of potentially misclassified cases of subclinical hypothyroidism and hypothyroxinemia in pregnant women was decreased by using trimester-specific reference ranges.

Many other authors from different parts of World have developed trimester specific reference intervals (refer ROL page 66 to 70). We

have observed vide variation in these reference intervals also, apart from 3 factors which we have mentioned other factors also contribute to variation in thyroid hormone reference intervals. These are storage of thyroid hormones, race and ethnic variation, dietary habits, presence and absence of iodine deficiency, data transformation (log or square root), sample size etc.

Distribution of pregnant women, according to 3 different reference intervals in shown in Figure 4.2.22 (present study reference interval), 4.2.23 (international guidelines for TSH during pregnancy) 4.2.24 (Marwah et al reference intervals for Indian pregnant women) and Table 4.2.17.

When we categorized subjects according to 3 different methods, we found that during first trimester, if we use method 2 then 27.4% subjects will have increased TSH and if we use method 1 and 3 only 4.1% subjects will have raised TSH. Similarly if we use method 3 then 68.5% subjects will have decreased FT4 and if we use method 2 then 13.7% and with method 1 only 1.4% subjects will have decreased FT4.

During second trimester, if we use method 2 then 19.2% subjects will reflect increased TSH and if we use method 1 and 3 only 4.1% subjects will have raised TSH. Similarly if we use method 3 then 50.1% subjects will have decreased FT4 and if we use method 2 then 30.1% and with method 1 only 1.4% subjects will have decreased FT4.

During third trimester, if we use method 2 then 26% subjects will have increased TSH and if we use method 1 and 3 only 5.5% subjects will have raised TSH. Similarly if we use method 3 then 33% subjects will have decreased FT4 and if we use method 2 then 6.8% and with method 1 only 4.1% subjects will have decreased FT4 (Table 4.2.18).

**Table 4.2.18: Distribution of subjects according to 3 different reference intervals**

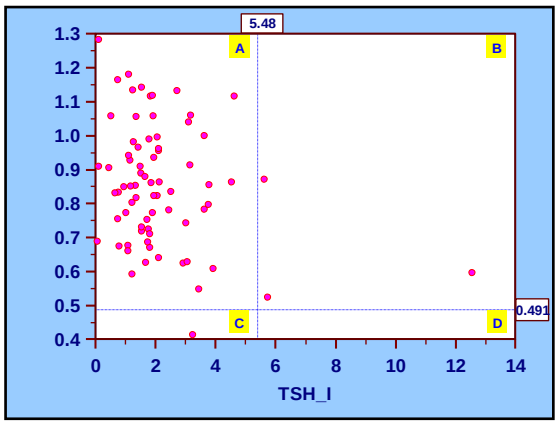
| Trimester | Method 1* | | Method 2** | | Method 3*** | |
|---|---|---|---|---|---|---|
| | TSH >upper limit | FT4 <lower limit | TSH >upper limit | FT4 <lower limit | TSH >upper limit | FT4 <lower Limit |
| **First** | 4.1% (3) | 1.4% (1) | 27.4% (20) | 13.7% (10) | 4.1% (3) | 68.5% (50) |
| **Second** | 1.4% (1) | 1.4% (1) | 19.2% (14) | 30.1% (22) | 1.4% (1) | 50.1% (37) |
| **Third** | 5.5% (4) | 4.1% (3) | 26% (19) | 6.8% (5) | 5.5% (4) | 33% (24) |

*present study reference interval, **international guidelines (reduced TSH upper limit and FT4 kit value)***reference interval for Indian women

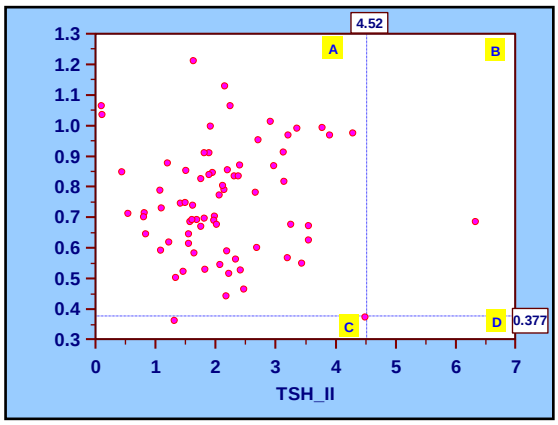
Figure in parenthesis denote actual number

From these figures it is concluded that, using different trimester-specific reference interval will result in different prevalence of thyroid dysfunction. Hence, choosing a right method is very important. Our reference intervals were self-sequential longitudinal reference intervals. In 2011 Wang et al have assessed thyroid function of pregnant Chinese women using self-sequential longitudinal reference intervals. They have screened 1,744 pregnant women with 3 different reference intervals: 1) self-sequential longitudinal reference intervals 2) Gestation-specific reference interval 3) non pregnant reference range. After comparing the results and pregnancy outcome, Authors have found that self-sequential longitudinal reference intervals had the best clinical specificity among the three reference intervals. Use of self-sequential longitudinal reference intervals can decrease the percentage of misclassification of thyroid dysfunction.

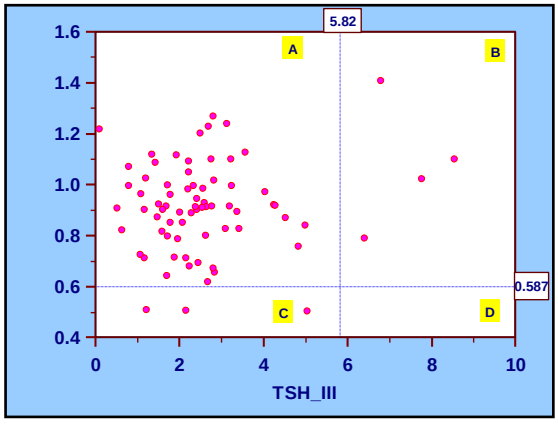
**Figure 4.2.22: Distribution of pregnant women according to trimester-specific reference internal of present study (method 1)**




**First trimester**



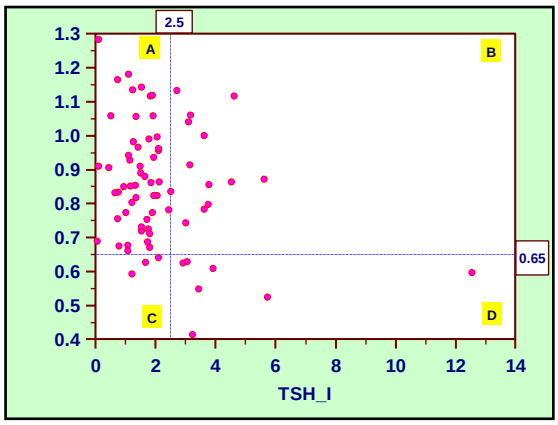

**Second trimester**



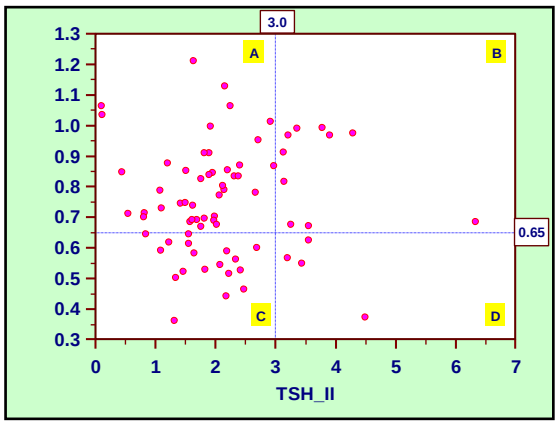

**Third trimester**

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (µIU/ml) upper limit

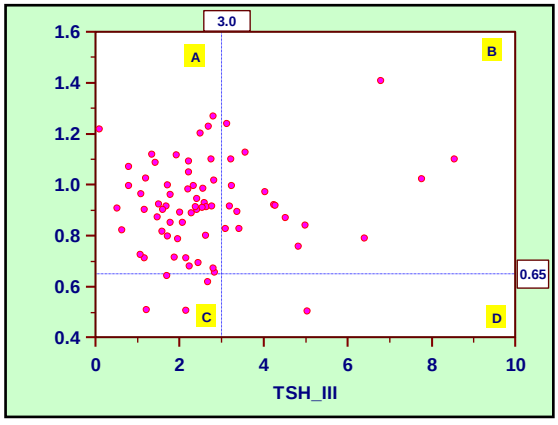
**Figure 4.2.23: Distribution of pregnant women according to International guidelines (method 2) (Albanovich et al, 2007)**




**First trimester**



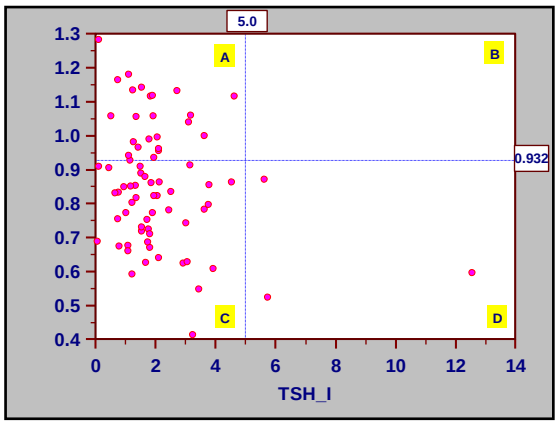

**Second trimester**



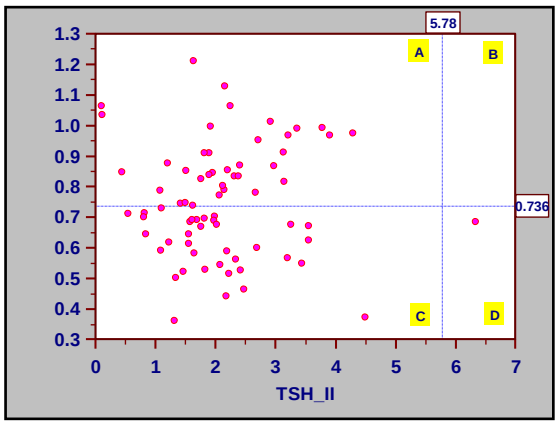

**Third trimester**

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (μIU/ml) upper limit

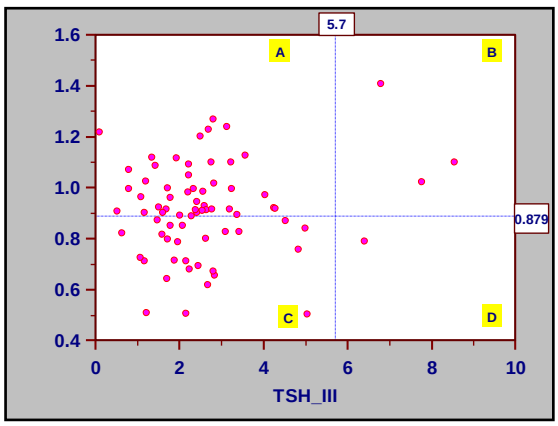
**Figure 4.2.24: Distribution of pregnant women according to trimester-specific reference interval (method 3) [Marwah et al, 2007]**




**First trimester**




**Second trimester**




**Third trimester**

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (μIU/ml) upper limit

### 4.2.8 Correlation and regression analysis

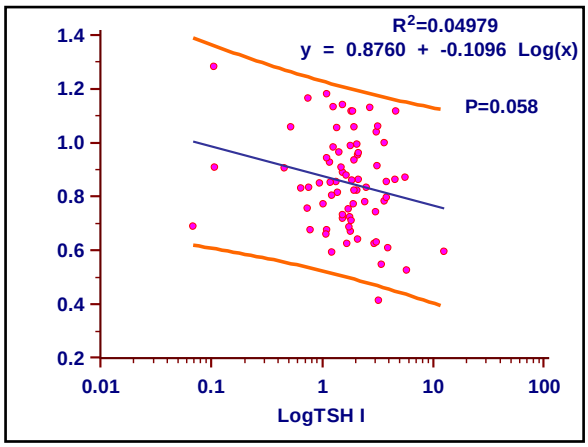
When correlation analysis was performed, we found positive correlation between per capita income and energy intake (r=0.2, p=0.012) and between gestation week and weight gain (r=0.3, p=0.008). A significant positive correlation was found between first trimester TSH and second trimester TSH (rho=0.77, p<0.0001), second trimester TSH and third trimester TSH (rho=0.677, p<0.0001) and between third trimester TSH and first trimester TSH (rho=0.665, p<0.0001). In case of FT4 a significant correlation was found between first and second trimester (r=0.388, p<0.0001) and between second and third trimester (r=0.520, p<0.0001) but not between third and first trimester (r=0.156, p=0.187).

We did not find any significant correlation between TSH, FT4, TT4 and UI, in contrast to other studies. One possibility is that our subjects were too homogenous regarding the presence and severity of iodine deficiency to show such a correlation. Correlation of thyroid hormones is shown in Figure 4.2.25 (TSH and FT4) and 4.2.26 (TSH and TT4). Log TSH had negative (non significant) correlation with FT4 and TT4 during each trimester. Correlation coefficient between log TSH and FT4 was found to be -0.22 during first trimester, -0.12 during second trimester and -0.008 during third trimester. Between log TSH and TT4, correlation coefficient during first, second and third trimester was -0.25, -0.012 and -0.091 respectively (Figure 4.2.26). A negative non significant association was found between TSH and urinary iodine during first (rho=-0.179, p=0.129), second (rho=-0.119, p=0.317) and third trimester (rho=-0.037, p=0.757).

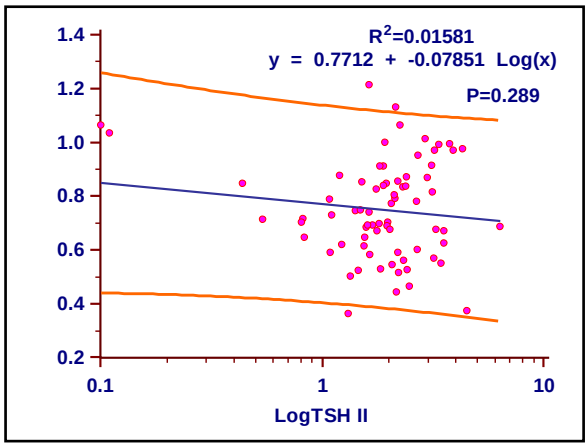
Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism (Hess et al, 2002). The mechanism by which iron status influences thyroid and iodine metabolism is unclear. IDA could impair thyroid metabolism through anemia and lowered oxygen transport (Surks et al, 1969; Galton 1972). When we correlated TSH with HB, we did not find any significant association (Figure 4.2.27).

**Figure 4.2.25: Correlation (regression line) between TSH and FT4 during each trimester**

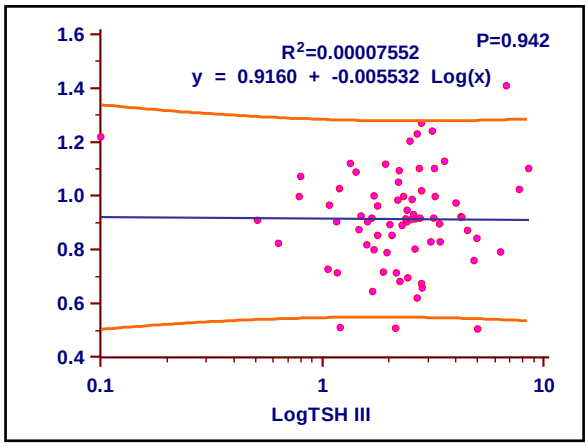
[Orange line denotes 95% prediction interval]




**a) First Trimester**



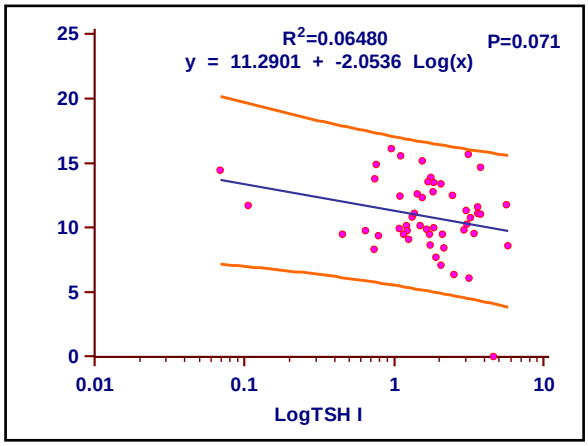

**b) Second Trimester**



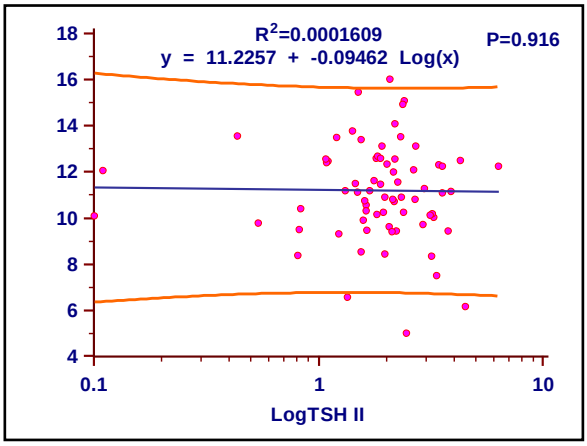

**c) Third Trimester**

**Figure 4.2.26: Correlation (regression line) between TSH and TT4 during each trimester**

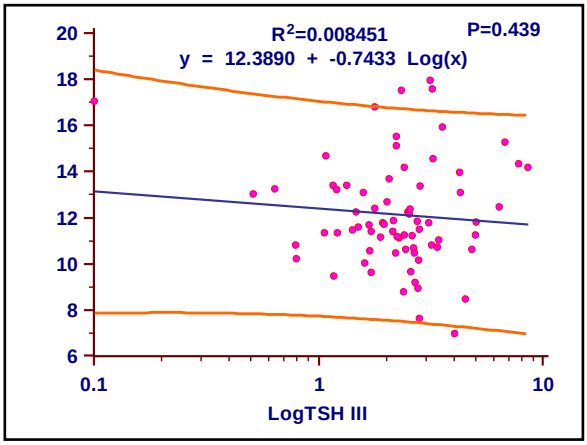
[Orange line denotes 95% prediction interval]




**a) First Trimester**



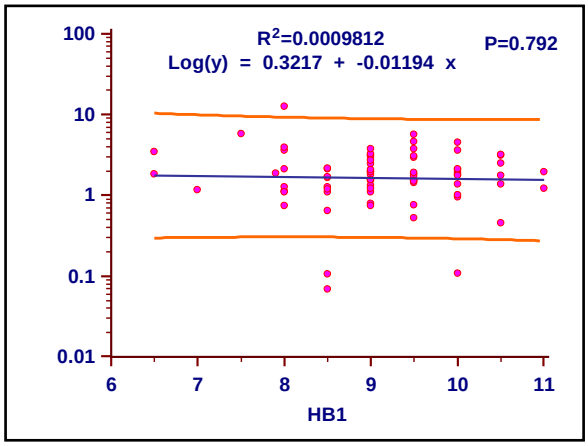

**b) Second trimester**



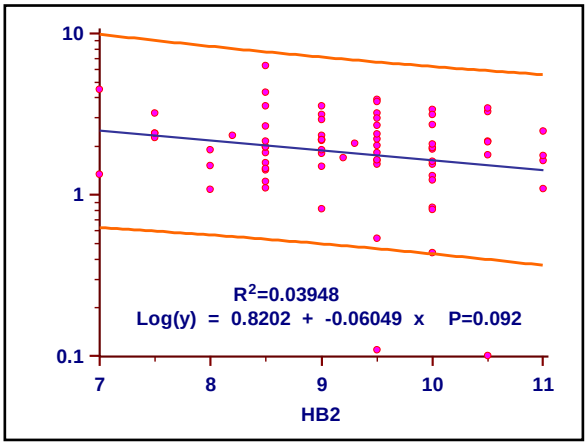

**c) Third trimester**

**Figure 4.2.27: Correlation (regression line) between TSH and HB during each trimester**

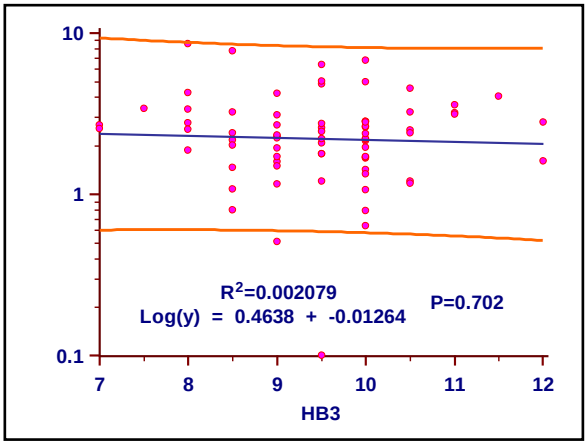
[Orange line denotes 95% prediction interval]




**a) First trimester**




**b) Second trimester**




**c) Third trimester**

### 4.2.9 Knowledge, Attitude and Practices

For improving Knowledge, Attitude and Practices (KAP) of pregnant women; Nutrition Health Education (NHE) was given to pregnant women during first trimester and pre data was collected. During second trimester reinforcement was carried out and post data was collected in third trimester.

Knowledge of pregnant women regarding critical role of iodine and iron during pregnancy was poor before intervention (Table 4.2.19). Percentage of women who have ever heard about iodized salt (IS) and IFA was 39.7% and 28.2% respectively. NHE has shown improvement in knowledge, attitude and practices of pregnant women. Percentage of women who could recognize IS increased by 62.8 % after providing NHE (Figure 4.2.28).

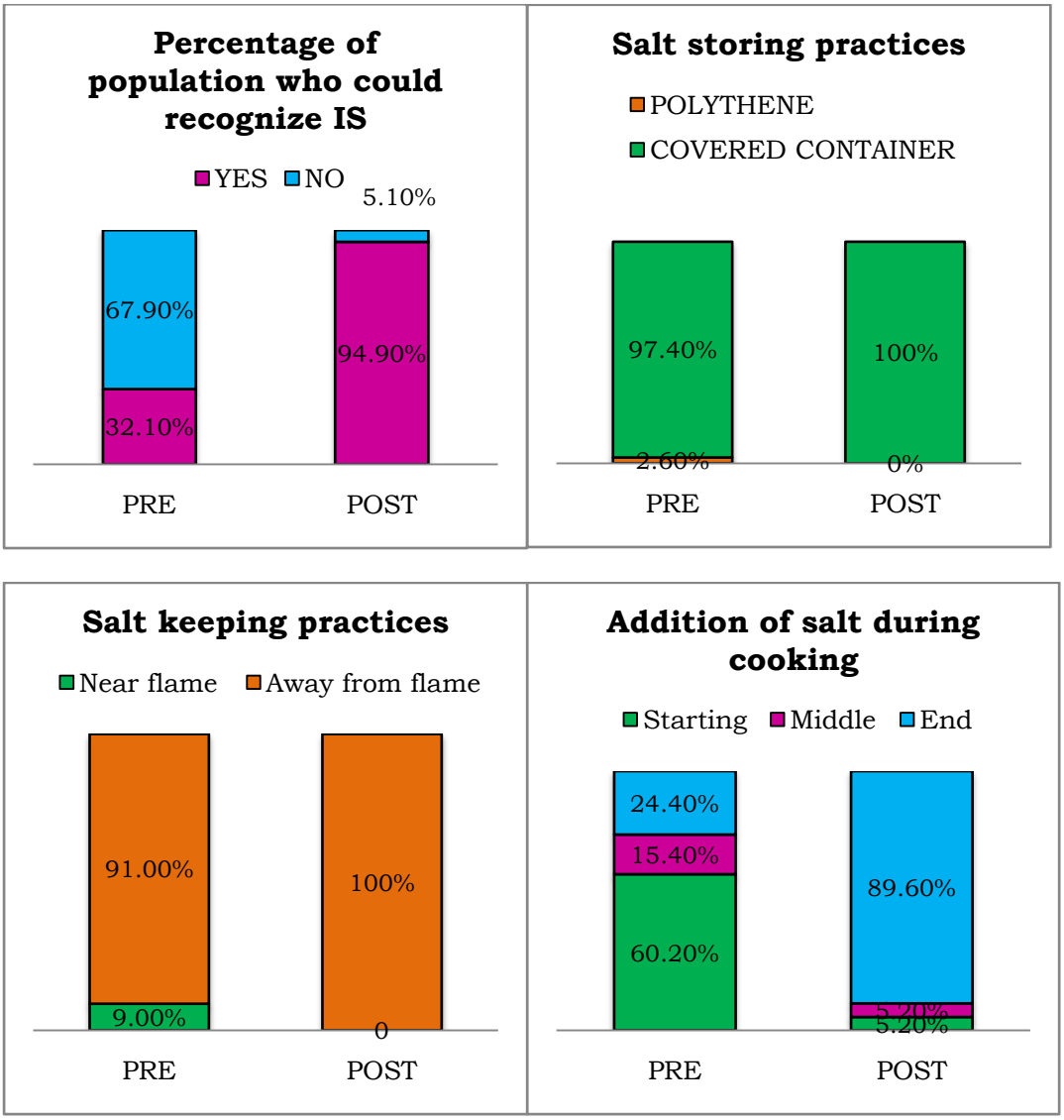
**Table 4.2.19: Knowledge of pregnant women regarding iodine and iron**

| Knowledge about | Response |
|---|---|
| **Iodine -** Percentage of population who have heard about IS | 39.7 % |
| **Iron-** Percentage of population who have heard about IFA | 28.2% |

According to Rana et al (2009) cooking losses of iodized salt ranges from 6.58- 51.08%. In order to get maximum iodine from our salt, it is necessary to take care of few small but important things like- proper storage of iodized salt, healthy cooking practices etc. A marked improvement was found in salt keeping practices of pregnant women. After intervention, everyone has started keeping iodized salt away from flame. Salt storing practices of the population were fair, and became good after NHE (Figure 4.2.28). A favourable change was observed in practices of pregnant women after NHE on addition of salt

during cooking. We observed that better cooking practices were followed by mothers like- adding salt after 75 % cooking is done, closing the lid while cooking etc. These small changes in their practices of storing, keeping and cooking iodized salt will definitely increase the iodine content of their diet.

**Figure 4.2.28: Outcome of intervention (NHE) on Knowledge and Practices of population**



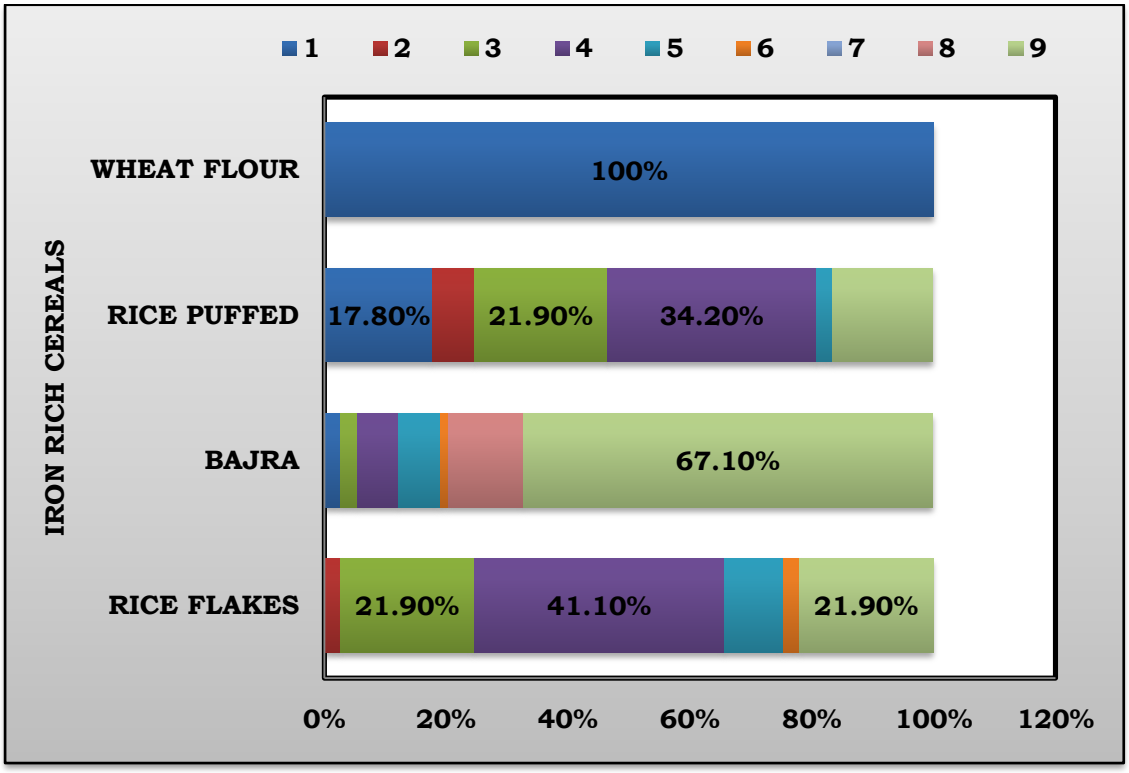

Since most of our subjects were educated till primary level (66%) only, our intervention in the form of NHE could help them improve their iodine and iron status. After receiving NHE, all pregnant women

became curious regarding brand name of their iodized salt. Earlier only few (19%) women were aware of the brand name of salt which they were consuming at home. Remembering brand name will definitely not increase the iodine content but it is an indicator of knowledge of pregnant women regarding importance of iodized salt.

### 4.2.10 Food Frequency

Data on consumption of iron rich foods, vitamin C rich foods and non vegetarian food items was obtained from pregnant women. In all frequency distribution figures legend were used, where 1 stands for daily, 2 specify three-four times a week, 3 stands for twice a week, 4 stands for weekly, 5 indicate bimonthly, 6 indicate monthly,7 means seasonally,8 means occasionally and 9 stands for never.

**Figure 4.2.29: Frequency of consumption of iron rich cereals**




Data on iron rich foods was obtained using a list of iron rich cereals, legumes and pulses, vegetables and iron rich fruits.

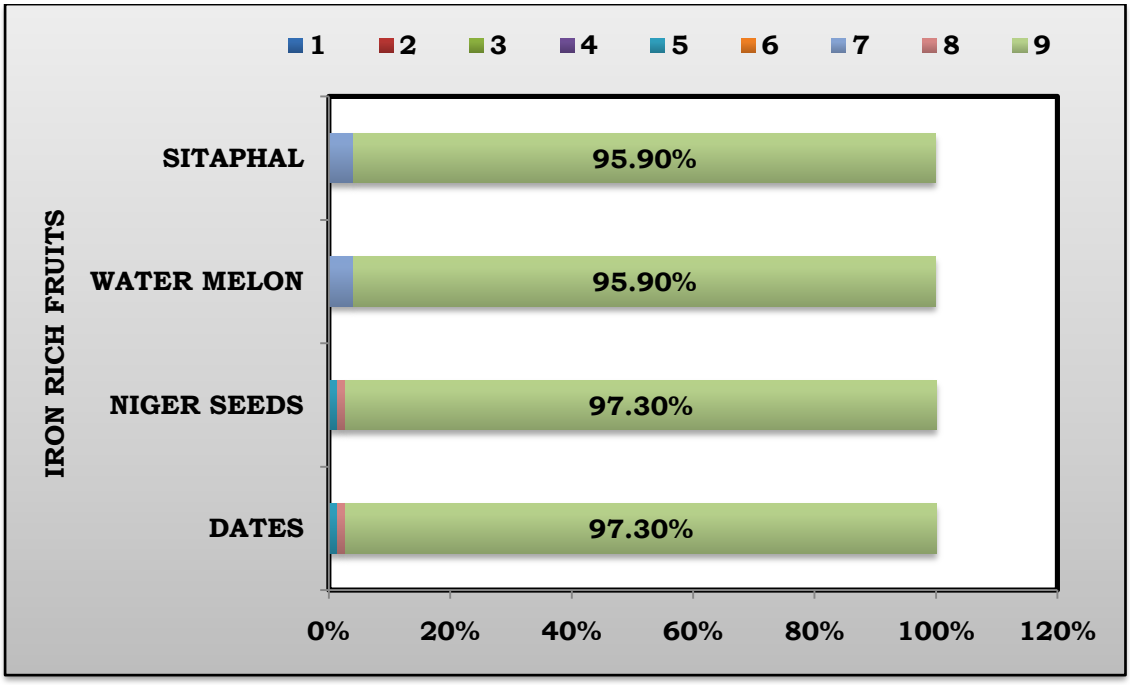
**Iron rich cereals**

Among the four cereals picked up, rice flakes contains maximum iron followed by bajra, puffed rich and wheat. Frequency distribution data reveals that 41.1% subjects consumed rice flakes once a week, 67.1% subjects never consumed bajra, 21.9% subjects consumed puffed rich twice a week while 34.2% subjects consumed it once in a week, and wheat flour was consumed daily by all the subjects (Figure 4.2.29).

**Iron rich fruits**

Among iron rich fruits that were selected, dates contain maximum iron followed by niger seeds, water melon and sitaphal. Though dates and niger seeds contain more iron than watermelon and sitaphal but amount of dates and niger seeds consumed daily by any person is generally less than the other two. Hence we can say that on an average these four food items will by and large provide same amount of daily iron. Frequency distribution reveals that only few subjects were consuming these iron rich fruits (Figure 4.2.30).

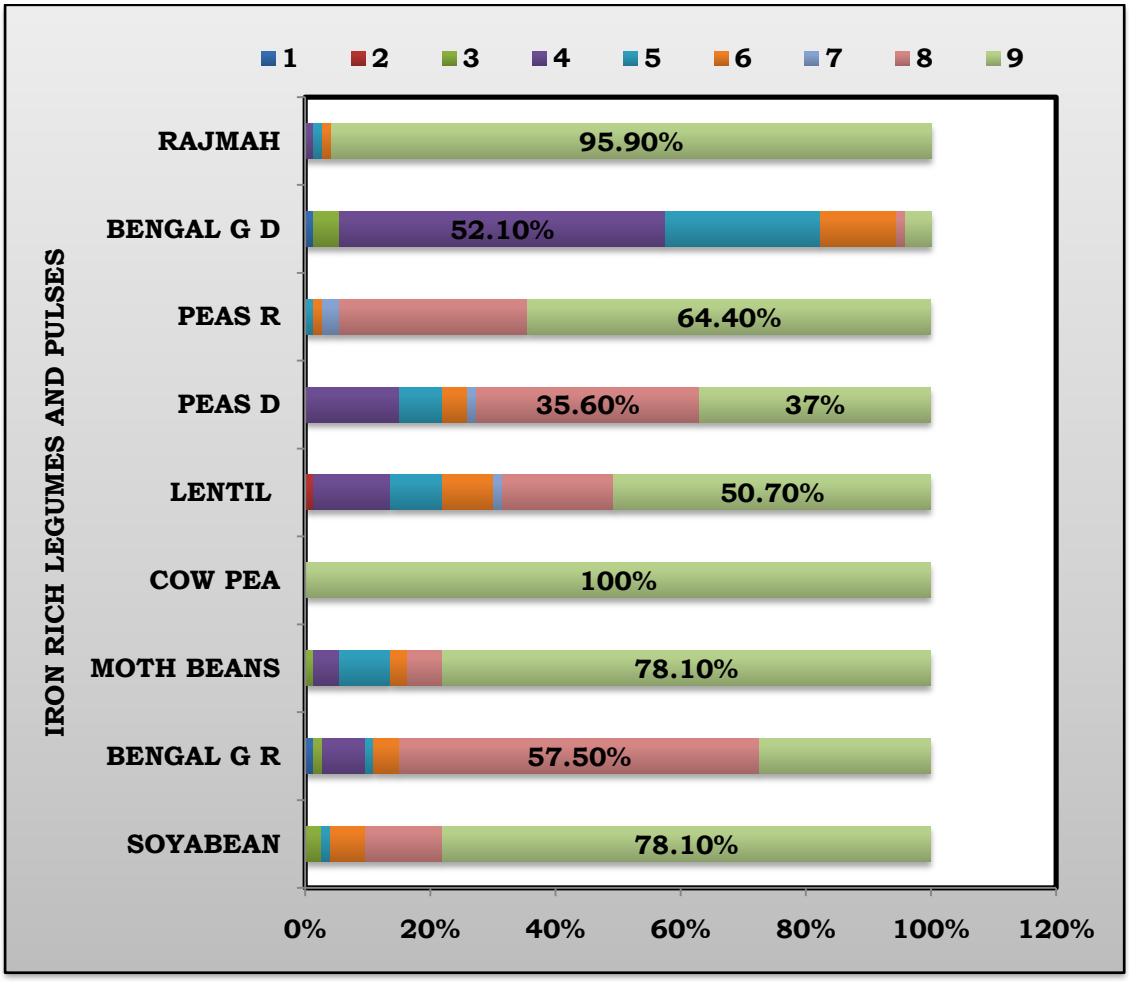
**Figure 4.2.30: Frequency of consumption of iron rich fruits**



**Iron rich legumes and pulses**

Among iron rich legumes and pulses, soyabean contained maximum iron followed by bengal gram roasted, moth beans, cow pea, lentil, peas dry, peas roasted, bengal gram dhal and rajmah. Soyabean and moth beans were never consumed by 78.1% subjects, bengal gram roasted and peas dry were occasionally consumed by 57.5% and 35.6% subjects respectively. Lentil, peas dry and peas roasted were never consumed by 50.7%, 37% and 64.4% subjects respectively. Bengal gram dhal was weekly consumed by almost half of the subjects. Rajmah was found to be the least consumed pulse, while none of the subjects consumed cow pea (Figure 4.2.31).

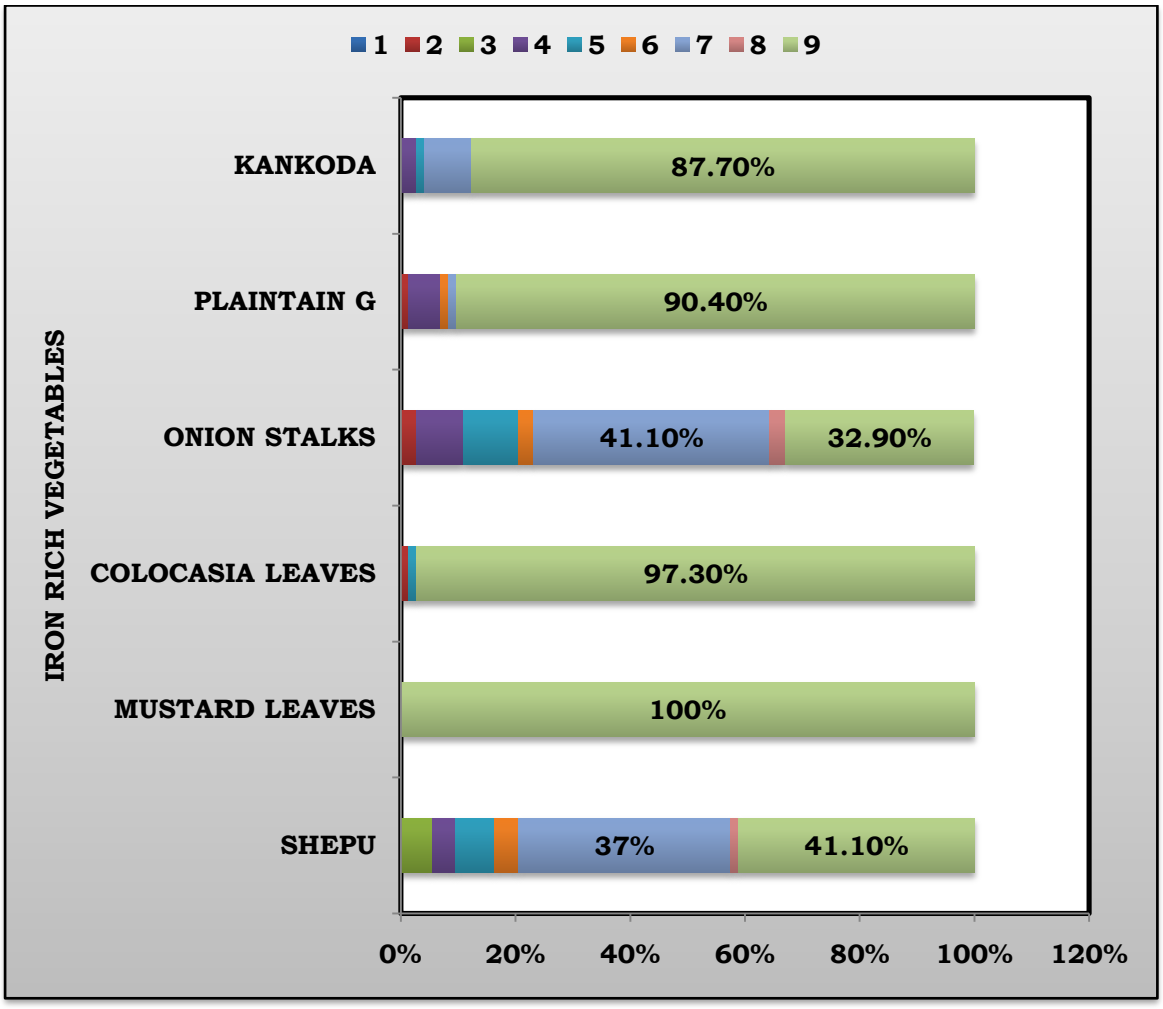
**Figure 4.2.31: Frequency of consumption of iron rich legumes and pulses**



**Iron rich vegetables**

Iron rich vegetables that were included in the list were, shepu with maximum iron content followed by mustard leaves, colocasia leaves, amaranth leaves, onion stalks, plantain green and kankoda. Shepu was seasonally consumed by 37% subjects, while 41.1% subjects never consumed it. Onion stalks were never consumed by 32.9% subjects, while 41.1% subjects consumed it seasonally. None of the subjects consumed mustard leaves. Vegetables like kankoda, plantain green and colocasia leaves were also never consumed by most of the subjects (Figure 4.2.32).

**Figure 4.2.32: Frequency of consumption of iron rich vegetables**



**Vitamin C rich foods**

It is a proven fact that vitamin C rich foods in the diet are enhancers of iron absorption, especially for those people who depend on vegetarian food items to fulfill their iron requirements.

Data on consumption of vitamin C rich fruits was obtained using a list of vitamin C rich fruits. Among the seven fruits which were included in the list, amla has maximum vitamin C followed by guava, sweet lime, pineapple, lemon, orange and tomato ripe. Ripe tomatoes were daily consumed by all the subjects. Like tomatoes, lemon was also consumed daily by most of the subjects, while fruits like amla and pineaaple were never consumed by 64.4% and 80.8% of the subjects respectively. Oranges, sweet lime and guavas were seasonally consumed by most of the subjects (Figure 4.2.33).

**Figure 4.2.33: Frequency of consumption of Vitamin C rich foods**

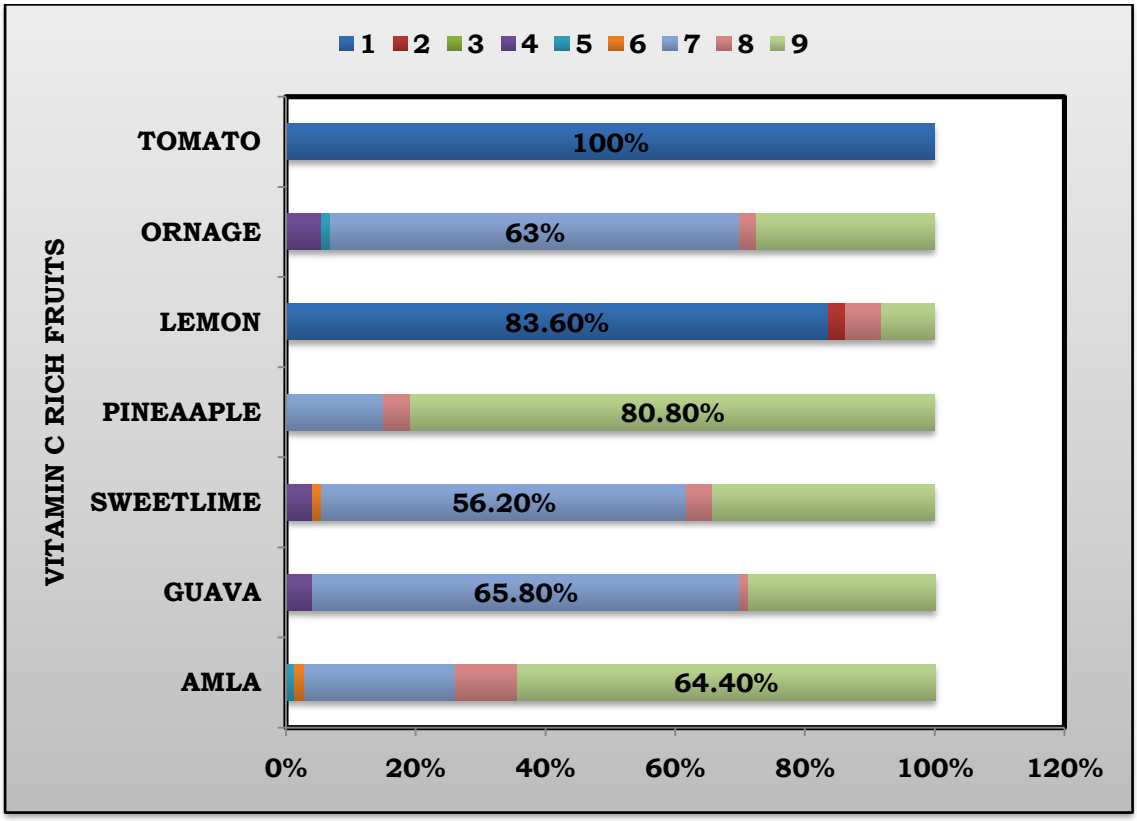
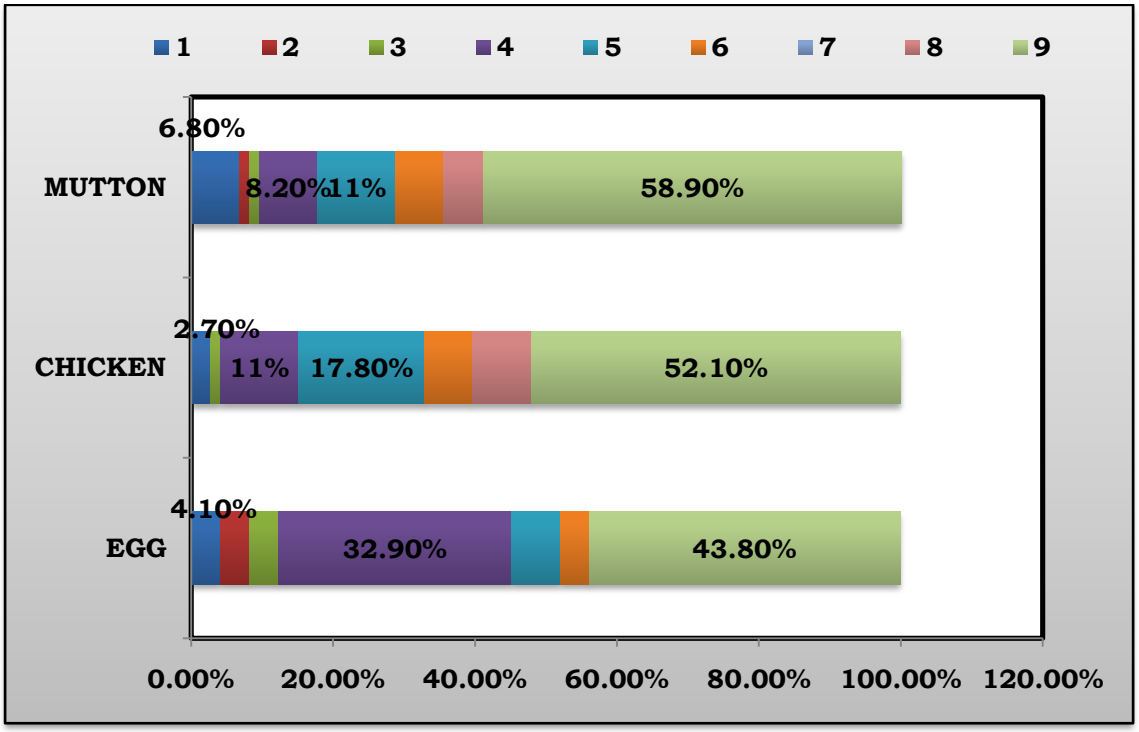


**Figure 4.2.34: Frequency of consumption of non vegetarian food items**




**Non vegetarian food items**

For obtaining data on frequency of consumption of non vegetarian food items- egg, chicken and mutton were used. Eggs, chicken and mutton were daily consumed by only few of the subjects. Percentage of subjects who consumed eggs, chicken and mutton weekly was 32.9%, 17.8% and 8.2% respectively (Figure 4.2.34). Many subjects (33%) reported that during pregnancy they have left non vegetarian food items. Reason given was they feel like vomiting due to smell of non vegetarian food. Percentage of subjects who were never consuming eggs, chicken and mutton was 43.8%, 52.15 and 58.9% respectively. Chicken was bimonthly consumed by 17.8% of subjects and mutton by 11%.

From the above discussed results it can be concluded that consumption of iron rich foods, vitamin C rich fruits and non vegetarian food items were not appreciable. Poor dietary intake of bio-available iron can result in anemia during pregnancy and lactation.

Dietary requirements of pregnant and lactating women are greater as compared to requirements of women during any other stage of life. However, In India it is observed that diet of pregnant and lactating women more or less remains the same as it was before pregnancy. Respite giving nutrition health education to pregnant women on importance of extra nutrients for delivering a healthy baby and keeping herself healthy, a marked improvement in their dietary habits were not observed. There is a need to further motivate pregnant women (LIG) to improve their dietary habits. This can be achieved by distributing food items rich in calories, proteins and minerals to pregnant and lactating women. In Vadodara, Gujarat Government is providing energy dense foods to pregnant and lactating women in the form of *sukhdi, upma* and *sheera* under NRHM (National Rural Health Mission) programme. There is a need to ensure that pregnant and lactating women are receiving these three food items. After they receive these food items again we must ensure that these items are being consumed by pregnant and lactating women. Often it happens that these foods are also consumed by other family members.

### 4.2.11 Maternal health and child care

Pregnancy and the first year of life are critical periods for human health. Maternal and child health are closely linked and the results are greatest when interventions are combined as packages that address the period before and during pregnancy, through birth and the neonatal stage, and then through early childhood (up to five years of age). Coverage of effective health interventions varies greatly within and between countries and across the continuum. It is highest for interventions that can be scheduled (e.g. antenatal care and immunization), but lower for interventions dependent on 24-hour service availability (such as skilled attendance at birth and care for sick newborns or children) and for behavioral and social change.

**Maternal health indicators**

Table 4.2.20 summaries the health status of pregnant women who participated in present study. Percentage of anemia (first trimester) in our subjects was found to be very high as compared to state data. However, prevalence of anemia was reduced with advancing gestation, indicating improvement in mean hemoglobin values due to IFA consumption.

**Table 4.2.20: Maternal health indicators**

| Indicator | Present study Vadodara | NFHS3 Gujarat | NFHS2 Gujarat | NFHS1 Gujarat |
|---|---|---|---|---|
| Pregnant women with anemia | 92% | 61% | 47% | NA |
| Three antenatal checkups | 100% | 65% | 61% | 61% |
| Institutional deliveries | 97.3% | 55% | 46% | 36% |
| Deliveries conducted by health personnel | 97.3% | 65% | 53% | 43% |
| Mothers received postnatal care within 2 days of delivery | 97.3% | 54% | NA | NA |

For all four indicators except anemia better status was observed as compared to State data from NHFS 1, 2 and 3. In achieving these near to three digit figures for four maternal health indicators, NHE has played an important role. During their first visit to hospital (booking visit) we explained all components of MAMTA CARD to pregnant women.

**Performance indicators for maternal health services**

Coverage of antenatal services (Tetanus toxoid injection, completing three antenatal checkups, received IFA tablets) was more than 90 %.

**Table 4.2.21: Performance indicators for maternal health services**

| Indicator | Present study | NFHS3 | NFHS2 | NFHS1 |
|---|---|---|---|---|
| **Coverage of antenatal services** | | | | |
| Tetanus toxoid injection (2 or more) | 100% | 80% | 73% | 63% |
| Completed 3 antenatal care visits(with abdominal examination and BP checkup) | 97% | 65% | 60% | 61% |
| Received IFA tablets | 94% | 82% | 78% | 69% |
| **Place of delivery** | | | | |
| Institutional delivery | 94% | 55% | 46% | 36% |
| Domiciliary delivery | 6% | 45% | 54% | 64% |
| **Institutional deliveries** | | | | |
| Government | 45% | 14% | 11% | 15% |
| NGO/trust | NA | 2% | 3% | NA |
| Private | 55% | 37% | 32% | 20% |
| **Type of delivery** | | | | |
| Vaginal delivery | 82.2% | 91.1% | 91.5% | 97% |
| Caesarean section | 17.8% | 8.9% | 8.5% | 3% |
| **Assistance during delivery** | | | | |
| Doctor | 17.4% | 52% | 37% | 29% |
| ANM/nurse/midwife/LHV | 76.6% | 11% | 16% | 14% |
| Dai | 6% | 37% | 46% | 47% |

Percentage of institutional delivery was 94% and domiciliary delivery was 6%. Among institutional deliveries 45% were performed at government hospitals and 55% were at private hospitals. Table 4.2.21

gives us the comparison of performance indicators for maternal health services between present study and NFHS 1, 2 and 3. Of the above mentioned three performance indicators better results were observed in our study as compared to NFHS data.

Among type of deliveries, cases of caesarean section were more in our study compared to NFHS data (Table 4.2.21). When we compared our data with NFHS data on percentage of deliveries assisted by doctors we found that the percentage was less in our study. In our study 76.6% deliveries were assisted by nurses.

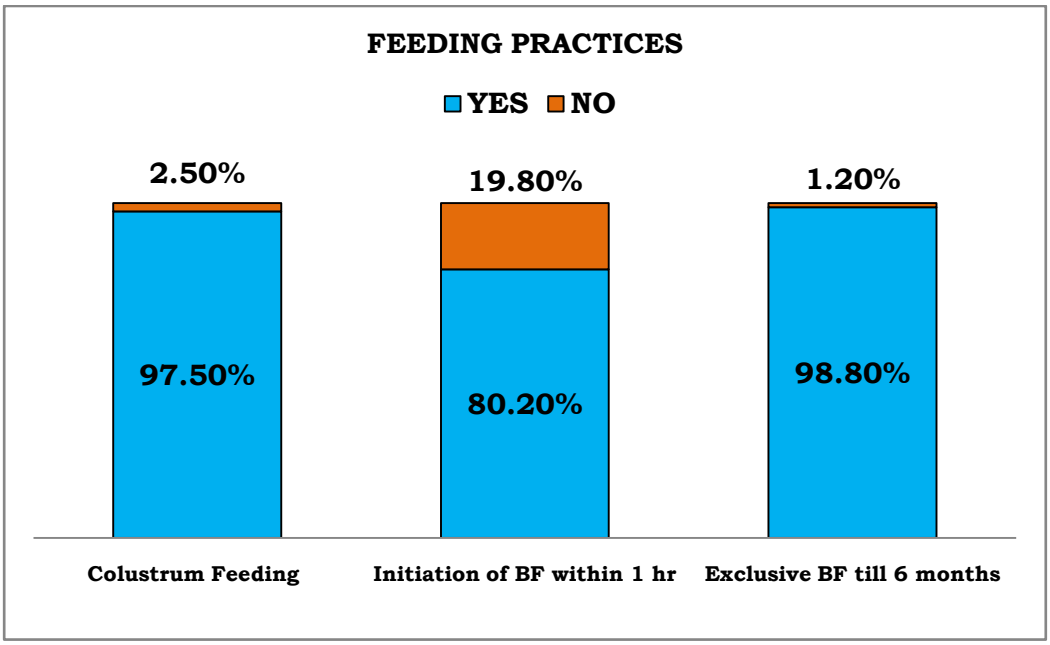
**Child care indicators**

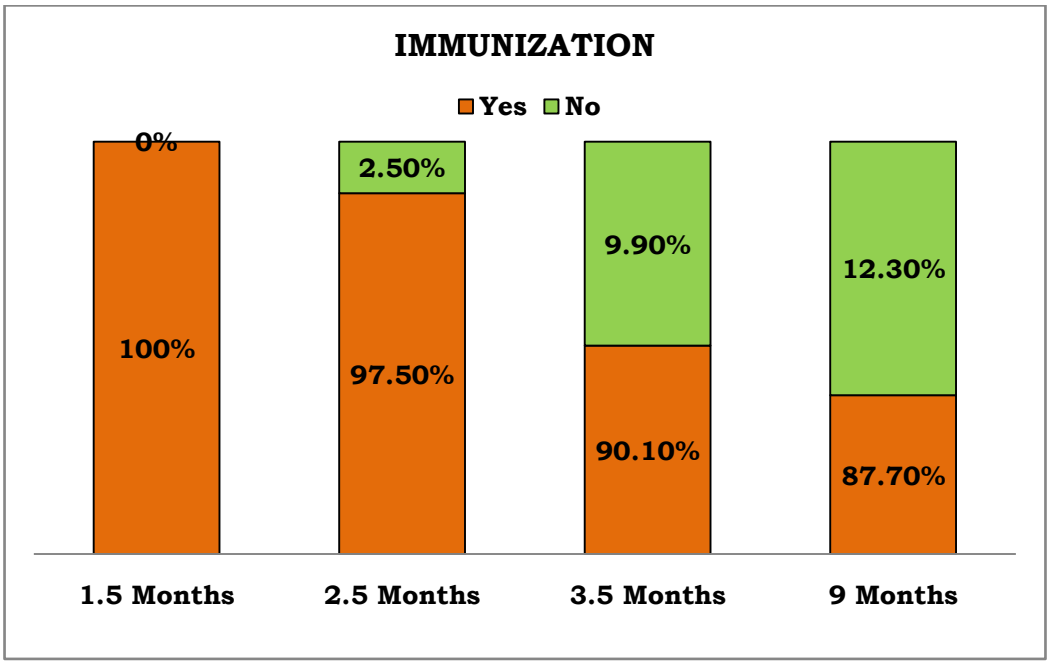
Colostrum feeding was done by 97.5% of mothers and the rest who did not feed the child responded that their relatives (elder females) had asked them not to give this yellow milk to child. Initiation of breast feeding within one hour was missed by 19.8% mothers (Figure 4.2.35). These mothers gave the reason that due to caesarean delivery they were not able to feed the child during first hour. Exclusive breast feeding till six months was done by 98.8% mothers. Respite giving counseling to these mothers, 1.2% mothers introduced water and biscuits to their child during fifth month.

Data on availing immunization services for child reveals that, during first immunization period (1.5 months) all mothers got their child fully immunized. As time progressed mothers became careless in availing immunization services for their child. During second (2.5 month), third (3.5 month) and fourth (9 month) period of immunization percentage of mothers who got their child immunized was 97.5%, 90.1% and 87.7% respectively. When asked the reason for delay in immunization, mothers reported that they often forget taking MAMTA CRAD with them during immunization and hence the nurse refuses to

immunize the child. Few mothers (3 subjects) reported that their elder children have torn the MAMTA CARD so she was not able to produce that during immunization. These reasons reflect that few mothers were careless regarding immunization of their child.

**Figure 4.2.35: Child care indicators**




[1.5 months-BCG, Polio 1, DPT 1, Hepatitis B-1; 2.5 months- Polio 2, DPT 2, Hepatitis B-2; 3.5 months-Polio 3, DPT 3, Hepatitis B-3 and 9 months-Measles, Vitamin A]

# *RESULTS*

# Phase III

Screening of neonates

### 4.3.1 Characteristics of neonates

Out of the 73 pregnant women, 60 had normal delivery and remaining 13 had a cesarean section (Table 4.3.1). Among neonates percentage of females (58.9%) were more compared to males (41.1%). Mean gestational age at birth was found to be 35.57 (2.3) weeks. Mean birth weight, birth length and head circumference at birth were 2.81 (0.4) kg, 47.59 (2.6) cm and 32.82 (1.2) cm respectively (Table 4.3.2).

**Table 4.3.1: Characteristics of neonates**

| Characteristics | | Percentage |
|---|---|---|
| **Type of delivery** | Normal | 82.2% (60) |
| | Cesarean | 17.8% (13) |
| **Gender of neonate** | Female | 58.9% (43) |
| | Male | 41.1% (30) |

Figure in parenthesis denote number of subjects

**Table 4.3.2: Gestational age at birth and Anthropometric measurements of neonates**

| Variable | N | Mean (sd) |
|---|---|---|
| Gestational age at birth (weeks) | 73 | 35.57 (2.3) |
| Birth weight (kg) | 73 | 2.81 (0.4) |
| Birth length (cm) | 39 | 47.59 (2.6) |
| Head circumference at birth (cm) | 39 | 32.82 (1.2) |

When we compared birth weight of males with that of females, a significant difference of 0.23 kg was observed. Male neonates had a mean weight of 2.9 kg while female neonates had 2.7 kg (Figure 4.3.1). Similarly when we compared birth weight of neonates which were born with normal delivery with those born with cesarean section, a difference of 0.2 kg was found (non significant). Mean birth weight was more in neonates born with cesarean section (2.9 kg) compared to neonates born with normal delivery (2.7 kg) (Figure 4.3.2).

**Figure 4.3.1: Mean birth weight of male and female neonates**

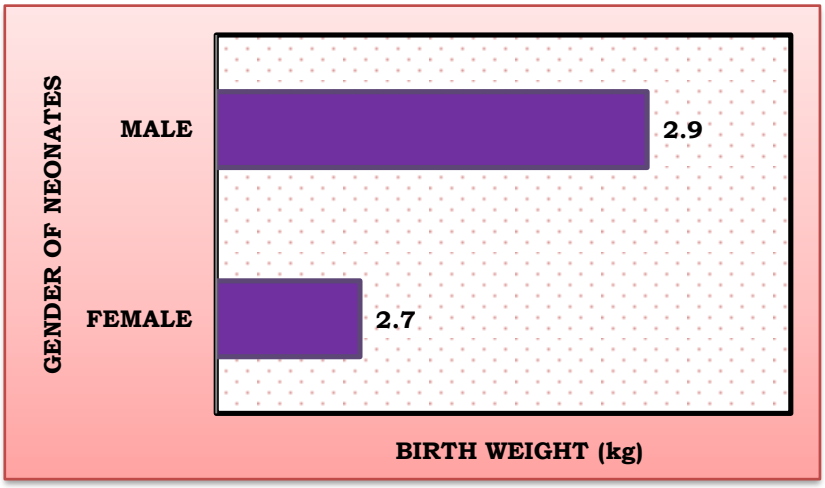



**Figure 4.3.2: Mean birth weight of neonates born with normal delivery and with cesarean section**

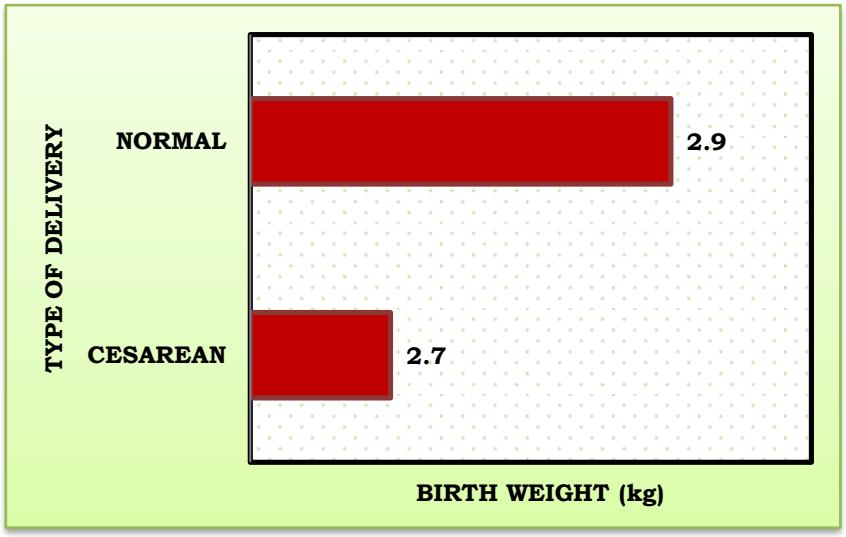



**Table 4.3.3: Bio-chemical profile of mothers during pregnancy**

| Parameter | I trimester | II trimester | III trimester |
|---|---|---|---|
| Hb (g/dl) | 9.18 | 9.31 | 9.0 |
| TSH (µIU/ml) | 2.41 | 2.33 | 2.66 |
| FT4 (ng/dl) | 0.88 | 0.77 | 0.92 |
| TT4 (µg/dl) | 7.6 | 11.0 | 12.0 |
| TG (ng/ml) | 1.6 | 4.2 | 11.3 |
| UI* (µg/L) | 301 | 247 | 275 |

*median value, (N=32)

Hemoglobin of mothers during pregnancy indicates iron deficiency anemia throughout gestation. Their thyroid hormone profile is given in Table 4.3.3. Median urinary iodine was found to be adequate in all three trimesters.

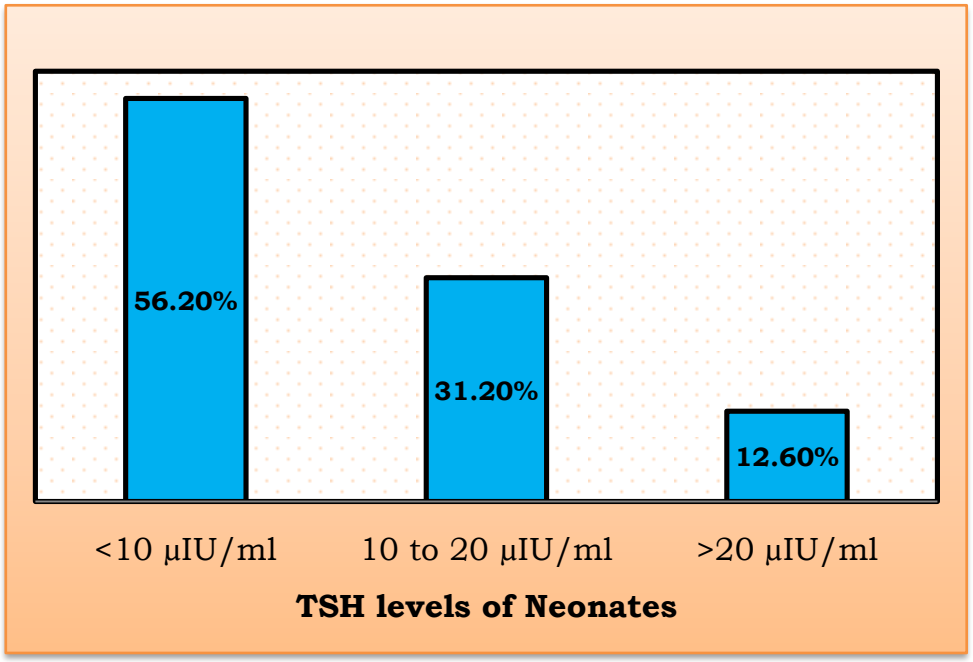
### 4.3.2 Thyroid profile of neonates

Mean cord blood TSH, FT4, TT4 and TG were 10.23 µIU/ml, 1.25 ng/dl, 9.36 µg/dl and 31.96 ng/ml respectively. Median and 95% CI for thyroid hormones is given in Table 4.3.4. Data on prevalence of raised TSH revealed that 43.8% neonates had CBTSH >10 µIU/ml, among these 31.2% had CBTSH between 10-20 µIU/ml and 12.6% had CBTSH >20 µIU/ml (Figure 4.3.3).

**Table 4.3.4: Thyroid hormone level of neonates**

| Thyroid hormones | N | Mean (sd) | SEM | Median |
|---|---|---|---|---|
| CBTSH (µIU/ml) | 39 | 10.23 (6.4) | 1.13 | 8.97 |
| CBFT4 (ng/dl) | 39 | 1.25 (0.1) | 0.02 | 1.24 |
| CBTT4 (µg/dl) | 39 | 9.36 (2.5) | 0.44 | 9.45 |
| CBTG (ng/ml) | 39 | 31.96 (13.0) | 2.30 | 33.20 |

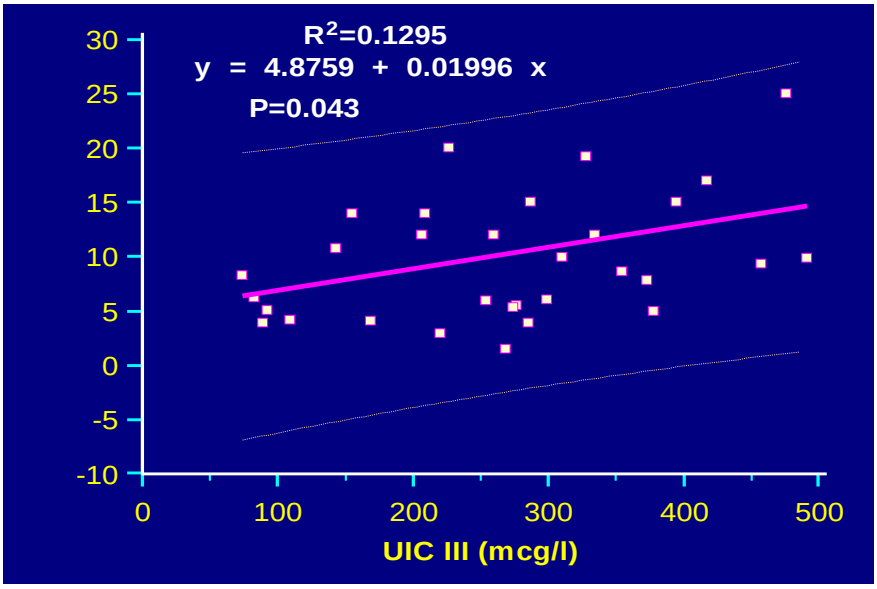
**Figure 4.3.3: Prevalence of raised TSH among neonates**



**Correlation and regression analysis**

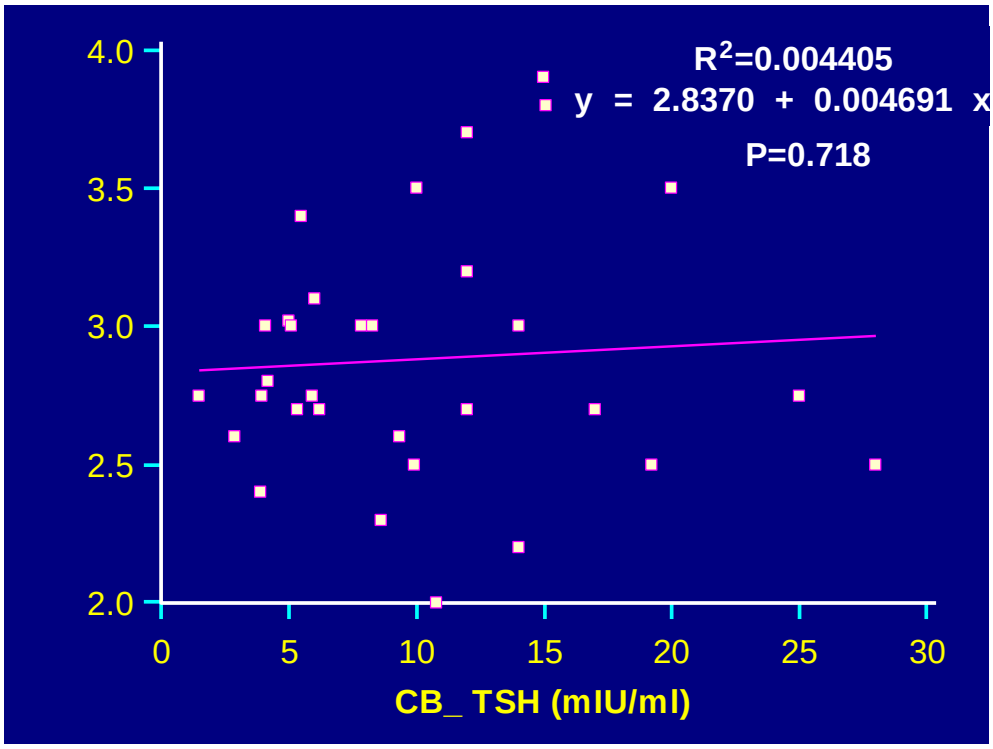
We did not find any significant correlation between CBTSH (r=0.066, p=0.718) and birth weight and between CBFT4 and birth weight (r=-0.091, p=0.619) [Figure 4.3.5]. However, a significant positive association was found between birth weight and birth length (r=0.666, p<0.001) and between birth weight and head circumference at birth (r=0.530, p=0.001) [Figure 4.3.6]. Further significant association was also observed between CBFT4 and FT4 during third trimester (positive, r=0.446, p=0.010) but not between CBTSH and third trimester logTSH (negative, r=-0.092, p=0.616) [Figure 4.3.7]. Additional to this, a significant relation was also found between CBTSH and UI during third trimester (rho=0.360, p=0.043) [Figure 4.3.4]. However, other authors Jaruratanasirikul et al (2009) and Chan et al (2003) did not find a significant association between maternal urinary iodine content and CBTSH. Our results on association between CBTSH and birth weight were echoed in studies of Shields et al (2011) and Jaruratanasirikul et al (2009). Unlike Shields et al (2011) we did not find positive association between CBFT4 and birth weight. Association between maternal FT4 and CBFT4 was also observed by Shield et al (2011).

**Figure 4.3.4: Correlation between CBSTH and UI during third trimester**

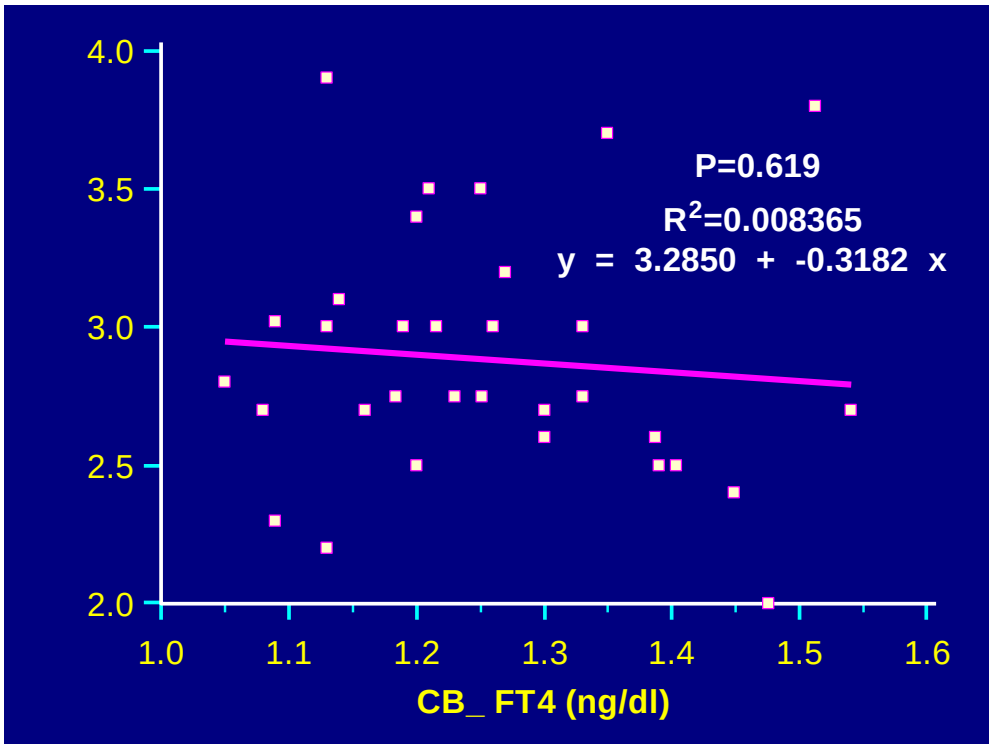



[Pink dotted line denotes 95% prediction interval]

**Figure 4.3.5: Correlation between birth-weight and CBTSH and CBFT4 and birth-weight**



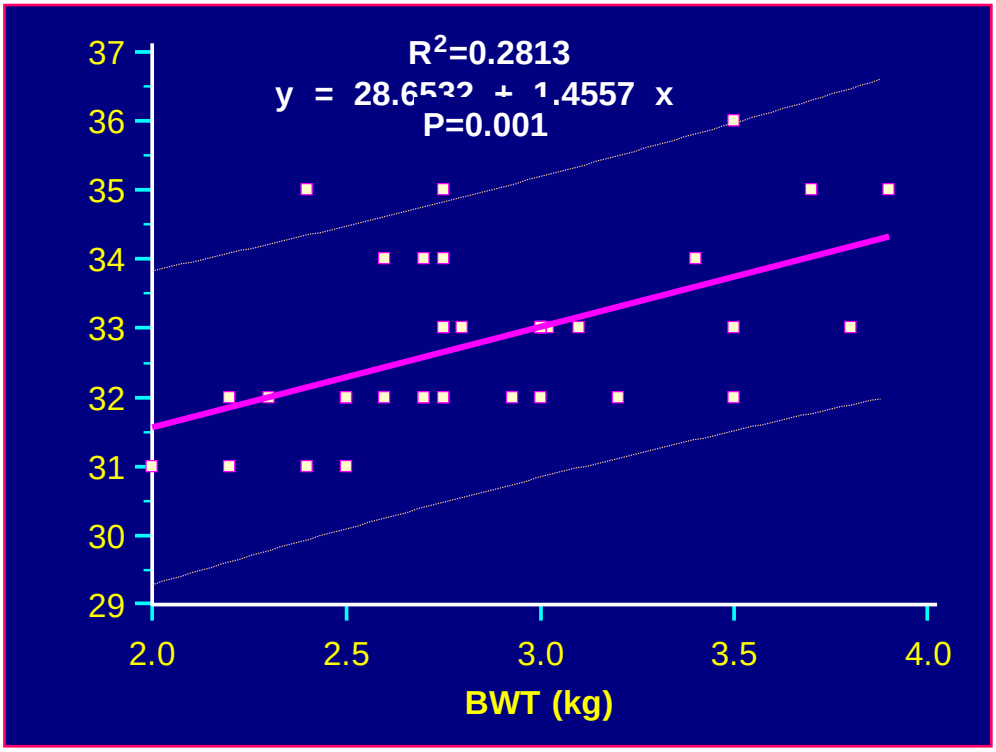

**CBTSH and BWt**



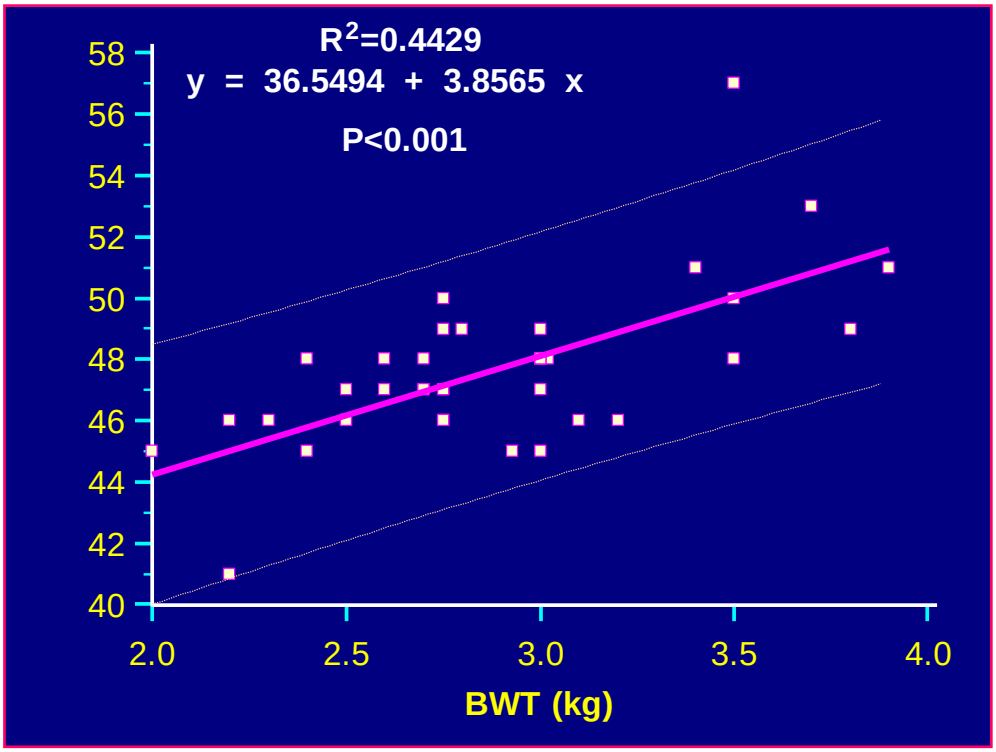

**CBFT4 and BWt**

**Figure 4.3.6: Correlation between birth-weight and head circumference and length and birth-weight**

[Pink dotted line denotes 95% prediction interval]



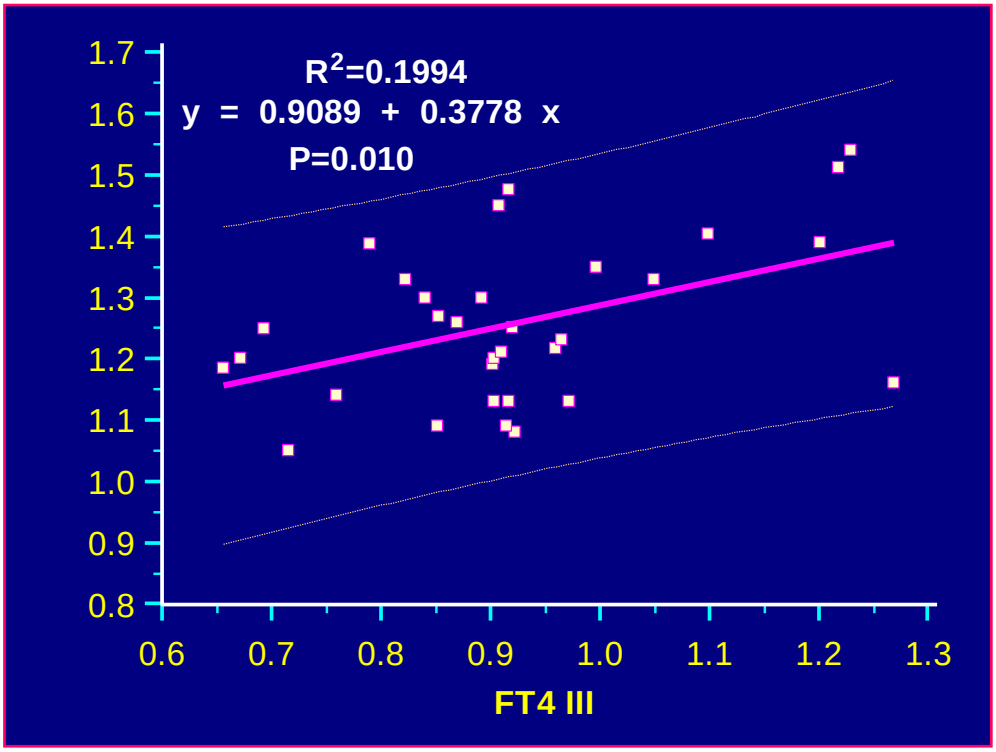

**BWt and HC at birth**



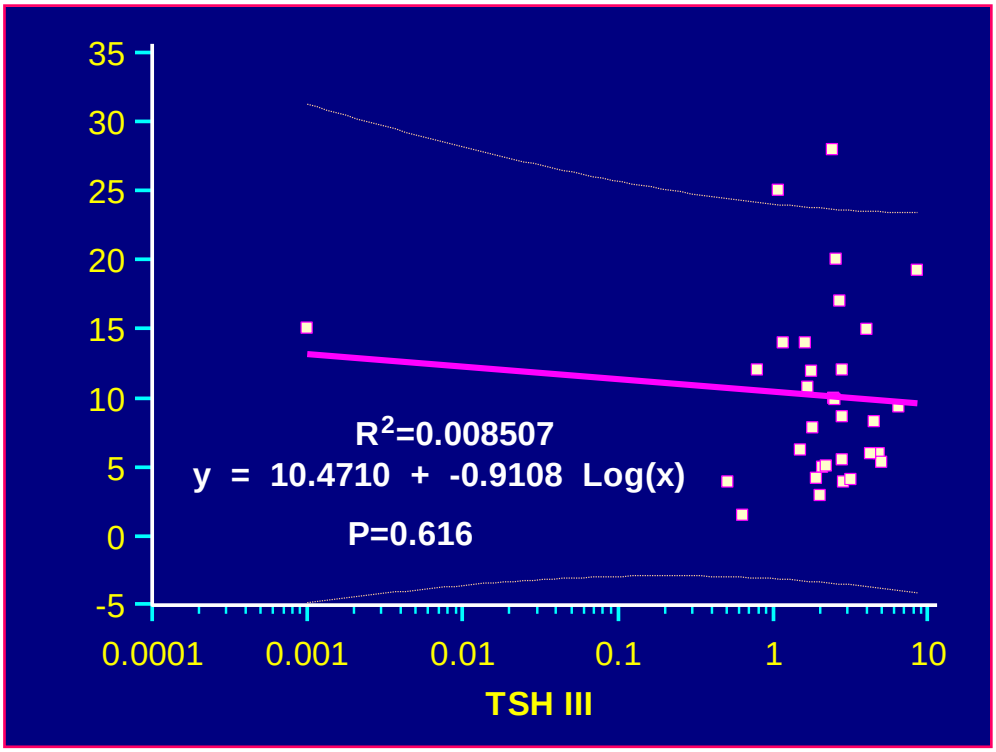

**BWt and Length at birth**

**Figure 4.3.7: Correlation between CBFT4 and FT4 during third trimester and CBTSH and TSH during third trimester**

[Pink dotted line denotes 95% prediction interval]




**CBFT4 and FT4 during third trimester**




**CBTSH and TSH during third trimester**

### 4.3.3 Newborn screening

After birth, the term baby experiences a surge of TSH as a physiological response to cold environment. The TSH concentration rises to 60-80 μIU/ml within 30 to 60 minutes after delivery and falls quickly in the first 24 hours to about 20 μIU/ml, followed by a slower decrease to below 10 μIU/ml after the first postnatal week. The rise in TSH initiates increase of T4 and free T4 to peak levels of 17 μg/dl and 3.5 ng/dl, respectively at 24 to 36 hours after birth with a slow decline to adult values over 4-5 weeks.

Congenital hypothyroidism (CH) is a major preventable cause of mental retardation. In most of the screening programs blood samples are collected at 5-6 days of age, but with large number of babies being discharged early, cord blood samples are being used as well (Wu et al, 1999; Ordookhani et al, 2003). In our country, it is very difficult to call back babies once discharged. Also, an effective social system whereby babies could be reached at home is practically non-existent. Thus cord blood remains a very practical alternative for screening purposes, and thus is the practice in some Asian countries (Wu et al, 1999;Ordookhani et al, 2003). Mixed cord blood samples for TSH values have compared well with filter paper samples taken in the first few days of life (Fuse et al, 1991;Walfish, 1976). The Indian Academy of Pediatrics recommends the use of cord blood samples for screening for CH.

Universal newborn screening for CH is currently being done in many parts of the world including Western Europe, North America, Japan, Australia, and parts of Eastern Europe, Asia, South America, and Central America. Three approaches are being used for screening:

1. Primary TSH, back upT4

2. Primary T4, back up TSH

3. Concomitant T4 and TSH

In the first approach, TSH is measured first. T4 is measured only if TSH is >20μIU/ml. This approach is likely to miss central hypothyroidism, thyroid binding globulin deficiency and hypothyroxinemia with delayed elevation of TSH. In the second approach, T4 is checked first and if low TSH is also checked. This is likely to miss milder/subclinical cases of CH in which T4 is initially normal with elevated TSH. Concomitant measurement of T4 and TSH is the most sensitive approach but incurs a higher cost. Screening programs use either percentile based cut-offs e.g., T4 below 10th percentile or TSH above 90th percentile or absolute cut-offs such as T4 <6.5 ug/dl and TSH >20 μIU/ml. In present study absolute cut-offs are used, we have used approach no. 3 (Concomitant T4 and TSH).

Three (9.2 %) neonates out of 32 were found to have low (<6.5 ug/dl) TT4, whereas 4 (20.6 %) neonates had TSH level >20 μIU/ml. Very few reports of cord blood values of TSH or T4 exist in Indian literature. Desai et al (1987) and Khadilkar et al (2002) had reported results on screening of neonates for congenital hypothyroidism. Desai et al screened 12,407 newborns for CH using cord blood TSH measurements, 2.8% babies were called for retesting and the incidence extrapolated was 1: 2481. In 1994, the same group screened 25,244 neonates at 24-94 hours and measured filter paper T4. The babies recalled were 18.91% and the extrapolated incidence was 1:2804. Khadilkar, et al found a mean cord TSH value of 12.3 μIU/ml, which is similar to our mean CBTSH value.

# *RESULTS*

# Phase IV

- Postpartum status of women
- Effect of DFS supplementation on maternal Iron and Iodine status
- Effect of early gestation thyroid dysfunction on infant development

### 4.4.1 Characteristic of women during postpartum

Mean weight of women was 47.5 (10.2) kg. Mean energy, protein and fat intake were 1,115.6 (274) kcal, 35.6 (11.8) g and 24.5 (9.6) g respectively. Mean TSH, FT4, TT4 and TG were 2.13 µIU/ml, 1.08 ng/dl, 9.6 µg/dl and 28.5 ng/ml respectively. Values of all thyroid hormones were falling under normal range. Median urinary iodine was found to be 218.8 µg/L and mean hemoglobin was 10.34 (1.2) g/dl (Table 4.4.1).

**Table 4.4.1: Characteristic of women during postpartum (6 months)**

| Variable | Mean (sd) |
|---|---|
| Weight (kg) | 47.5 (10.2) |
| Energy intake (kcal) | 1115.6 (274) |
| Protein intake (g) | 35.6 (11.8) |
| Fat intake (g) | 24.5 (9.6) |
| TSH (µIU/ml) | 2.13 (1.7-2.6)** |
| FT4 (ng/dl) | 1.08 (0.27) |
| TT4 (µg/dl) | 9.6 (2.3) |
| TG (ng/ml) | 28.5 (12.1) |
| HB (g/dl) | 10.34 (1.2) |
| UI (µg/L) | 218.8 (189.7-273.6)* |

** Geometric Mean (95% CI), * Median (95% CI)

Mean weight of women during postpartum period was similar to their early pregnancy weight (45.7 kg). According to RDA for lactating women (sedentary, Indian) calorie, protein and fat intake should be 2,500 kcal, 77.9 g and 30 g respectively. Data on dietary intake reveals that these women were not meeting the RDA for three major macro nutrients. Deficit in calorie, protein and fat intake was -1,384.4 kcal, -42.3 g and -5.5 g respectively; with 9.1% women having calorie intake between 1,500-2,500 kcal, 11.5% women having protein intake between 50-80 g and 26.1% women having fat intake >30 g.

### 4.4.2 Comparison of dietary intake of subjects during pregnancy and postpartum

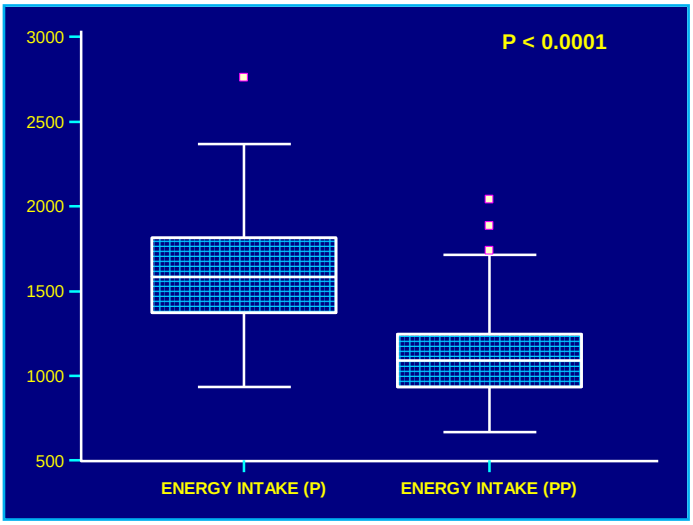
Mean energy, protein and fat intake of women reflected significantly low levels during postpartum period (Figure 4.4.1). The difference was 503.1 kcal in energy intake, 10.99 g in protein and 27.93 g in fat intake (Table 4.4.2).

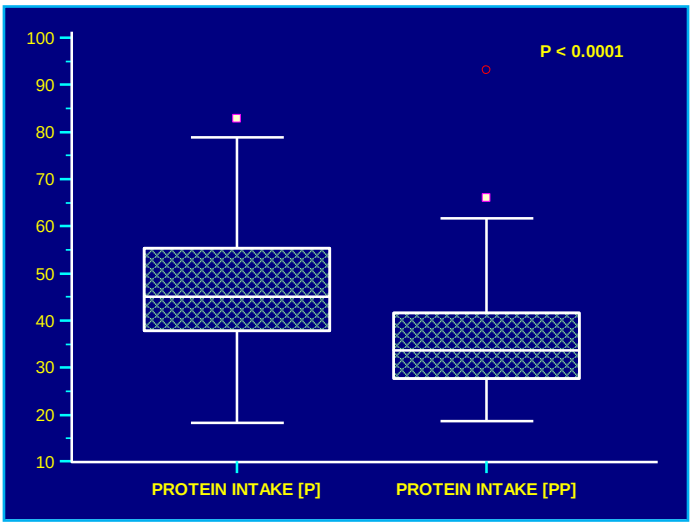
**Table 4.4.2: Comparison of maternal dietary intake**

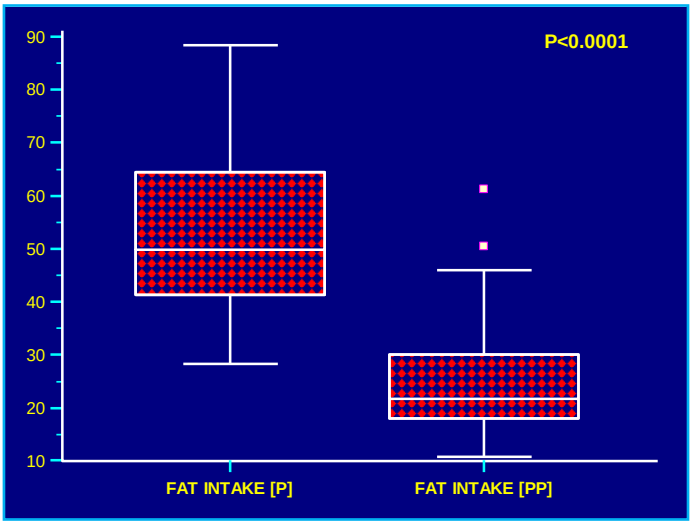
| Parameter | Mean (sd) during pregnancy | Mean (sd) during postpartum | |
|---|---|---|---|
| Energy (kcal) | 1,617(367) | 1115.6 (274) | |
| Protein (g) | 46.5(13.3) | 35.6 (11.8) | |
| Fat (g) | 52.3(14.7) | 24.5 (9.6) | |
| **Parameter** | **Test statistic** | **P** | **Difference** |
| Energy (kcal) | 9.718 | <0.0001 | 503.1 |
| Protein (g) | 5.419 | <0.001 | 10.99 |
| Fat (g) | 13.65 | <0.001 | 27.93 |

Only 1.2 % women were meeting the requirements for protein and 25.9 % for fat, whereas none of the women was meeting the requirements for calories according to RDA during lactation. However during pregnancy 1.4 % women were meeting the requirements for energy and protein intake and 97.3 % for fat intake. Hence we can conclude that dietary intake during postpartum period was even poor than during pregnancy. Also it can be stated that dietary intakes of women from LIG are more or less similar during pregnancy and lactation and hence there in widespread maternal malnutrition. Composition of breast-milk depends to some extent on maternal nutrition. In general, even the undernourished mothers can successfully breast-feed. But in the case of severe malnutrition, both the quality and quantity of breast-milk may be affected. Trace element composition of breast-milk, however, is not affected by the mother's nutritional status. Fat content of breast milk is much affected in malnutrition as compared to protein.

**Figure 4.4.1: Comparison of energy, protein and fat intake of women during pregnancy and postpartum**



### 4.4.3 Micronutrient deficiency [iodine and iron] during pregnancy and postpartum period

Median UI indicated adequate iodine intake. After categorizing women according to WHO/UNICEF/ICCIDD classification for UI, 18.5% of women were found to be iodine deficient (table 4.31). Mean hemoglobin indicated moderate IDA among these women and 96.3 % of women were anemic. Prevalence of severe, moderate and mild IDA was 1.2%, 29.6% and 65.4% respectively (Table 4.4.3).

**Table 4.4.3: Prevalence of IDA and ID**

| **Iron status** | **Cut-off (g/dl)** | **Percentage** |
|---|---|---|
| Severe | <7 | 1.2 (1) |
| Moderate | 7-9.9 | 29.6 (24) |
| Mild | 10-11.9 | 65.4 (53) |
| Normal | ≥12 | 3.7 (3) |
| **Iodine status** | **Cut-off (µg/L)** | **Percentage** |
| Inadequate | <100 | 18.5% (15) |
| Adequate | >100 | 81.5% (66) |

Figure in parenthesis denote actual number

On comparing prevalence of IDA during pregnancy and lactation, maximum prevalence was found during first trimester and minimum during third trimester (Figure 4.4.2). This highest prevalence during first trimester could be due to low maternal hemoglobin stores and lowest prevalence during third trimester could be due to improvement in hemoglobin stores due to IFA consumption. Similarly on comparing prevalence of ID during pregnancy and lactation, maximum prevalence was found during second trimester and minimum during postpartum period (Figure 4.4.3).

**Figure 4.4.2: Prevalence of IDA during pregnancy and lactation**

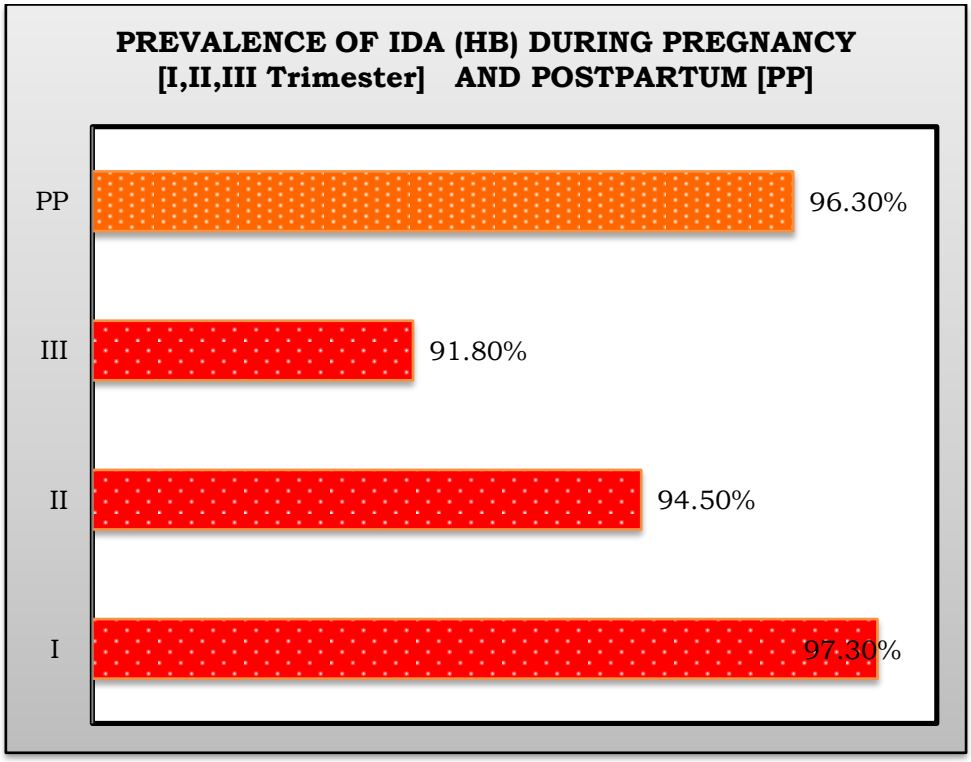
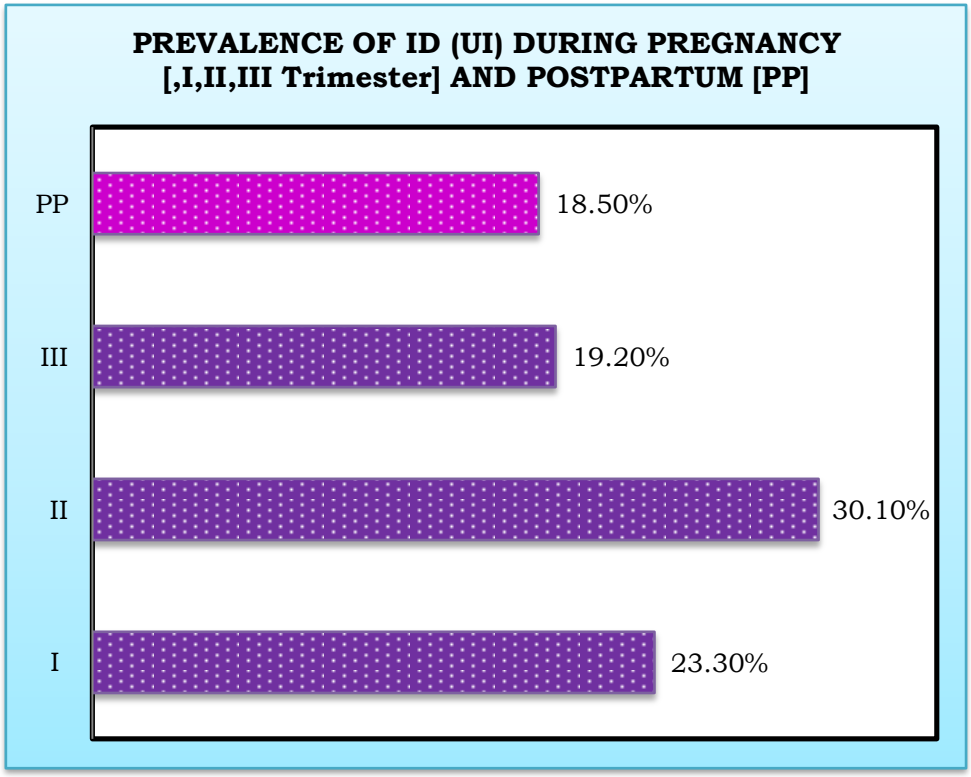



**Figure 4.4.3: Prevalence of ID during pregnancy and lactation**



### 4.4.4 Prevalence of thyroid dysfunction during postpartum period

During postpartum period only 9.9% women were found to have thyroid dysfunction. After applying normal (non pregnant) ranges for TSH and FT4, we found 2.5% women having overt hypothyroidism and 7.4% with subclinical hypothyroidism. Not a single woman was found to be hypothyroxinemic during postpartum period (Table 4.4.4).

**Table 4.4.4: Comparison of thyroid dysfunction**

| Thyroid function | Pregnancy [n=73] | | | Lactation [n=81] |
|---|---|---|---|---|
| | I Trimester | II Trimester | III Trimester | Postpartum 6 months |
| Overt hypothyroidism | 10.96(8) | 5.48(4) | 1.37 (1) | 2.5 (2) |
| Subclinical hypothyroidism | 17.81(13) | 13.7(10) | 24.66(18) | 7.4 (6) |
| Hypothyroxinemia | 4.11(3) | 24.66(18) | 5.48(4) | - |
| Normal | 67.12(49) | 56.16(41) | 68.49(50) | 90.1 (73) |

Figure in parenthesis denote number

When we compared thyroid function of women during pregnancy and lactation (postpartum) we found that most (90%) of the women became normal after pregnancy (Table 4.4.4). Thus we can state that due to pregnancy there were fluctuations observed in thyroid hormones, especially TSH and FT4. A euthyroid state of mother during early pregnancy is very important for proper development and differentiation of the fetal brain. However, most of the women were not able to maintain euthyroid state during pregnancy (Table 4.4.4).

### 4.4.5 Comparison of diagnostic test

We have evaluated thyroid hormones of our subjects during early gestation (first trimester) using two different upper limits for TSH (μIU/ml), 1) upper TSH cut off as 2.5 and 2) upper TSH cut off as 5.0. After comparing these two diagnostic methods with normal (non

pregnant) thyroid TSH values during postpartum period for same women, we found that the upper TSH cut off as 2.5 was better indicator of thyroid status. Table 4.4.5 shows the comparison of these two diagnostic methods.

**Table 4.4.5: Comparison of 2 diagnostic methods for hypothyroidism**

| | Upper TSH limit as 2.5 μIU/ml | Upper TSH limit as 5 μIU/ml |
|---|---|---|
| Sensitivity | 73.33% | 33.33% |
| Specificity | 55.30% | 48.67% |
| Disease prevalence | 18.52% | 7.41% |
| Positive predictive value | 27.16% | 4.94% |
| Negative predictive value | 90.12% | 90.12% |

From the above discussion, the need to assess thyroid function during pregnancy is justified. Also proper diagnosis of thyroid dysfunction during pregnancy is important to avoid both fetal and maternal complications. As discussed earlier thyroid activity undergoes many changes during normal pregnancy, including 1) a significant increase in serum TBG, thyroglobulin, TT4, 2) an increase in renal iodine clearance 3) stimulation of the thyroid by hCG. Taken together, these changes can make diagnosis of thyroid dysfunction during pregnancy difficult.

### 4.4.6 Comparison of biochemical parameters of subjects during pregnancy (I, II and III trimester) and postpartum

Mean FT4, TG and HB during postpartum period were significantly higher than during first, second and third trimester (Table 4.4.6). Difference in FT4 during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 0.23 ng/dl, 0.33 ng/dl, 0.17 ng/dl respectively (Figure 4.4.7).

Difference in Hb during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 1.23 g/dl, 1.11 g/dl, 0.88 g/dl respectively (Figure 4.4.4). Median UI during postpartum period was significantly lower than during first, second and third trimester value. Difference in UI during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was -51.3 µg/L (non significant), -73.9 µg/L, -65.9 µg/L respectively (Figure 4.4.5). However, no significant difference in mean TSH was observed during postpartum period and pregnancy (table 4.34). Difference in TSH during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 0.5 µIU/ml, 0.31 µIU/ml, -0.07 µIU/ml respectively (Figure 4.4.6).

**Table 4.4.6: Comparison of biochemical parameters of subjects during pregnancy (I, II and III trimester) and postpartum**

| Parameter | Test statistic (ANOVA) | P | Difference |
|---|---|---|---|
| Hb (g/dl) | F=23.2 | <0.001 | I,II, III trimester, P<0.05 |
| TSH (µIU/ml) | F=2.25 | 0.082 | NS difference |
| FT4 (ng/dl) | F=34.9 | <0.001 | I,II, III trimester, P<0.05 |
| TT4 (µg/dl) | F=104.733 | < 0.001 | I,II, III trimester, P<0.05 |
| TG (ng/ml) | F=27.195 | < 0.001 | I,II, III trimester, P<0.05 |
| UI (µg/L) | 8.5 | 0.03 | II, III trimester, P<0.05 |

Mean TT4 was significantly low during postpartum then during pregnancy (Table 4.4.6 and Figure 4.4.8). Mean FT4, TG and Hb were highest during postpartum period (Figure 4.4.4, 4.4.7 and 4.4.9). Median UI (Figure 4.4.5) and mean TT4 (Figure 4.4.8) were low during postpartum period and mean TSH (Figure 4.4.6) was highest during third trimester.

**Figure 4.4.4 Mean HB during pregnancy and postpartum**

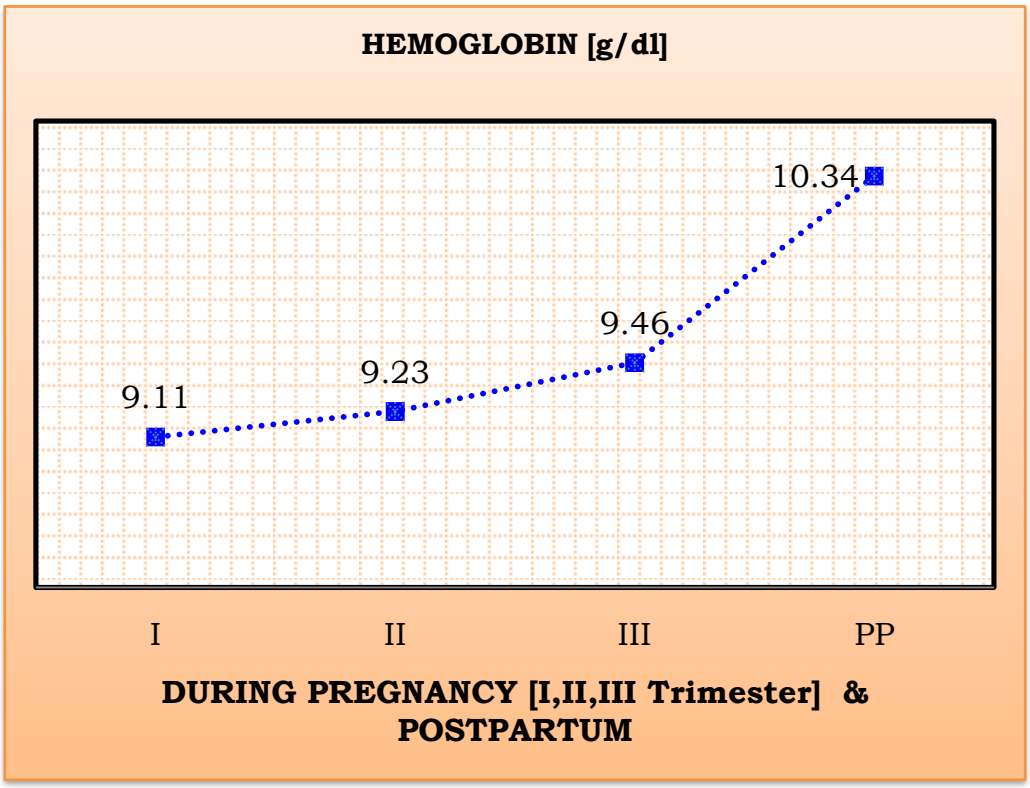



**Figure 4.4.5: Median UI during pregnancy and postpartum**

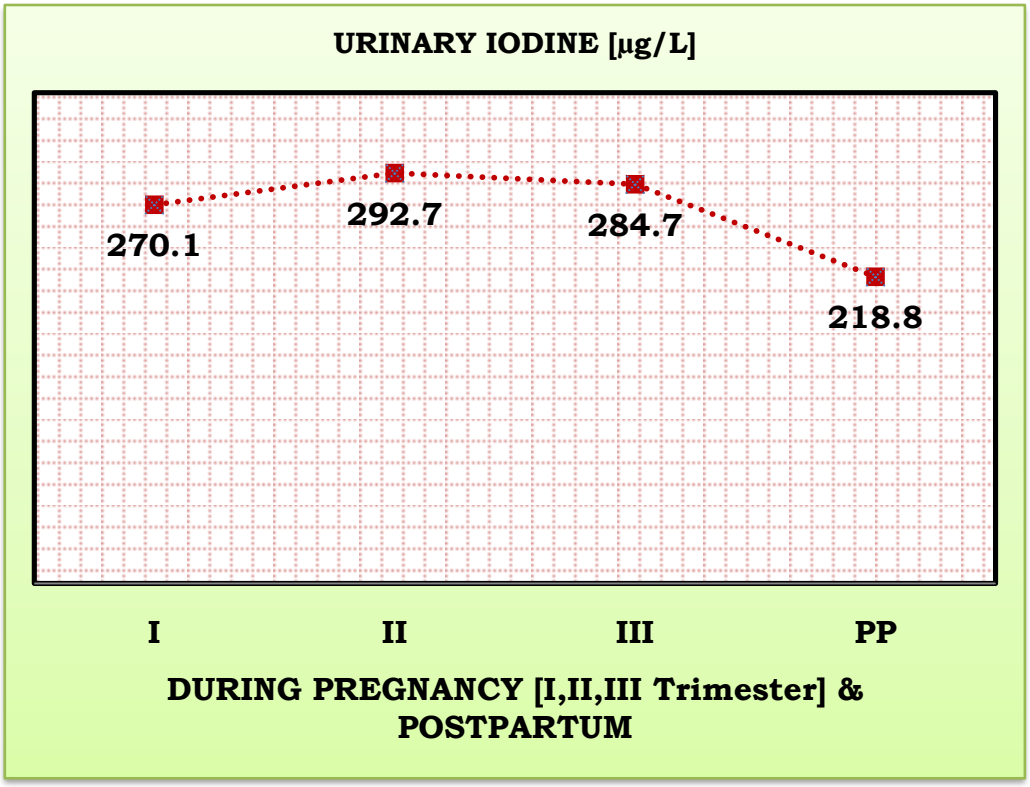


**Figure 4.4.6: Mean TSH during pregnancy and postpartum**

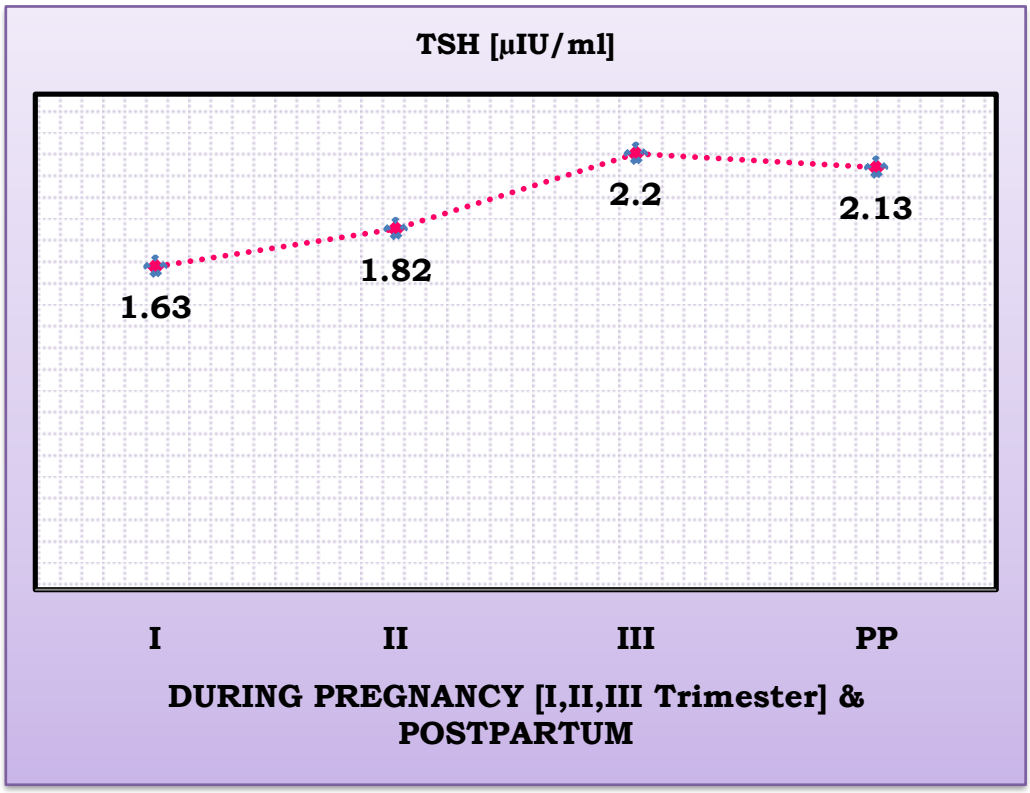



**Figure 4.4.7: Mean FT4 during pregnancy and postpartum**

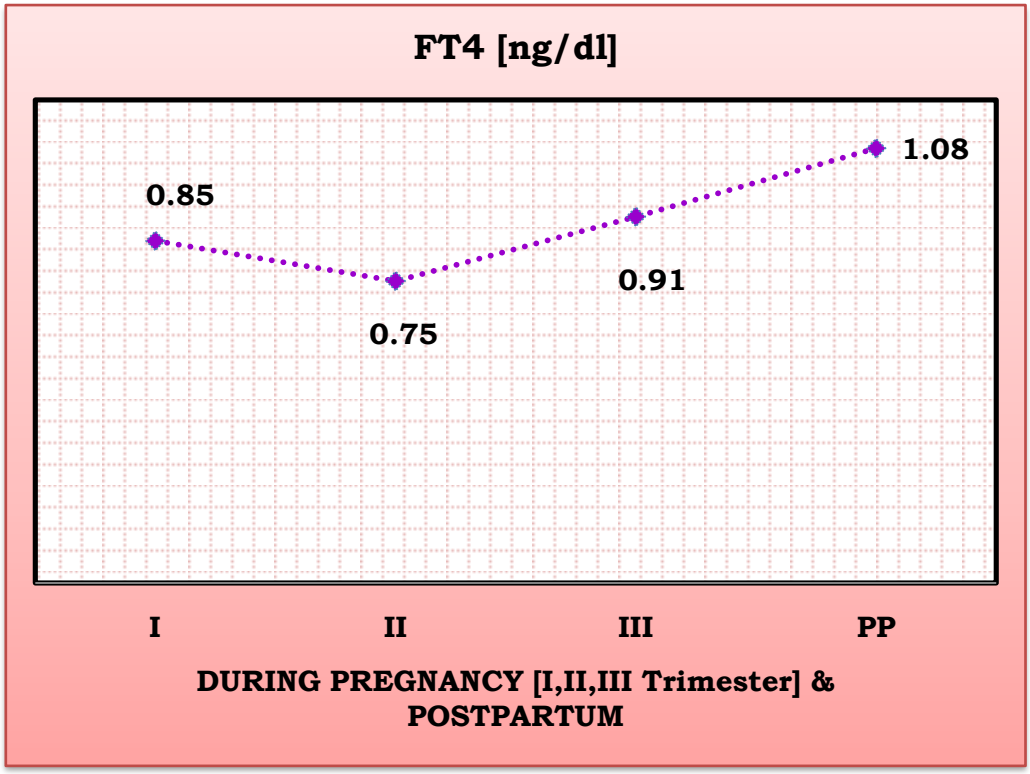


**Figure 4.4.8: Mean TT4 during pregnancy and postpartum**

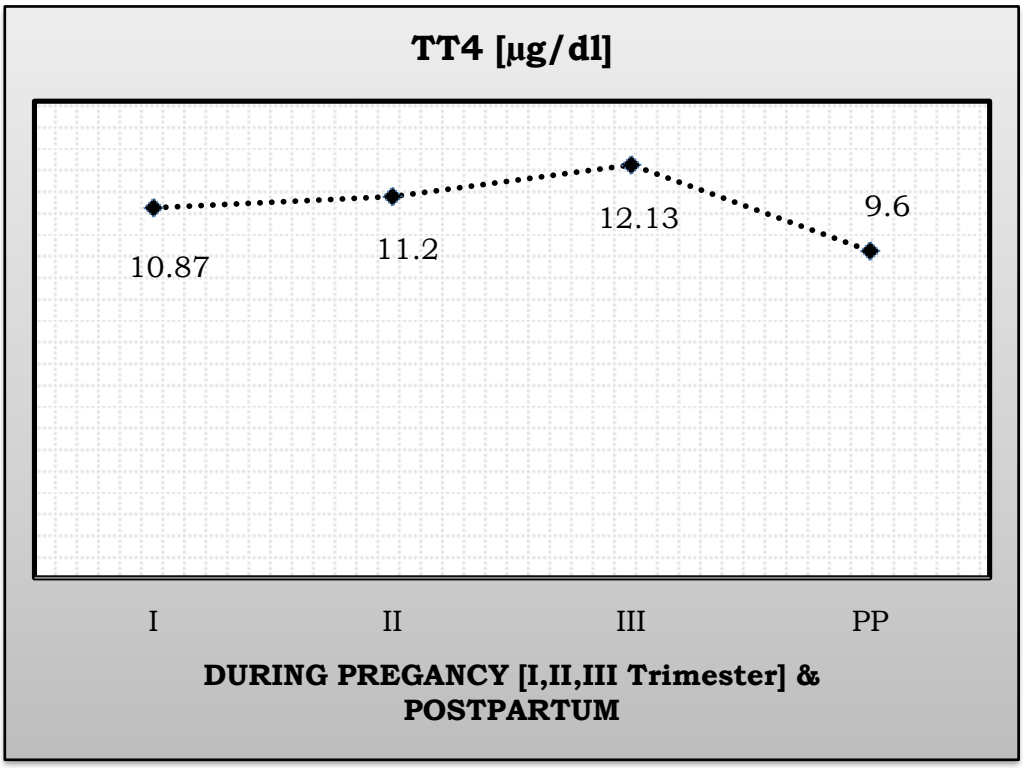
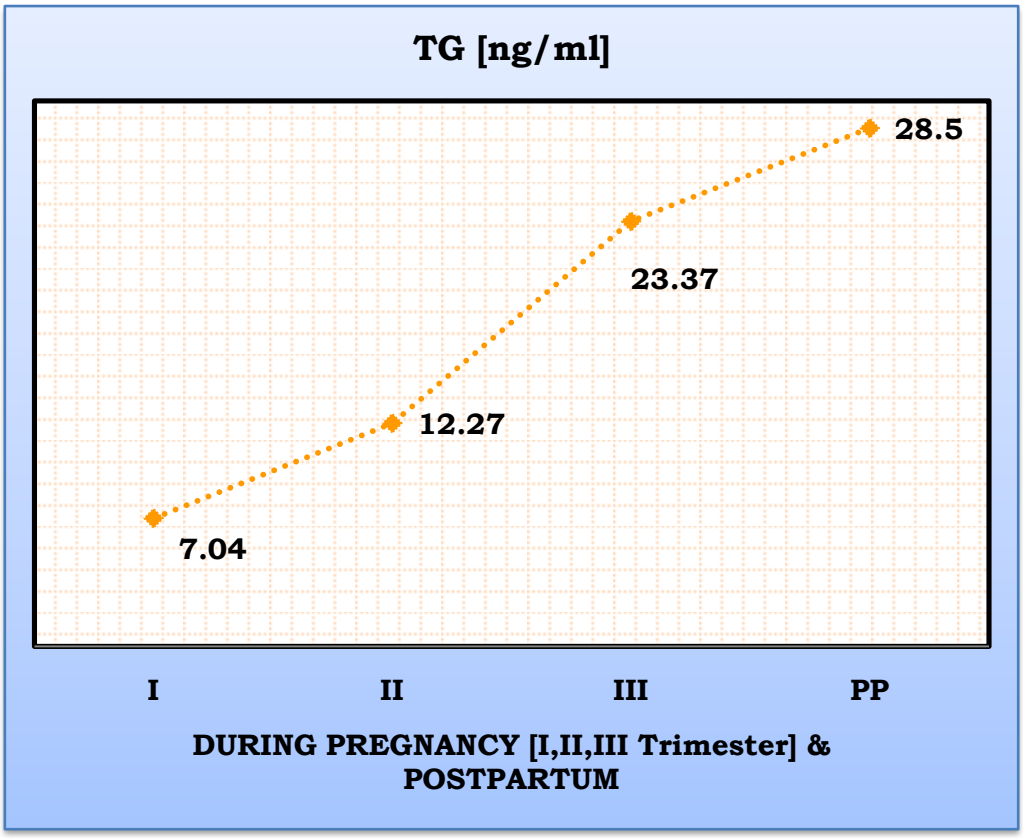



**Figure 4.4.9: Mean TG during pregnancy and postpartum**



Similar to our results, studies from China (Wang et al, 2009), Sweden (NHNES, 2007) and Hungary (Toldy et al, 2004) had also reported a fall in TSH during postpartum period. A reduction in TT4 was also found by Wang et al (2009), NHNES (2007), Toldy et al (2004) and Kung et al (2000). An increase in FT4 was also reported by NHNES (2007), Dhatt et al (2006), Kurioka et al (2005), Toldy et al (2004), Panesar et al (2001), Kung et al (2000) and Eltom et al (2000). Eltom et al (2000) had also reported an increase in TG from pregnancy to postpartum period. A fall in urinary iodine during lactation (postpartum period) was also mentioned by Eltom et al (2000), Kung et al (2000) and Yeo et al (2001).

The above discussed results confirm the reversibility of pregnancy induced changes in the iodine status and thyroid function of subjects. Glinoer el at (1997) found that the restoration of thyroid function to the pre-pregnancy state occurred at about six months after delivery, with exception of TG, which persisted in some of the cases till 1 year post-natally.

The organ most vulnerable to iodine and thyroid hormone is the central nervous system. Iodine is also necessary during the first few months of life for neurological development and myelination in order to achieve optimum intellectual development. Elevated levels of TSH and reduced level of FT4 and UI indicate deterioration of maternal iodine status. This deterioration during the post natal period may be due to breastfeeding, which may increase the demand for extra iodine intake (Delange et al, 1988). Since we have not found any deterioration in iodine status of women during post natal period we can assume that these women would have met the extra requirements of iodine during lactation and hence they delivered adequate iodine to their infants via breast milk. In case when lactating women could not meet the extra requirements, they are expected to lose some of their iodine in breast milk, this may lead to a reduced maternal iodine pool and consequently reduced thyroid hormone production.

### 4.4.7 Characteristics of Infants

Mean birth weight of infants was 2.8 (0.4) kg. Median urinary iodine value was 370.6 µg/l. Mean weight, length and head circumference during six months was 6.6 (0.8) kg, 66 (2.6) cm and 41.6 (1.3) cm respectively. During twelve months weight, length and head circumference increased to 7.4 (0.7) kg, 70.9 (2.8) cm and 44.2 (1.3) cm respectively. Mean BDSTI at six and twelve months were found to be 17.8 (0.5) and 34.7 (0.6) (Table 4.4.7).

**Table 4.4.7: Characteristic of infants**

| Variable | Mean (sd) |
|---|---|
| Birth Weight (kg) | 2.8 (0.4) |
| UI (µg/l) | 370.6 (251.9-438.7)* |
| Weight at 6 M (kg) | 6.6 (0.8) |
| Weight at 12 M (kg) | 7.4 (0.7) |
| Length at 6 M (cm) | 66.0 (2.6) |
| Length at 12 M (cm) | 70.9 (2.8) |
| Head circumference at 6 M (cm) | 41.6 (1.3) |
| Head circumference at 12 M (cm) | 44.2 (1.3) |
| BDSTI at 6 M | 17.8 (0.5) |
| BDSTI at 12M | 34.7 (0.6) |

*median (95% CI), M-months

Despite a low weight gain by the mother during pregnancy, mean birth weight was >2.5 kg. Median UI indicated optimal iodine status among infants. None of the infant had UI <100 µg/L. BDSTI at six month and twelve month were less than normal. Nutritional status of infants at six months and twelve months of age is given in Table 4.4.8.

**Table 4.4.8: Nutritional status of infants (at six and twelve months)**

| **Nutritional status of infants at 6 months based on *z*- scores** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Weight-for-age** | | | | **Height-for-age** | | | |
| **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** | **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** |
| 4 (9.4%) | 13 (16%) | 64 (79%) | - | 1 (1.2%) | 9 (11.1%) | 70 (86.4%) | 1 (1.2%) |
| **Weight-for height** | | | | **Head circumference-for-age** | | | |
| **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** | **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** |
| 5 (6.2%) | 13 (16%) | 62 (76.5%) | 1 (1.2%) | 1 (1.2%) | 7 (8.6%) | 72 (88.9%) | 1 (1.2%) |
| **Nutritional status of infants at 12 months based on *z*- scores** | | | | | | | |
| **Weight-for-age** | | | | **Height-for-age** | | | |
| **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** | **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** |
| 2 (2.5%) | 25 (30.9%) | 52 (64.2%) | 2 (2.5%) | 7 (8.6%) | 23 (28.4%) | 51 (63%) | - |
| **Weight-for height** | | | | **Head circumference-for-age** | | | |
| **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** | **<-3SD** | **>-3SD to <-2SD** | **-2SD to +2SD** | **≥2SD** |
| 2 (2.5%) | 11 (13.6%) | 64 (695) | 4 (4.9%) | 1 (1.2%) | 11 (13.6%) | 68 (84%) | 1 (1.2%) |

Figure in parenthesis denote percentage

### 4.4.8 Nutritional status of infants

**Weight for age (under nutrition)**

Percentage of severely undernourished (<-3SD) infants was 9.4 % at six months which reduced to 2.5% at twelve months. Percentage of moderately undernourished (>-3SD to <-2SD) infants was increased from 16 % to 30.9% from six months to twelve months. Hence, we can conclude that there was shift from severe to moderate category from six to twelve months. None of the infant was falling in overweight category at six months, however a few (2.5%) infants were found to be overweight (>2SD) at twelve months.

**Weight for height (stunting)**

Cases of stunting (severe and moderate) increased from six months to twelve months. Percentage of infants who were severely stunted increased from 1.2 % to 8.6% and of moderately stunted increased from 11.1% to 28.4%. At six months a few (1.2%) infants were having length as above average (>2SD). However at twelve months none of the infant had length above average.

**Weight for height (wasting)**

Percentage of infants who were wasted was reduced. Severe wasting reduced from 6.2% at six months to 2.5% at twelve months and moderate wasting reduced from 16% at six months to 13.6% at twelve months. Percentage of infants who were above average increased from 1.2 % at six months to 4.9% at twelve months.

**Head circumference for age**

Percentage of infants who were <-3SD and >+2SD remained same at six months and at twelve months. Percentage of infants who were <-2SD were increased from 8.6% at six months to 13.6% at twelve months.

Overall nutritional status of infants with respect to under nutrition and wasting was improved. However, recovery in case of stunting and head circumference for age was not observed (Table 4.4.8). According to recent WHO/UNICEF statements for SAM, these infants are more prone to be affected by various morbidities and hence later mortality. Appropriate interventions at this time will cure these infants.

### 4.4.9 DFS supplementation to combat anemia among lactating women

There was no significant difference in energy, protein, fat and dietary iron intake in both experimental and control groups (Table 4.4.9). There was no significant difference in median UI of infants born to mothers who were given DFS and who received IS.

**Table 4.4.9: Comparison of maternal and infant characteristics in 2 groups**

| Variable | Mean (sd) | | P value |
|---|---|---|---|
| | Experimental group[DFS]N=48 | Control group[IS]N=33 | |
| **Infant Characteristics** | | | |
| UI | *385.7 | *319.5 | P = 0.513 |
| **Maternal Characteristics** | | | |
| Energy intake (kcal) | 1115.1 (268.5) | 1116.5 (286.0) | P = 0.982 |
| Protein intake (g) | 36.2 (10.7) | 34.7 (13.2) | P = 0.58 |
| Fat intake (g) | 24.0 (9.2) | 25.1 (10.4) | P = 0.611 |
| Dietary iron (mg) | 8.5 (4.1) | 8.5 (3.5) | P = 0.994 |
| UI at postpartum 6 M | *198.5 | *252.8 | - |
| UI at postpartum 12 M | *281.6 | *265.5 | - |
| Hb at postpartum 6 M | 10.44 (1.2) | 10.19 (0.9) | - |
| Hb at postpartum 12 M | 10.66 (1.3) | 10.02 (0.9) | - |

*median; M-months; UI-(µg/L); Hb-g/dl, RDA for iron=21mg; RDA for iodine=250µg; normal value for UI >100 µg/L

**DFS supplementation**

Double Fortified Salt (DFS) is an edible salt fortified with iodine and encapsulated iron. At 10 g/day of daily consumption of salt, a person can receive 10 mg of iron per day – that's around one third of their daily requirement of iron. Today, DFS has emerged as an exciting

intervention that works complementarily with other approaches to tackle iron and iodine deficiencies, which affect half the world's population. The technology is available in India and is transferable. Thus it is suggestive that supplementation of DFS is a powerful new solution which India and other nations can use to address anemia especially in developing countries.

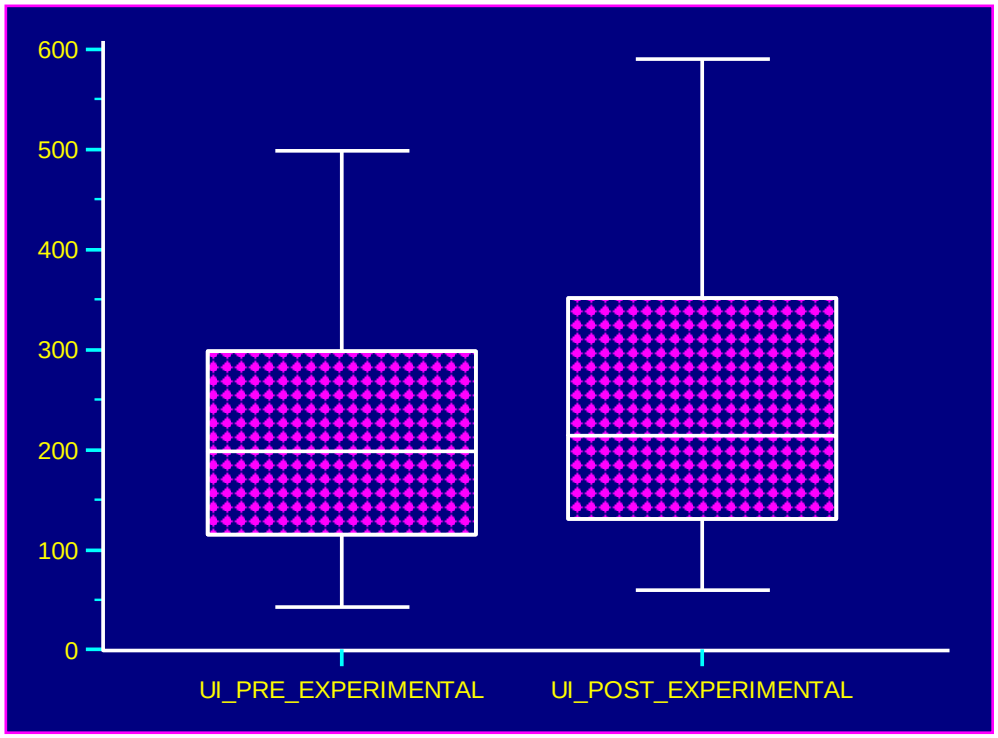
After a six months supplementation of DFS during lactation period, there was a significant increase (Figure 4.4.11) in hemoglobin of 0.22 g/dL [($p<0.05$), 10.66±1.3 (final)- 10.44±1.2 (initial)] in experimental group and in control group, there was a significant decrease in hemoglobin of 0.17 g/dL [($p<0.05$), 10.02±0.9 (final)- 10.19±0.9 (initial)]. Median urinary iodine increased (Figure 4.4.10) by 78 mcg/L [($p<0.05$), 274 (final) - 199 (initial) in experimental group and in control group it decreased by 16 mcg/L [($p=0.964$), 265 (final) - 281 (initial)]. Mean energy, protein fat and iron in experimental group was 1,115 kcal (±268), 36.2 g (±10.7), 24 g (±9.2) and 8.5 mg (±4.1) respectively. In control group mean energy, protein fat and iron was 1,116 kcal (±286), 34.7 g (±13.2), 25.1 g (±10.4) and 8.5 mg (±3.5) respectively (Table 4.4.9). Prevalence of IDA and ID before and after supplementation during lactation is recorded in Table 4.4.10.

**Table 4.4.10: Prevalence of IDA and ID before and after 6 months supplementation of DFS**

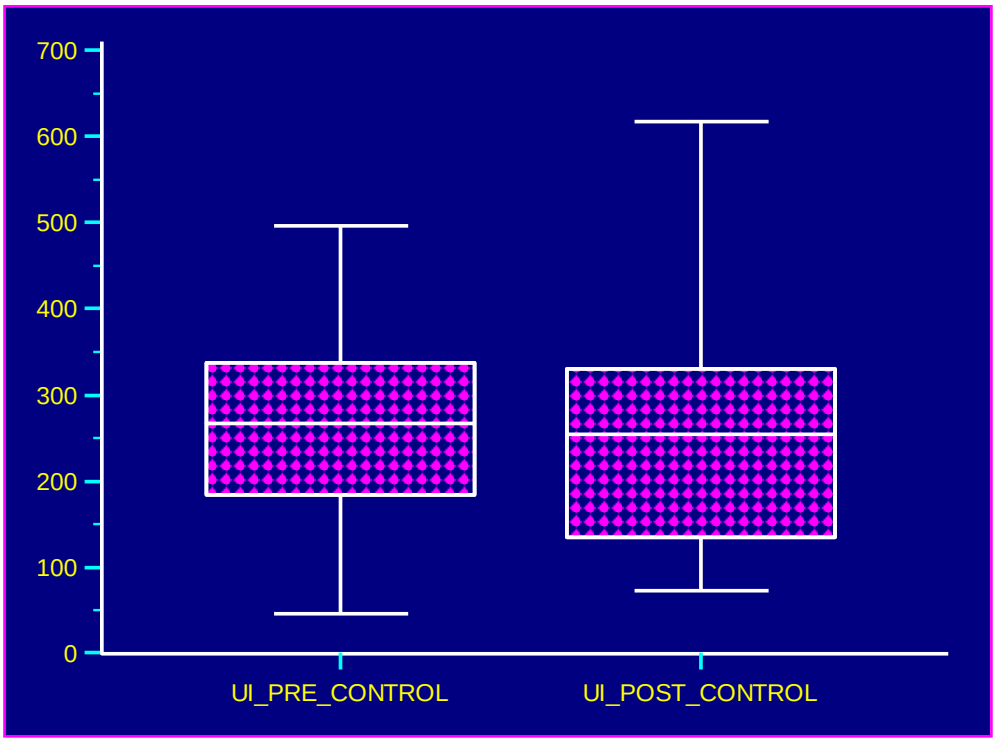
| Indicator | Category | Experimental group (n=48) | | Control group (n=33) | |
|---|---|---|---|---|---|
| | | Pre | Post | Pre | Post |
| Hb (g/dl) | <7 | 2.1% (1) | 2.1 % (1) | - | - |
| | 7-9.9 | 20.8 % (10) | 22.9 % (11) | 42.4 % (14) | 51.5 % (17) |
| | 10-11.99 | 72.9 % (35) | 54.2 % (26) | 54.5 % (18) | 48.5 % (16) |
| | >12 | 4.2 % (2) | 20.8 % (10) | 3.0 % (1) | - |
| UI (µg/L) | <100 | 20.8 % (10) | 6.3 % (3) | 15.2 % (5) | 6.1 % (2) |
| | >100 | 79.2% (28) | 93.7 % (45) | 84.8 % (28) | 93.9 % (31) |

Figure in parentheses denote actual number

**Figure 4.4.10: Median urinary iodine (UI) before (Pre) and after (Post) DFS supplementation in experimental group and control group**

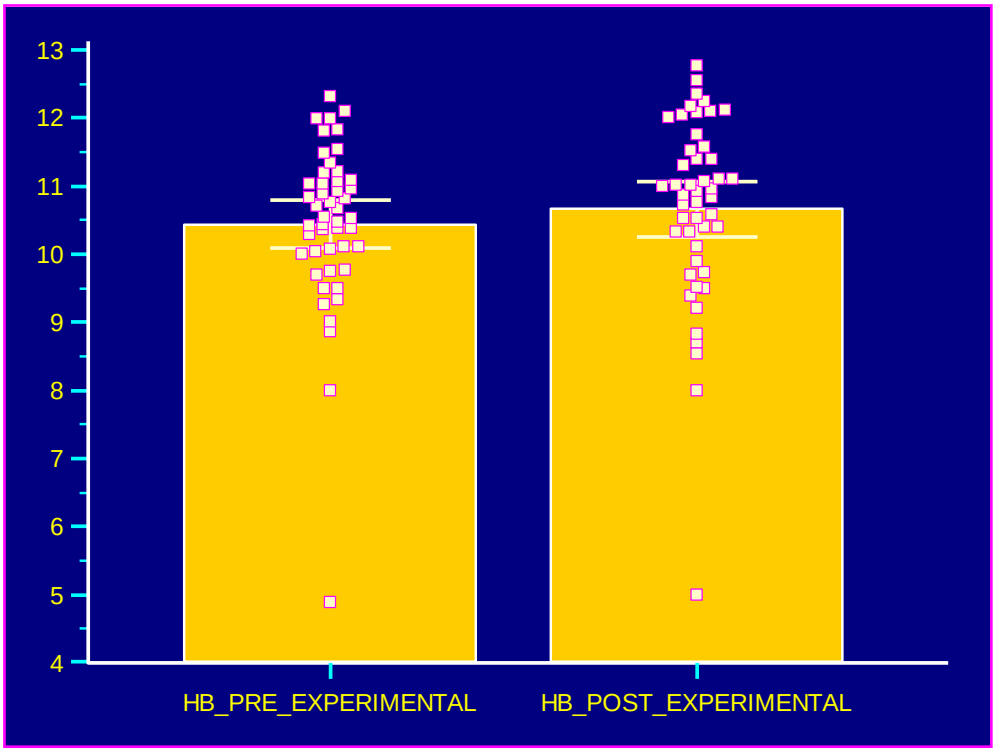



**↑ 78 µg/L [274 (final)-199 (initial), p=0.006*]**

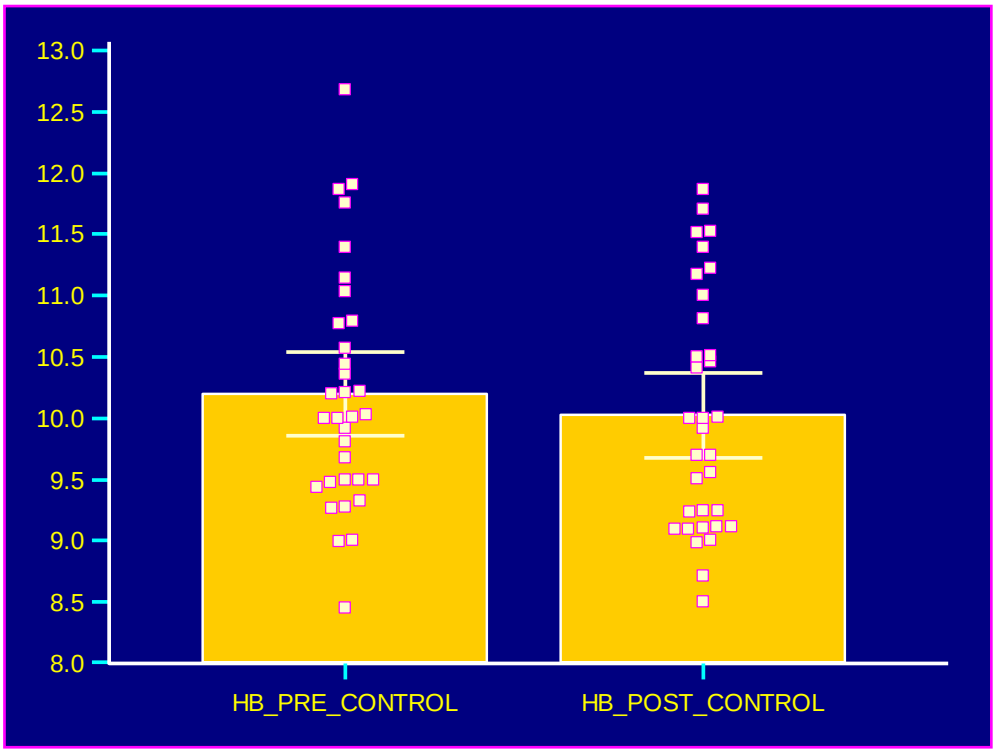



**↓16 µg/L [265 (final)-281 (initial), p=0.964$^{ns}$]**

**Figure 4.4.11: Mean hemoglobin before (Pre) and after (Post) DFS supplementation in experimental and control group**




**↑0.22 g/dl [10.66±1.3 (final)-10.44±1.2 (initial), p=0.020*]**




**↓ 0.17 g/dl [10.02±0.9 (final)-10.19±0.9 (initial), p=0.039*]**

NIN conducted an efficacy trial from 1989 to 1992 in the tribal areas of East Godavari (Andhra Pradesh), which is endemic for goitre as well as with a high prevalence of IDA. Four blocks were randomly selected; three blocks were allocated to experimental group (DFS supplementation for 2 years), while the fourth block served as control (IS for 2 years). There was a significant reduction in the prevalence of total goitre from an initial 28% to 14% after intervention of DFS in tribal areas in 2 years. Median urinary iodine excretion increases from 116 to 155 µg/L in DFS group and from 59 to 160 µg/L in IS group. Overall prevalence of anemia decreased from 78 % to 55 % in DFS group. The results demonstrated that the hemoglobin levels increased significantly in anemic subjects and there was a marginal or no improvement in non-anemic subjects (NIN, 2005).

In a multicentric study in India, the bio-efficacy of DFS was assessed in communities covering three states of the country. Over a period of one 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/L at baseline to 205 µg/L at the end of the study in the experimental group and from 225 mcg/L to 220 mcg/L in the control group (Vinodkumar et al, 2007).Zimmerman et al (2003) also studied the efficacy of DFS [containing 25 mcg iodine/g salt (as potassium iodide) and 1 mg iron/g salt (as ferrous sulfate hydrate encapsulated with partially hydrogenated vegetable oil)] supplementation to that of iodized salt in a 9-months, randomized, double-blind trial in iodine-deficient, 6–15-y-old children (n=377) in Morocco (Zimmermann et al, 2003). During the efficacy trial, urinary iodine levels and thyroid volumes improved significantly ($p<0.001$ and $<0.05$, respectively) from baseline in both groups. At 40 weeks, mean hemoglobin concentrations in the DFS group had increased by 1.4 g/dL ($p<0.01$). The prevalence of iron deficiency anemia in the DFS group decreased from 35% at baseline to 8% at 40 weeks ($p< 0.001$).

Concurrent to results in above mentioned studies, our study supplementation of DFS showed an improvement in both iron and iodine status. DFS 10 g/day provided 10 mg iron (40 % RDA for lactating women) and 200 µg iodine (80% RDA for lactating women).Six months supplementation of DFS (providing 10 mg of daily iron) could bring an increase of 0.22 g/dL in hemoglobin during lactation as compared to an increase of 0.44 g/dL in hemoglobin during pregnancy after six months supplementation of 60 mg of elemental iron. The prevalence of IDA in the DFS group decreased from 96% (before supplementation) to 79% (after supplementation) ($p<0.001$).DFS delivered less but crucial amount of iron to lactating women, which significantly contributed for sustained release of iron and iodine during breastfeeding and postpartum period.

This amount of iron from DFS was 1.5 mg more than what they were getting from their daily diet [8.5 mg (34% RDA)]. When experimental and control groups were compared, an increase in mean hemoglobin was found in experimental group and a decrease in control group. Hence we can state that, though the amount of iron in DFS is less but it could help lactating women to sustain their hemoglobin levels.

Median urinary iodine in experimental group increased after supplementation; however in control group a decrease in median urinary iodine was observed. The prevalence of iodine deficiency in the DFS group decreased from 21% (before supplementation) to 6% (after supplementation) ($p< 0.05$). As mentioned earlier iodine requirements during pregnancy and lactation are higher during non-pregnant and non-lactating state. Single iodized salt provides 150 µg of iodine/day and the requirement is 250 µg/day. DFS contains 400 µg iodine/g salt and it can provide 200 µg (80% RDA) iodine/g salt at consumer level, whereas single iodized salt contains 300 µg iodine/g salt and it can provide 150 µg (60% RDA) iodine/g salt at consumer level.

### 4.4.10 Effect of thyroid dysfunction during early gestation on infant development

For determining the effect of early gestation thyroid function on infant development, pregnant women were categorized into two groups- group-I [women with thyroid dysfunction] and group-II [women with normal thyroid function].

**Table 4.4.11: Mean BDSTI scores, anthropometric measurements and thyroid hormones in both groups**

| Variable | Mean (sd) | | P value |
|---|---|---|---|
| | Group IN=39 | Group IIN=42 | |
| **Maternal Characteristics** | | | |
| TSH (µIU/ml) | 3.08* (2.18-4.34) | 1.56* (1.25-1.94) | P=0.001* |
| FT4 (ng/dl) | 1.043 (0.28) | 1.122 (0.25) | P=0.194$^{ns}$ |
| **Infant Characteristics** | | | |
| BDSTI at 6 months | 17.7 (0.7) | 17.9 (0.1) | P = 0.032 |
| BDSTI at 12 months | 34.5 (0.9) | 34.9 (0.3) | P = 0.028 |
| Birth weight (kg) | 2.8 (0.4) | 2.7 (0.4) | P = 0.354 $^{ns}$ |
| Weight at 6 M (kg) | 6.7 (0.9) | 6.6 (0.7) | P = 0.858 $^{ns}$ |
| Weight at 12 M (kg) | 7.48 (0.8) | 7.47 (0.5) | P = 0.963 $^{ns}$ |
| Length at 6 M (cm) | 66.1 (2.6) | 65.9 (2.6) | P = 0.715 $^{ns}$ |
| Length at 12 M (cm) | 71.1 (3.0) | 70.8 (2.6) | P = 0.631 $^{ns}$ |
| HC at 6 M (cm) | 41.68 (1.5) | 41.63 (1.0) | P = 0.862 $^{ns}$ |
| HC at 12 M (cm) | 44.09 (1.6) | 44.39 (1.0) | P = 0.333 $^{ns}$ |

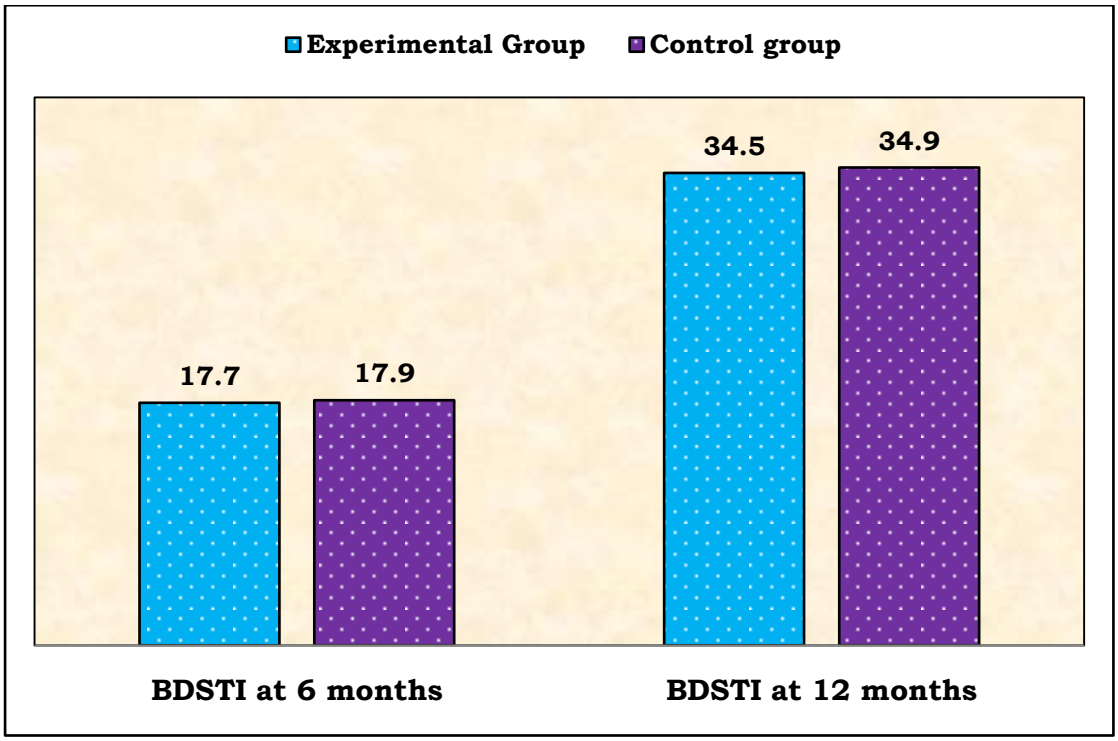
*Geometric Mean (95%CI), M –months, HC-head circumference

Mean TSH in both the groups was found to be significantly different. In group I mean TSH was higher (3.08) than in group II (1.56).

However no significant difference was found in mean FT4 values in both groups. Significant difference in both groups were not found with respect to anthropometric indices (birth weight, weight at 6 and 12 months, length at 6 and 12 months and head circumference at 6 and 12 months) (Table 4.4.11).

BDSTI at 6 months in Group I and Group II was found to be 17.7 (0.7) and 17.9 (0.1) respectively and BSDTI at 12 months in Group I and group II was 34.5 (0.9) and 34.9 (0.3) respectively. A significant difference of 0.2 and 0.4 was found between mean scores of both groups at 6 months and 12 months respectively. This difference is an indicator of effect of early gestation thyroid dysfunction on infant mental and psychomotor development (Figure 4.4.12).

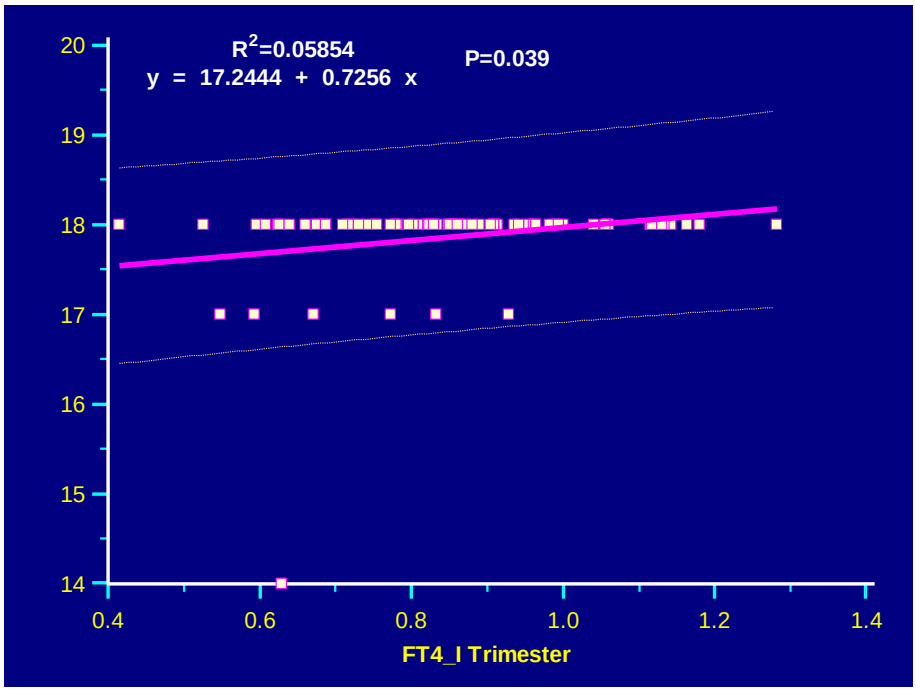
**Figure 4.4.12: Mean BDSTI in group I [experimental] and Group II [control]**




A positive significant (r=0.241, p=0.039) association was found between first trimester FT4 and BDSTI at six months (Figure 4.4.13) but not between first trimester TSH and BDSTI at six months (r=-0.06, p=0.588). BDSTI at twelve months was also not significantly

associated with first trimester TSH (r=-0.037, p=0.751) and FT4 (r=0.163, r=0.167).

**Figure 4.4.13: Association between first trimester FT4 and BDSTI at 6 months**




[Pink dotted line denotes 95% prediction interval]

**Thyroid hormones and brain development**

Thyroid hormones are essential for normal brain development. Extremely low levels of thyroid hormones during gestation result in mental retardation. In early pregnancy the embryo depends entirely on maternal thyroid hormone that crosses the placenta, and by about 12-14 wk gestation, fetal thyroid function begins (de Escobar et al 2004 and 2007). Even after the onset of fetal thyroid secretion, maternal transfer constitutes a fraction of circulation fetal T4, and continues to have a protective role in fetal neurodevelopment until birth. Mothers with hypothyroidism during first trimester of pregnancy, and even those with low-normal T4 levels or mild serum TSH elevations, have children with poorer neurocognitive function (Haddow et al 1999; Pop et al 1999 and 2003).

The development of fetal thyroid function is dependent on the embryogenesis, differentiation, and maturation of the thyroid gland. This is coupled with evolution of the hypothalamic-pituitary-thyroid axis and thyroid hormone metabolism, resulting in regulation of thyroid action, production, and secretion. Throughout gestation there is a steady supply of maternal thyroxine which has been observed in embryonic circulation as early as 4 weeks post-implantation. This is essential for normal early fetal neurogenesis. T4 concentrations are highly regulated to maintain low concentrations, essential for protecting the fetus and reaching key neurological sites such as the cerebral cortex at specific developmental stages.

Thyroid hormones primarily regulate genes involved in myelination and neuronal glial cell differentiation (Bernal, 2005). Delivery of thyroid hormones to the fetal brain is a complex process requiring, at different times, expression of brain thyroid hormone receptors, maternal-fetal thyroid hormone and iodine transport, an intricate system of endocrine feedback (HTP axis) and thyroid hormone metabolism by liver and brain deiodinase enzymes (D2) and D3 to ensure basal levels are sustained (Zoller et al, 2007).

In 1969, Man and Jones suggested that mild maternal hypothyroidism alone was associated with lower IQ levels in the offspring. In 1990, Matsuura and Konishi documented that fetal brain development is adversely affected when both the mother and fetus have hypothyroidism caused by chronic autoimmune thyroiditis.

Pop et al (1999) reported that low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Neurodevelopment was assessed in 220 healthy infants at 10 months of age using Bayley Scales of Infant Development. Children of women with FT4 levels below 5$^{th}$ (0.76 ng/dl) and 10$^{th}$ (0.80 ng/dl) percentiles at 12 weeks gestation had significantly lower scores on Bayley Psychomotor Development Index (PDI) at 10 months of age, compared to children of mothers

with higher FT4 levels. At 32 weeks gestation, no significant differences were found.

Kooistra et al (2006) confirmed that maternal hypothyroxinemia constitutes a serious risk factor for neurodevelopmental difficulties that can be identified in neonates as early as 3 weeks of age. The group examined 108 neonates who were born to mothers with low FT4 levels (<10$^{th}$ Percentile) at 12 weeks gestation and 96 neonates who were born to women whose FT4 values were between 50$^{th}$ to 90$^{th}$ percentiles. Newborn development was assessed using Neonatal Behavioral Assessment Scale (NBAS). Infants of women with low FT4 at 12 weeks had significantly lower scores on the NBAS orientation index compared to other infants.

In 2011, Su et al (China) studied 1027 serum samples of women with singleton pregnancy for TSH and FT4 during first 20 weeks of gestation. Clinical hypothyroidism was associated with congenital circulation system malformations; the adjusted odds ratio (95% (CI) was 10.44 (1.15–94.62). Subclinical hypothyroidism was associated with poor vision development, and neurodevelopment delay; the adjusted odds ratios (95% CI) were 5.34 (1.09–26.16), and 10.49 (1.01–119.19), respectively. Isolated hypothyroxinemia was related to musculoskeletal malformations; the adjusted odds ratios (95% CI) was 9.12 (1.67–49.70). Wang et al (2011) reported that maternal thyroid disorders during early pregnancy can influence pregnancy outcome and fetal development.

From the above discussion and from results of present study it is evident that thyroid hormones during early gestation play an important role in brain development. It is indicative from the data, that these hormones being a major metabolite hormone have its influence in regulating the system. The data further suggests, all pregnant women should be subjected to thyroid screening at the onset of pregnancy and government should take initiative in implementing the same.

**General discussion**

The high global prevalence of iodine deficiency and thyroid disorders, the mental and physical consequences of these disorders create a huge human and economic burden that can be prevented by early detection and therapeutic measures. Over the past several years it has been proved that maternal thyroid disorders influence the outcome of both mother and fetus, during pregnancy and after pregnancy. However, currently there are no recommendations for universal screening for thyroid disorders in women before or during pregnancy.

In present study we have made an attempt to justify early screening of pregnant women for thyroid disorders. We have screened pregnant women using two TSH cut-offs [2.5 and 5.0 μIU/ml]. Screening results indicated that, 28% women were at low risk (TSH >2.5) and 5.5% women were at high risk (TSH >5.0) of developing hypothyroidism.

We have followed (sub sample) these pregnant women till delivery and one year postpartum. TSH cut-off of 2.5 (first trimester) and 3.0 (second and third trimester) μIU/ml, with FT4 cut-off of 0.65 ng/dl (first, second and third trimester) was used for defining thyroid dysfunction during pregnancy. However, during postpartum period when thyroid status of these women was again tested, normal adult reference range for TSH (0.25-5.0 μIU/ml) and FT4 (0.65-2.10 ng/dl) was used. Results indicated that, mean values for all four thyroid hormones (TSH, FT4, TT4 and TG) were normal, but thyroid dysfunction was found in 32.88%, 43.84% and 31.51% women during first, second and third trimester respectively. Thyroid hormones were compared using different trimester specific reference intervals. Results of comparison revealed that, use of different trimester specific reference interval resulted in different prevalence of thyroid dysfunction. Hence, we concluded that choosing a right method is very essential.

During postpartum period only 9.9% (2.5% overt hypothyroidism and 7.4% subclinical hypothyroidism) women were found to have thyroid

dysfunction. Not a single woman was found to be hypothyroxinemic during this period. After comparing thyroid function of women during pregnancy and lactation (postpartum period) we found that, 90% women become normal after pregnancy. Thus we concluded, due to pregnancy there were fluctuations observed in thyroid hormones especially TSH and FT4. A euthyroid state of mother during early pregnancy is very important for proper development and differentiation of fetal brain. However, most of them were not able to maintain euthyroid state during pregnancy.

After obtaining thyroid hormone status of women during postpartum period (when there was no effect of pregnancy induced changes on thyroid gland), we compared our two diagnosis methods (TSH >2.5 and TSH >5.0 μIU/ml). Our result revealed that, TSH cut-off >2.5 μIU/ml was proved to be a better indicator of thyroid status with high sensitivity and specificity as compared to TSH cut-off >5.0 μIU/ml.

Hence, considering our results of pregnancy and postpartum period, the need to asses thyroid function during pregnancy is justified.

We have also observed the effect of thyroid dysfunction during early gestation on infant development at 6 and 12 months. A significant difference of 0.2 and 0.4 was found between mean BDSTI scores of both groups (with thyroid dysfunction and with normal thyroid function) at 6 and 12 months respectively. This difference is an indicator of effect of early gestation thyroid dysfunction on mental and psychomotor development of infant.

Iodine Deficiency is the world's leading cause of preventable intellectual disability and Iron Deficiency Anemia is the most common and wide-spread nutritional disorder in the world. Iodine plays a critical role in the neuropsychological development of the fetus throughout gestation and in the first two years of life. Iron is critical for cognitive and motor development in childhood and for physical activity in all humans. The requirements for these two micronutrients are increased during pregnancy and lactation as compared to non

pregnant state. These increased requirements are higher to meet the physiological changes and increased nutritional needs during pregnancy and lactation.

In present study, double fortified salt (DFS) was considered as an additional strategy (along with IFA supplementation) to combat IDA and ID during lactation. During lactation, women were randomized into experimental group (DFS supplementation for 6 months) and control group (single iodized salt for 6 months).

We found a significant increase of 0.22 g/dl in hemoglobin in experimental group compared to a significant decrease of 0.17 g/dl in hemoglobin in control group. Median urinary iodine level significantly increased by 78 µg/l in experimental group, while in control group it decreased by 16 µg/l (non significant). DFS delivered crucial amount of iodine and iron to these (experimental group) women through their diets. Hence we conclude that, DFS helps in sustaining iron and iodine levels in lactating women.

# SUMMARY AND CONCLUSION

**Phase I**

**Screening for thyroid dysfunction**

- Screening of pregnant women for thyroid dysfunction during early gestation revealed that 28% women were at low risk (TSH >2.5 mIU/l) and 5.5% women were at high risk (TSH >5.0 mIU/l) of developing hypothyroidism.

**Phase II**

**Dietary intake of pregnant women**

- Calorie and protein intake of pregnant women was quite low, which resulted in low weight gain (5.4 kg) during entire pregnancy.

**Iron status**

- Mean hemoglobin during first, second and third trimester indicated moderate IDA. Prevalence of IDA during first, second and third trimester was found to be very high (I-97.26%, II-94.52% and III-91.78%). An improvement in mean hemoglobin and iron status was found with advancing gestation.

**Iodine status**

- Median urinary iodine during first, second and third trimester showed adequate intake by the population. Prevalence of iodine deficiency during first, second and third trimester was found to be low (I-23.29%, II-30.14% and III-19.18%). An increase in median urinary iodine levels was found from first to second trimester, while from second to third trimester a minor decrease was found which was non-significant. With urinary iodine data we can presume that median urinary iodine levels more or less remained same during pregnancy with slight fluctuations.

**Thyroid dysfunction**

- Mean TSH, FT4, TT4 and TG were falling under normal range (adult reference value). Thyroid dysfunction was found in 32.88%, 43.84% and 31.51% during first, second and third trimester respectively. There was an increase in mean TSH, FT4 and TG with advancing gestation; however FT4 decreased from first to second trimester and then increased from second to third trimester. These fluctuations in FT4 levels could be due to changing concentrations of TBG during entire pregnancy.

**Importance of trimester specific reference intervals**

- Different trimester specific reference intervals resulted in different prevalence of thyroid dysfunction. With method 1, most of the pregnant women were falling under normal category, with method 2, most of pregnant women were found to have hypothyroidism and with method 3 most of pregnant women were found to have hypothyroxinemia. Hence choosing a right method is very important.

**Impact of KAP**

- Knowledge of pregnant women regarding two major micronutrients (iodine and iron) was poor (knowledge regarding iodized salt-39.7% and IFA-28.2%).
- A positive impact of NHE was found with respect to Knowledge, Attitude and Practices of pregnant women regarding iodized salt.

**Food frequency**

- Consumption of iron rich foods, vitamin C rich foods and non vegetarian food items were not appreciable.

**Maternal and child health indicators**

- Maternal health indicators and performance indicators for maternal health services revealed a better status and performance than NHFS 3 findings.
- Colostrum feeding and exclusive breast feeding was done by almost all women. Child care indicators reveal that as time

progressed mothers became careless in availing immunization services for their child.

**Phase III**

**Screening of neonates**

- Raised CBTSH was found in 12.60% neonates and low CBFT4 was found in 9.25% neonates.
- Respite a low weight gain by pregnant women, mean birth weight was above normal. However, mean gestational age was found to be 35.57 weeks; this low mean gestational age could be due to wrong reporting of last menstrual period by pregnant women.

**Phase IV**

**Postpartum status of women**

- Dietary intake of women during lactation was even poorer than during pregnancy.
- Like pregnancy, moderate IDA was also found during lactation.
- Median urinary iodine levels during lactation indicated adequate iodine intake by the population.
- There was a huge difference in prevalence of thyroid dysfunction during pregnancy and postpartum period. Most of women became normal after pregnancy. This indicates that these women were not able to maintain euthyroid state during pregnancy.
- After comparing 2 diagnostic tests for testing hypothyroidism; 1) with TSH upper limit as 2.5 and 2) with TSH upper limit as 5.0, we found that method 1 had better sensitivity, specificity and positive predictive value.
- Mean hemoglobin, FT4 and TG were found to be highest during postpartum period. After pregnancy there was a slight fall in mean TSH and TT4, which is indicative of reversibility of pregnancy induced changes in thyroid status.

**Infant status**

- Median urinary iodine of infants indicated adequate iodine intake and none of the infants were found to have urinary iodine levels below normal value.
- Overall nutritional status of infants was found to be below normal, this could be due to poor dietary intake of their mothers during pregnancy and lactation. However, their status with respect to under nutrition and wasting was improved from six months to twelve months but recovery in case of stunting and head circumference for age was not observed, thereby indicating compromised brain development and therefore lower IQs as the child grows.

**Impact of DFS supplementation**

- There was a significant increase of 0.22 g/dl in hemoglobin in experimental group, while in control group there was a significant decrease in hemoglobin of 0.17 g/dl. Median urinary iodine increased by 78 μg/l ($p<0.05$) in experimental group and in control group it decreased by 16 μg/l ($p=0.964$).
- It can be concluded that, DFS helps in sustaining iron and iodine levels in lactating women. DFS, if supplemented from the adolescent period till twelve months postpartum, along with modifications in dietary habits, will improve the iron and iodine status of women during the most critical periods of their life, that is, pregnancy and lactation.

**Effect of thyroid dysfunction during early gestation on infant development**

- A significant difference of 0.2 and 0.4 was found between mean BDSTI scores of both groups (with thyroid dysfunction and with normal thyroid function) at 6 months and 12 months respectively.
- This difference is an indicator of effect of early gestation thyroid dysfunction on mental and psychomotor development of the infant.

# RECOMMENDATION

- Screening of pregnant women during early gestation using a lower TSH cut-off can result in identifying pregnant women who may be at risk of developing hypothyroidism.
- Adequate iodine status (UI) during pregnancy in not an indicator of thyroid sufficiency as respite an adequate iodine intake during pregnancy as reflected by urinary iodine values (UI>150 μg/l) pregnant women were not able to maintain euthyroid state. Hence it is recommended that spot UI should not be used as a sole indicator to identify iodine sufficiency during pregnancy. Instead of spot UI, it is advisable to use 24 hour sample.
- Use of appropriate reference intervals or cut-offs which are method and trimester specific is essential for monitoring thyroid function during pregnancy.
- DFS can be used as an additional strategy to combat anemia among women along with IFA supplementation and dietary modification.
- Since thyroid dysfunction during early gestation effects infant development, it is of great importance to diagnose thyroid dysfunction during early gestation and start appropriate treatment.
- All pregnant women should be subjected to thyroid screening at the onset of pregnancy and government should take initiative in implementing the same.

# LIMITATIONS

TPO-Ab and Tg-Ab were not analyzed because of cost constrains.

Lost to follow up during cord blood collection was due to poor responsible behavior from subjects, respite informing them before time, they did not inform us regarding delivery details like the actual date, time and place (During third trimester we asked for permission from hospital management to collect the blood of infant using heel prick method, unfortunately we did not get the permission, the hospital management gave explanation that from our side we will take care but what will happen if the infant gets infection after going home).

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# APPENDICES

**Appendix-i**

**FORM (pregnant women)**

**General information**

1. **Code:**
2. **Contact no.:**
3. **Address:**
4. **Name:**
5. **Age (y):**
6. **Religion:**

   Hindu: Muslim: Sikh: Christian:
7. **Qualification:**

   Illetrate: Primary: X pass: XII pass: Graduate:
8. **Occupation:**

   Working: Housewife:
9. **LMP:** **EDD**
10. **Gravida:** **Parity:** **Abortion:** **Live:** **Dead:**

11. Type of family:

    Nuclear: Joint: Extended:
12. **Total family members:**

    2: 3-4: 5-6: >6:
13. **Total family income (rs.):**

    <2,000: 2,000-5,000: 5,000-10,000: >10,000:
14. **Percapita income:**
15. **Morbidity profile:**

| **Family member** | **Type of disease** | **Duration** |
|---|---|---|
| | | |

**Anthropometric measurement**

| Anthropometric Measurements | Trimester | | |
|---|---|---|---|
| | First | Second | Third |
| Weight | | | |
| Height | | | |

**Biochemical parameters**

| Trimester | First | Second | Third |
|---|---|---|---|
| Serum & Urine Sample Collection | | | |
| UI | | | |
| HB | | | |
| Thyroid hormone | Trimester | | |
| | First | Second | Third |
| TSH | | | |
| FT4 | | | |
| TT4 | | | |
| TG | | | |
| Cord Blood TSH at birth | | | |

**Supplements (received from hospital)**

| Supplements | Months | | | | | | |
|---|---|---|---|---|---|---|---|
| | III | IV | V | VI | VII | VIII | IX |
| Iron | | | | | | | |
| Folic Acid | | | | | | | |
| Calcium | | | | | | | |
| Multivitamin | | | | | | | |
| Zinc | | | | | | | |
| Other | | | | | | | |

**Salt supplementation**

| | Trimester I | | | Trimester II | | | Trimester III | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | III | IV | V | VI | VII | VIII | IX |
| DFS | | | | | | | | | |

**Antenatal Information**

1. **First antenatal visit (month):**
2. **Total no. of antenatal visits (month):**
3. **Abdominal examination:**

| Trimester I | | Trimester II | | Trimester III | |
|---|---|---|---|---|---|
| 3rd M | | 7th M | | 9th M | |

4. **Blood pressure measurement:**

| Trimester I | | Trimester II | | Trimester III | |
|---|---|---|---|---|---|
| 3rd M | | 7th M | | 9th M | |

5. **Weight check-up:**

| Weight Check-up (kg) | 1st Visit | Months | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4th | 5th | 6th | 7th | 8th | 9th |
| | | | | | | | |
| | | | | | | | |

6. **Iron folic acid supplementation:**

| Trimester II | | | | | |
|---|---|---|---|---|---|
| 4th M | | 5th M | | 7th M | |

7. **TT injection:**

| Month | | | |
|---|---|---|---|
| 4th | | 6th | |

8. **Intestinal parasite drug:** Yes- No-
9. **Information of specific pregnancy complications:**

Yes- No-

Difficulty with vision during daylight-

Night blindness-

Convulsions not from fever-

Swelling of the legs or face-

Excessive fatigue-

Vaginal bleeding-

**KAP**

1. **Have you ever heard about iodized salt:**

   Yes- No-

2. **Can you recognize iodized salt:**

   Yes- No-

   If yes, how will you recognize?

   Smiling sun logo- other-

3. **Do you know the brand name of salt that is used in your family:**

   Yes- No- if yes, specify-

4. **Cost of salt (per kg):**

   2 3-5 6-10 >10

5. **Amount of salt used in the family (kg):**

   1 2 >2

6. **Where do you keep your salt:**

   Near flame- Away from flame-

7. **How do you store salt:**

   Covered contained- Uncovered contained- Polythene-

8. **At what time you add salt during cooking:**

   Starting- Mid- End-

9. **Have you ever heard about IFA-**

   Yes- No-

10. **Are you under any of the services provided by government:**

    Yes- No-

    If no, what are the reasons behind not availing the services?

    Not interested- not aware- cant answer-

11. **From where do you get supplements:**

    Gov. Hospital- Private hospital- AWC-

12. **Do you regularly take supplements:**

    Yes- No-

    If yes, how many tablets are provided each time?

<15 15-30 >30

Frequency of consuming tablets:

Daily- 2-3 times a wk- weekly- once in 15 days-

If no, what are the reasons behind not taking supplements?

Not necessary- side effects- dislike- often forget-

13. **Have you faced any side effects after consuming the tablets:**

Yes- No- If yes, specify-

14. **What do you do in case of side effects:**

Continue- Discontinue- Reduce the dose-

15. **Have you felt any beneficial change after receiving the supplements:**

Yes- No- if yes, specify-

**<u>Dietary Information</u>**

1. **Is there any change in your diet patter during pregnancy:**

Yes- No- if yes, specify-

2. **Any special food items consumed during pregnancy:**

Yes- No- if yes, specify-

3. **Type of food consumed:**

V- NV- OV-

**Appendix-ii**

**FORM (Lactating women)**

**Delivery care**

1. Place of delivery
2. Delivery type

**Postpartum care**

1. Postnatal check-up
2. Within 48 hours

**Feeding**

1. Colostrum feeding
2. Initiation of breastfeeding within 1 hour
3. Exclusive breastfeeding (6 months)
4. Complementary feeding (+BF >6 months)

**Complications**

1. Postpartum haemorrhage
2. Eclampsia
3. Genital infection
4. Postpartum blues
5. Postpartum depression

**Anthropometric measurements and biochemical parameters (PP period)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Weight | (6 months PP) | | | | | |
| HB | 6 months PP | | | 12 months PP | | |
| Urine sample | 6 months PP | | | 12 months PP | | |
| Serum sample | | | | | | |
| Supplementation | 6 m PP | 7 m PP | 8 m PP | 9 m PP | 10 m PP | 11 m PP |

**Appendix-iii**

**FORM (infant)**


Contact: Code:
Mother Name: Infant Name:
DOB: _____3 (m) _____6 (m) _____9 (m) _____12 (m) _____

| **Details (infant)** | | **Explanation** |
|---|---|---|
| Cord blood | | √/x |
| Sex | | M/F |
| Term | | Full term/ Pre term |
| Birth weight | | Kg |

**Details of Immunization** **Growth Chart**

| **Month** |  | | **Month** | **Weight (kg)** | **Length (cm)** | **HC (cm)** |
|---|---|---|---|---|---|---|
| 1.5 | BCG | | 1 | | | |
| 1.5 | Polio 1 | | 2 | | | |
| 1.5 | DPT 1 | | 3 | | | |
| 1.5 | Hepatitis B-1 | | 4 | | | |
| 2.5 | Polio-2 | | 5 | | | |
| 2.5 | DPT 2 | | 6 | | | |
| 2.5 | Hepatitis B-2 | | 7 | | | |
| 3.5 | Polio-3 | | 8 | | | |
| 3.5 | DPT 3 | | 9 | | | |
| 3.5 | Hepatitis B-3 | | 10 | | | |
| 9 | Measles | | 11 | | | |
| 9 | Vitamin A | | 12 | | | |
| 16-24 | DPT | | | | | |
| 16-24 | Polio | | | | | |
| 16 | Vitamin A | | | | | |
| 24 | Vitamin A | | | | | |
| 30 | Vitamin A | | | | | |
| 36 | Vitamin A | | | | | |

| Age (M) | Sr. No. | Items | BDSTI score |
|---|---|---|---|
| 1 | 1 | Arms and legs thirst in play | Child score at 6 months<br>$\frac{itemspassed}{totalitems}$<br>Child score=$\frac{\mathbf{items}}{\mathbf{18items}}$ |
| | 2 | Momentary regard | |
| | 3 | Lateral head movement (prone) | |
| 2 | 4 | Responds to sound | |
| | 5 | Follows moving person | |
| | 6 | Free inspection of surroundings | |
| 3 | 7 | Social smile/vocalizes | |
| | 8 | Eye co-ordination | |
| | 9 | Head erect and steady | |
| 4 | 10 | Holds head steady | |
| | 11 | Recognises mother | |
| | 12 | Elevates on arms | |
| 5 | 13 | Play with rattle/hand play | |
| | 14 | Reaches for dangling ring | |
| | 15 | Sits with slight support | |
| 6 | 16 | Turns head to sound | |
| | 17 | Turns from back to side | |
| | 18 | Exploitive paper play | |
| 7 | 19 | Discriminates strangers | Child score at 12 months<br>$\frac{itemspassed}{totalitems}$<br>Child score=$\frac{\boldsymbol{items}}{\mathbf{35}\boldsymbol{items}}$ |
| | 20 | Pulls to sit | |
| 8 | 21 | Bangs to play | |
| | 22 | Sits alone steadily | |
| 9 | 23 | Retails two things in two hands | |
| | 24 | Pulls to stand | |
| | 25 | Playful response to mirror images | |
| | 26 | Sits with good coordination | |
| 10 | 27 | Pulls string-secures toy | |
| | 28 | Co-operates in play | |
| | 29 | Crawling (pre-walking) | |
| 11 | 30 | Rings bell purposefully | |
| | 31 | Fine prehension | |
| | 32 | Raises to sit | |
| | 33 | Stands by furniture | |
| 12 | 34 | Adjusts to words | |
| | 35 | Says da-da | |

**Appendix-iv**

**24 HOUR DIETARY RECALL**

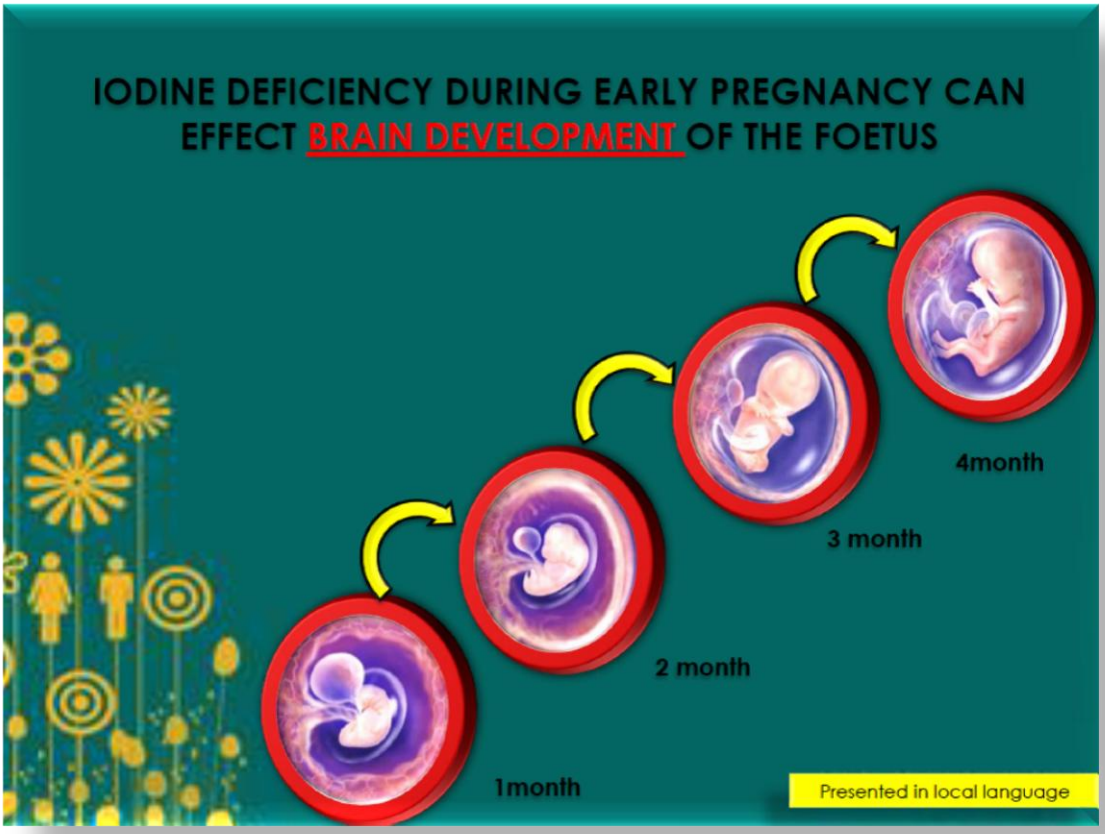
**Code :** **Name :**

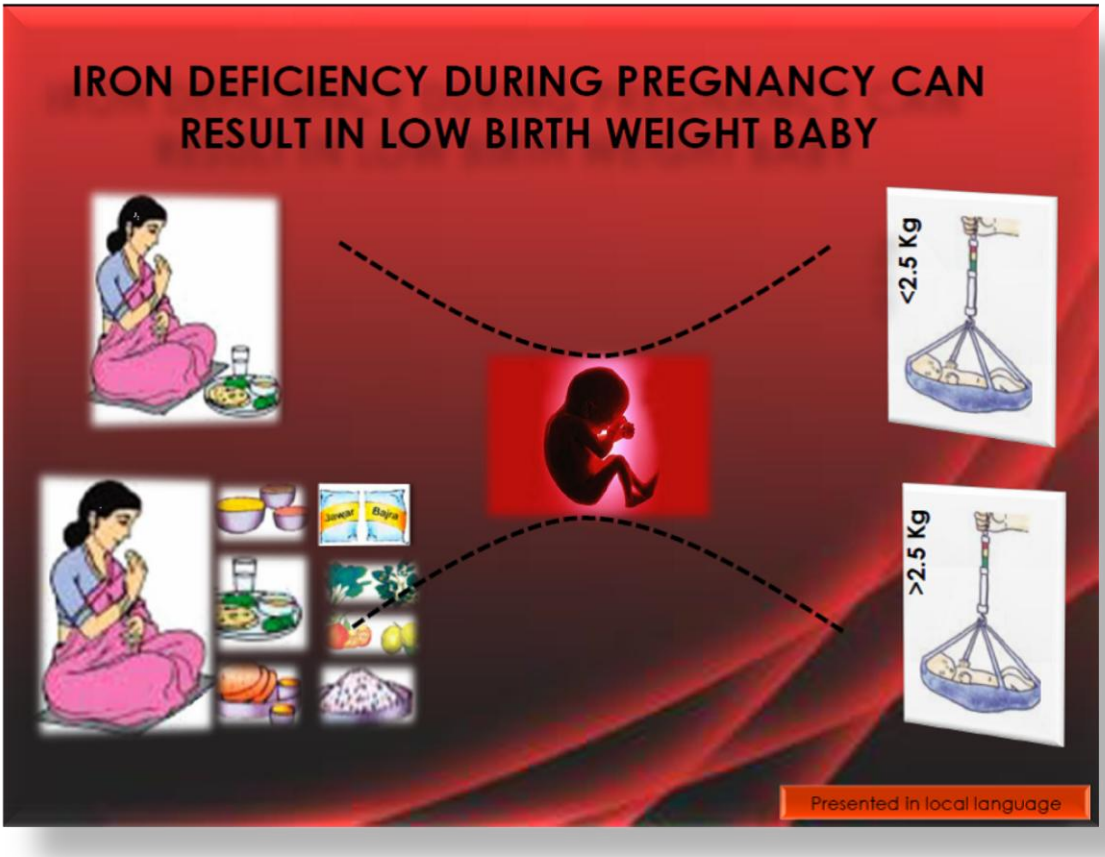
| Meal | Item | Ingredients | Amt |
|---|---|---|---|
| | | | |

## Appendix-v

| | FOOD FREQUENCY | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Food Groups** | Daily | 3-4 times wk | 2ce wk | 1ce Wk | Once 15 days | Mon-thly | Seaso -naly | Occasio-nally | Never |
| **Cereals** | | | | | | | | | |
| Rice flakes (pohe) | | | | | | | | | |
| Bajra (bajri) | | | | | | | | | |
| Rice puffed (murrmura) | | | | | | | | | |
| Wheat flour (ato) | | | | | | | | | |
| **Pulses** | | | | | | | | | |
| Soyabean | | | | | | | | | |
| Bengal gram, roasted (phutana) | | | | | | | | | |
| Moth beans (mut) | | | | | | | | | |
| Cow pea (chorap) | | | | | | | | | |
| Lentil (masur) | | | | | | | | | |
| Peas, dry (vatana) | | | | | | | | | |
| Peas, roasted | | | | | | | | | |
| Bengal gram dhal (chaneki dhal) | | | | | | | | | |
| Rajmah (phanasi) | | | | | | | | | |
| **GLVs** | | | | | | | | | |
| Shepu (suvanibhaji) | | | | | | | | | |
| Mustard leaves (sarsokasaag) | | | | | | | | | |
| Colocasia leaves (arvikasaag) | | | | | | | | | |
| Amaranth leaves (chaulikasaag) | | | | | | | | | |
| **Other** | | | | | | | | | |
| Onion stalks (dunglinadakkadi) | | | | | | | | | |
| Plantain, green (kela) | | | | | | | | | |
| Kankoda | | | | | | | | | |
| Dates (khajoor) | | | | | | | | | |
| Niger seeds (kalatil) | | | | | | | | | |
| Water melon (tarbooj) | | | | | | | | | |
| Sitaphal | | | | | | | | | |
| **Fruits** | | | | | | | | | |
| Amla | | | | | | | | | |
| Guava (jam phal) | | | | | | | | | |
| Sweet lime (musambi) | | | | | | | | | |
| Pineaaple (ananas) | | | | | | | | | |
| Lemon (limbu) | | | | | | | | | |
| Orange (santra) | | | | | | | | | |
| Tomato ripe | | | | | | | | | |
| **Non veg.** | | | | | | | | | |
| Egg (murgi nu indu) | | | | | | | | | |
| Chicken (murga) | | | | | | | | | |
| Mutton (ghetanugos) | | | | | | | | | |

## Appendix-vi: IEC

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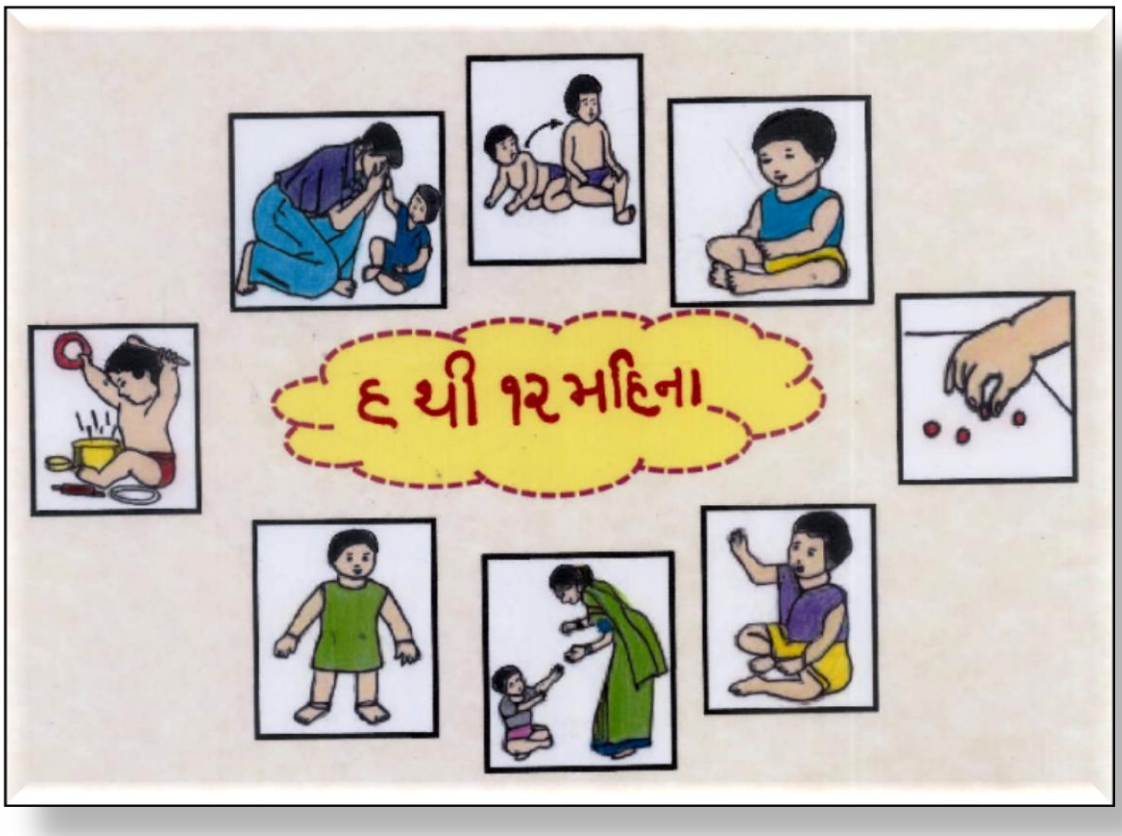
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## Appendix-vii

## REPORT FORMAT

**DEPARTMENT OF FOODS AND NUTRITION**
**FACULTY OF FAMILY AND COMMUNITY SCIENCES**
**THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA**
**VADODARA 390 002 - INDIA**

**Research Work**

**Screening for Iodine Deficiency in Pregnant Women**

**(Clinical Hypothyroidism/Subclinical Hypothyroidism/Hypothyroxinemia)**

**Patients Name: _____Code No. _____**

| **Thyroid Function Tests** | **Normal range** | **Pregnancy** |
|---|---|---|
| TSH : _____ µIU/mL | 0.25-5.1 | <2.5 |
| FT4 : _____ ng/dL | 0.65-2.1 | |

Assay technique: **RadioImmuno Assay** (BARC, Mumbai)
The above figures are laboratory values and do not indicate clinical diagnosis. This report is for use by qualified medical professionals only.

| **Condition Status** | **TSH** | **FT4** | **Patient** |
|---|---|---|---|
| Clinical Hypothyroidism | ↑ | ↓ | _____ |
| Subclinical Hypothyroidism | ↑ | N | _____ |
| Hypothyroxinemia | N | ↓ | _____ |
| Normal | N | N | _____ |

**Remarks**

_____

**Referral:** Yes _____ No _____

**Date:**

## Appendix-viii

## Consent Form (Local Language)

### સંમતિ પત્રક

**"આયોડિન અને લોહતત્વની ઊણપ અંગેનો સંશોધન કાર્યક્રમ "**

વ્હાલી બહેનો,

અમો મહારાજા સયાજીરાવ યુનિવર્સિટીની ફુડ્સ એન્ડ ન્યટ્રિશન શાખા દ્વારા ગર્ભવતી બહેનો અને તેમના આવનાર બાળકમાં આયોડિન અને લોહતત્વની ઊણપ અંગેનું સંશોધન કરી રહ્યા છીએ.

આયોડીન અને લોહતત્વ બે મહત્વના તત્વો છે કે જેની ઊણપથી માતા અને બાળકના સ્વાસ્થ્ય પર માઠી અસર થાય છે. બાળકનો માનસિક અને શારિરિક વિકાસ પણ રુંધાય છે આથી આપના પેશાબ અને લોહીના નમૂના દ્વારા આપનામાં આ ઊણપ અંગેની તપાસ અમારા આ સંશોધનમાં હાથ ધરવામાં આવશે.

આપના નમૂનાની તપાસના રિપોર્ટની કોપી પણ આપને આપવામાં આવશે.

તદ્ઉપરાંત આપના ખોરાક અને પોષકને લગતી માહિતી આ સંશોધન કાર્ય દરમ્યાન પૂરી પાડવામાં આવશે અને આપની ગર્ભાવસ્થાના પૂરાગાળા સુધી આયોડિન અને આયર્નયુકત મીઠું પૂરું પાડવામાં આવશે જે આપના કુટુંબ માટે પણ વાપરી શકાશે.

અમારા આ સંશોધન કાર્યમાં સંમતિ આપવા વિનંતી આભાર

સંશોધક

કેજલ જોષી

રીતુ રાણા

આપની સહી

_____

**Appendix –ix**

**Development curve -BDSTI**

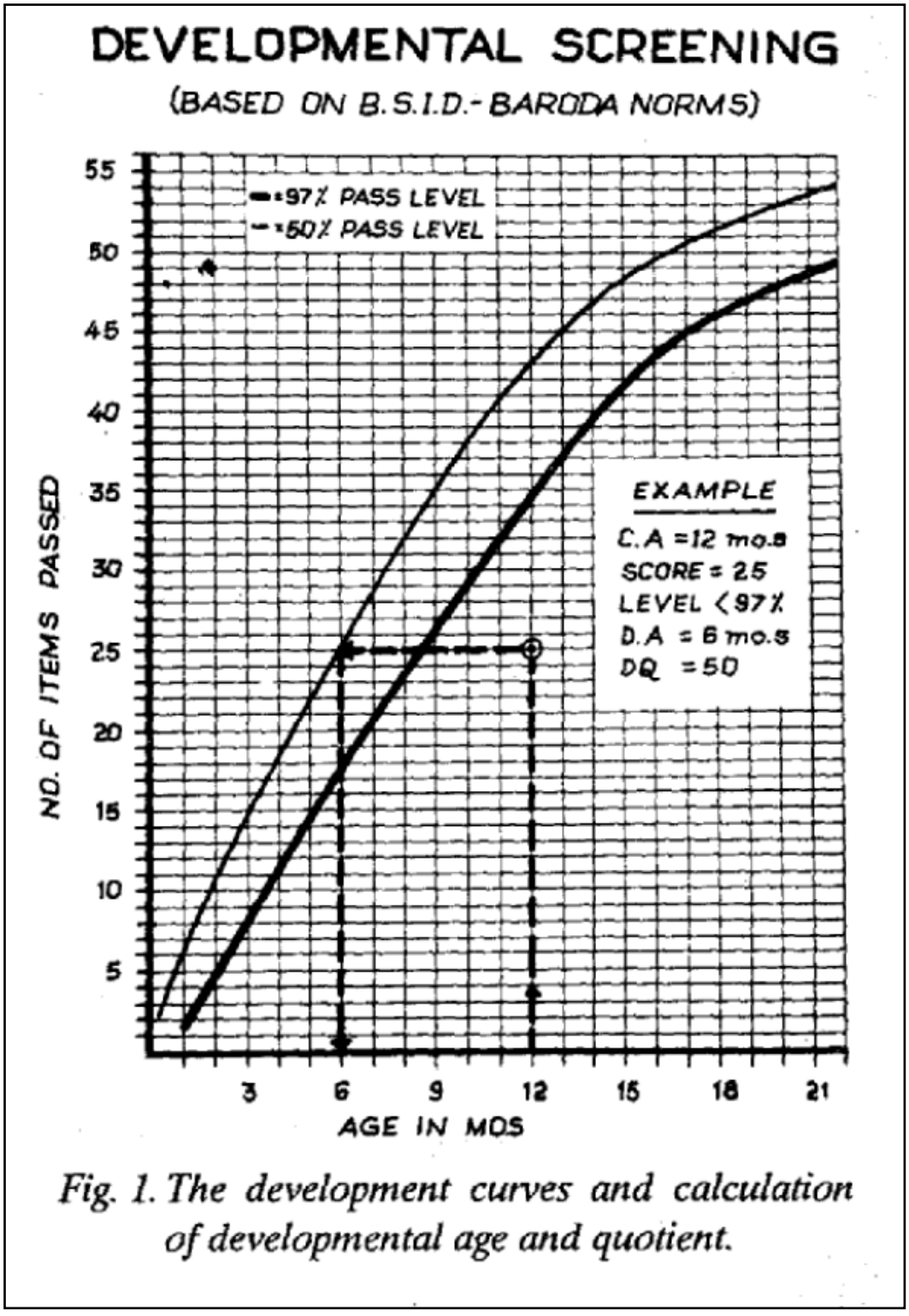


*Fig. 1. The development curves and calculation of developmental age and quotient.*

## SPECIAL THANKS TO:

All doctors, especially gynaecologist *Dr. G N Patel* and *Dr. Rajendra Chauhan* and Paediatrician *Dr. Dilip Vaghera*, PPTCT counsellors *Mrs. Nimisha* and *Mr. Nilay* and other staff of Jamnabai General Hospital, Vadodara.

All *pregnant women*, who made most valuable contribution to this study by agreeing to give their blood and urine samples 4 times, along with other general and diet related information.

All *lactating women*, who responded to our calls, turned to hospital as and when required and who took out special time for providing us urine samples of their infants.

All *infants* who have participated in this study by taking out time from their playing and sleeping hours during INFANT DEVELOPMENT TESTING at six months and twelve months.