SCREENING OF THYROID DISORDERS FOR EARLY PREVENTION

Ph.D. THESIS

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Certificate

This is to certify that the research work presented in the results of this thesis has been carried out independently by Ms. Juhi Agarwal in pursuit of a Doctoral Degree in Foods and Nutrition and represents her original work.

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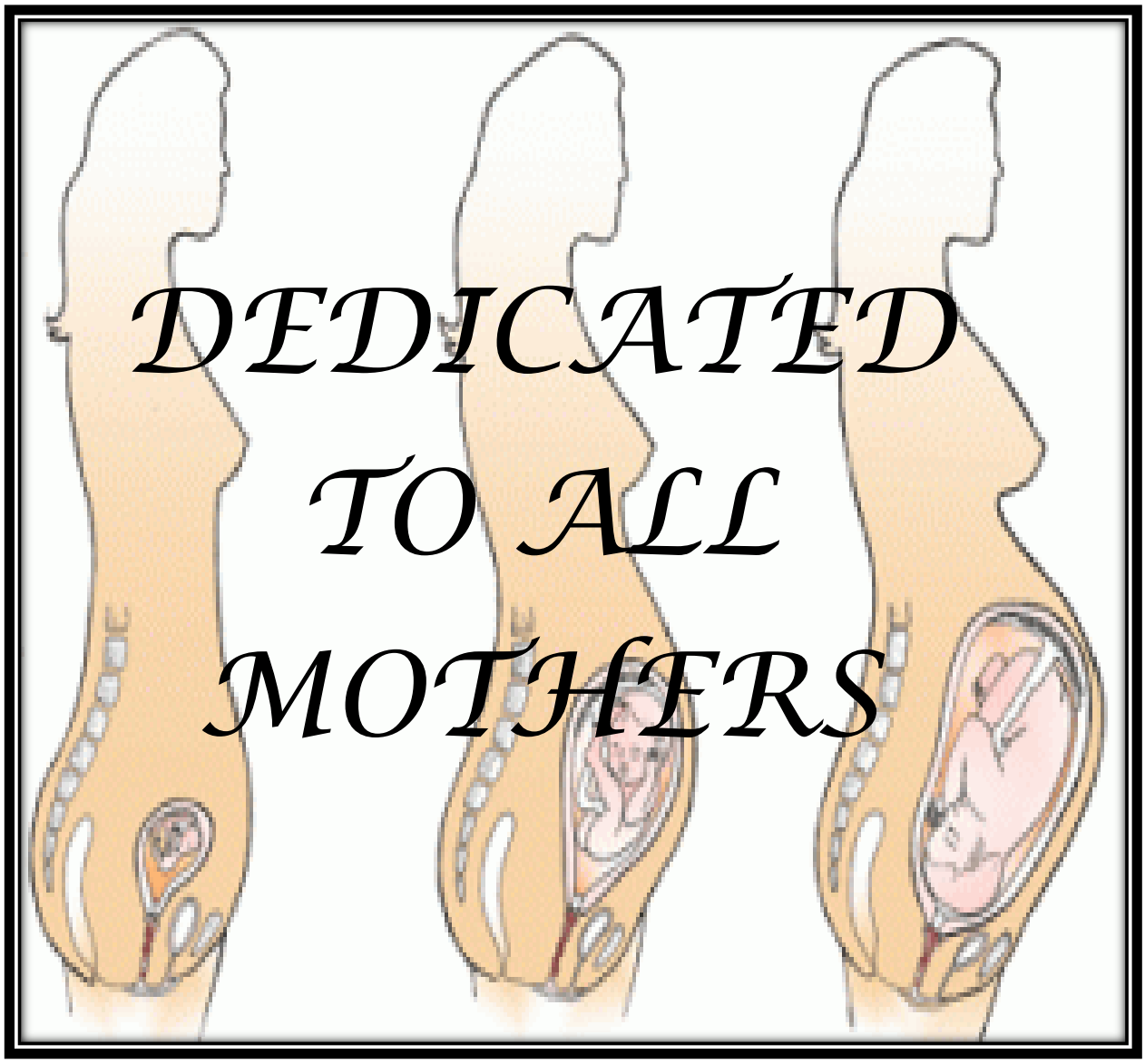
DEDICATED
TO ALL
MOTHERS

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Juhi Agarwal

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LIST OF ABBREVIATIONS

µg	Micrograms (millionths of a gram)
AITD	Autoimmune thyroid disease
AOAC	Association of Official Analytical Chemists
BAT	Brown adipose tissue
BMI	Body mass index
CFT_4	Cord blood free thyroxine
cm	Centimeters
CNS	Central nervous system
CTSH	Cord blood thyrotropin
ECL	Electrochemiluminiscence
FT_3	Free triiodothyronine
FT_4	Free thyroxine
g	Grams
GTT	Gestational transient thyrotoxicosis
Hb	Hemoglobin
HC	Head circumference
hCG	Human chorionic gonadotropin hormone
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine deficiency disorders
IH	Isolated hypothyroxinemia
IQ	Intelligence Quotient
IU	International unit
IUGR	Intra uterine growth restriction
KAP	Knowledge, attitudes and practice
L-T4	Levothyroxine
MDI	Monodeiodinase
MS	Mass spectrometry
N	Number
NACB	National Academy of Clinical Biochemistry
NIS	Sodium Iodide Symporter
OH	Overt hypothyroidism

pM	Picomoles
PP	Postpartum
PPT	Postpartum thyroiditis
PTU	Propylthiouracil
RDA	Recommended dietary allowance
RIA	Radioimmunoassay
SCH	Subclinical hypothyroidism
SD	Standard deviation
SGA	Small for gestational age
T_3	Triiodothyronine
T_4	Thyroxine
TAI	Thyroid autoimmunity
TBG	Thyroxine binding globulin
Tg	Thyroglobulin
TPO-Ab	Thyroperoxidase antibody
TRH	Thyrotropin releasing hormone
Trim	Trimester
TSH	Thyroid stimulating hormone
TTR	Transthyretin
UIC	Urinary iodine concentration
UNICEF	United Nations Children's Fund
UNU	United Nations University
WHO	World Health Organization

Abstract

ABSTRACT

Pregnancy is a euthyroid state that is normally maintained by complex changes in thyroid physiology. Thyroid disease is the second most common endocrine disorder (after diabetes mellitus) affecting women of reproductive age. Over the past several years it has been proved that maternal thyroid disorder influence the outcome of mother and fetus, during and also after pregnancy. The most frequent thyroid disorder in pregnancy is maternal hypothyroidism. Worldwide, particularly in mountainous regions and in Central Africa, South America and northern Asia, the most common cause of hypothyroidism is iodine deficiency. In areas of iodine sufficiency, the most common cause of hypothyroidism in pregnant women is Hashimoto's (chronic thyroiditis), an autoimmune disease where the bodies own antibodies attack the thyroid.

The objective of the study was to screen pregnant women so as to prevent brain damage of the fetus, 341 pregnant women in the first trimester of pregnancy [189 women with non specific thyroid status comprised group I and 145 women who were already on hormone replacement therapy comprised group II] were enrolled from various hospitals of Delhi. The subjects in both the groups were serially followed throughout pregnancy and upto six months postpartum. Anthropometric and physiological parameters like height, weight, thyroid gland examination were recorded and serum was analyzed for free triiodothyronine (FT_3), free thyroxine (FT_4), thyroid stimulating hormone (TSH), thyroperoxidase antibody (TPO-Ab) and hemoglobin (Hb). Urine was also collected for estimation of urinary iodine concentration (UIC). All the examinations were carried out in the three trimesters and 6 weeks, 12 weeks and 24 weeks postpartum (PP). Also the subjects were provided with nutrition health education (NHE). Their diet history, food frequency and knowledge, attitude and practices regarding iodized salt were recorded at the time of registration and in third trimester after providing NHE.

Only ninety two women in Group I and one hundred and two women in Group II regularly visited in all the three trimesters of the pregnancy, so they were further analyzed.

Out of 92 women in Group I, medicine of 24 subjects had to be started during the course of pregnancy and they formed group I B. Group I A (n=68) comprised of normal women. Majority (60%) of the subjects had normal hemoglobin. The median UIC of both the groups I A and I B showed iodine sufficiency with median UIC being 189.2 µg/L and 180.2 µg/L, respectively in first trimester. The median UIC was significantly ($p<0.05$) elevated during pregnancy when compared to postpartum and non-pregnant control group values. Only 7.4% of group I A subjects had TPO-Ab positivity whereas 67% of group I B were TPO-Ab positive.

In group I A, the mean FT_3 decreased significantly ($p<0.05$) from 4.23 ± 0.7 to 3.77 ± 1.12 pM/L in first and third trimester, respectively. It increased significantly to 4.83 ± 1.29 pM/L just after delivery (6 weeks PP). Similarly, FT_4 decreased significantly ($p<0.05$) from 14.19 ± 3.65 pM/L in first trimester to 12.83 ± 2.8 pM/L in second trimester and thereafter in third trimester decreased non-significantly to 12.69 ± 2.35 pM/L. At 6 weeks PP the FT_4 concentration increased significantly ($p<0.05$) to 15.36 ± 4.26 pM/L. The FT_3 and FT_4 concentrations remained lower in second and third trimester and reached to pre-pregnancy values postpartum. The TSH concentration (1.99 ± 1.23 µIU/ml) decreased significantly ($p<0.05$) during the first trimester by 20.7% when compared to non-pregnant state and increased significantly by 25.1 percent in second trimester (2.49 ± 1.16 µIU/ml) when compared to first trimester. Postpartum the TSH concentration showed non-significant decline but the mean remained higher than the first trimester value. A significant negative correlation ($r=-0.251$, $p<0.05$) was found between urinary iodine and TSH ($p<0.05$) in first trimester whereas in all the other trimesters the correlation was non-significant.

In group I B, the subjects had abnormal TSH in the first trimester ranging from 0.03 to 100 µIU/ml and it decreased significantly ($p<0.05$) in second and third trimester when compared to first after the initiation of levothyroxine (L-T4) treatment. The median TSH decreased significantly ($p=0.026$) by 29 percent from first to third trimester. The absolute percentage increase in dose during pregnancy was 45.58 µg/day and 25.09 µg/kg day.

In group II, out of 102 subjects, 92 were hypothyroidic (group II A) and were on levothyroxine. Rest of the subjects were hyperthyroidic, group II B (n=10). The pre-pregnancy mean FT_4 and TSH of group II A subjects was within the reference range.

Sixty four percent of the subjects had to increase their L-T4 dose one or more times during pregnancy. The mean L-T4 dose significantly ($p<0.05$) increased by 17.7 percent from initial stages of pregnancy till the end of pregnancy. On an average, the subjects whose dose was increased, required a cumulative increase in thyroid hormone dosage from baseline of 14.3 percent in the first trimester, 33 percent in the second trimester and 37.5 percent in the third trimester (Mann-Whitney U test, $p=0.02$, $p=0.00$, $p=0.00$, respectively). When these subjects were divided on the basis of etiology of hypothyroidism [subclinical hypothyroidic (SCH) and overt hypothyroidic (OH)], it was found that before pregnancy and final L-T4 dose (µg/day) was significantly higher by 33.9 percent and 20.7 percent in OH when compared to SCH. The increment in Δ% of absolute doses was higher in SCH than OH group. All the women in group II B were on propyl thio uracil (PTU) and had to decrease their dose as the pregnancy progressed.

Out of sixty eight women from Group I A with complete three trimesters data, women were excluded on the basis of laboratory results that revealed positive serum TPO-Ab. All the women having goiter, overt hypothyroidism or overt hyperthyroidism were also excluded. With these 46 women trimester- specific reference intervals for FT_3, FT_4 and TSH was established for pregnant Indian population using IA, Roche Elecsys 1010 analyzer method or similar methods. These reference intervals for FT_3, FT_4 and TSH for each trimester of pregnancy are [FT_3: 1.7-5.39, 2.38-5.12 and 2.21-5.18 pM/L; FT_4: 10.12-18.56, 8.29-19.02 and 9.27-17.69 pM/L; TSH: 0.03-4.31, 0.54-4.02 and 0.78-4.29 µIU/ml].

Pregnancy outcome of total 184 subjects (69 in group I A, 16 in Group I B, 90 in Group II A and 9 in Group II B) was available as some subjects had miscarriage or dropped out from the study or had home delivery. The parameters of the neonates showed that they had normal mean birth length, birth weight and head circumference. Approximately, twenty nine percent neonates had TSH level above 10 µIU/ml which indicates moderate iodine deficiency. A significant ($p<0.05$) positive correlation of cord FT_4 was found with birth weight, birth length and head circumference. Cord blood FT_4 was found to be positively correlated with maternal first trimester FT_4 and third trimester FT_4. Cord blood TSH was neither associated with neonatal parameters nor to maternal thyroid function tests.

A trial study on supplementation of iodine rich seaweed *Caulerpa racemosa* availed from Gujarat coast was conducted on iodine-deficient or thyroid-insufficient (n=10) pregnant women (Group III B). They were supplemented daily with 0.17 g of algae in 20 g wheat flour *ladoo* for one month so as to provide 50µg/day of iodine and 0.343 mg/day of iron. A slight non-significant increase (104.75 to 121.05 µg/L) in median UIC was observed after one month of supplementation. No significant effect of supplementation was observed on thyroid function parameters of the subjects. They also showed slight increase in hemoglobin level. Prolonged supplementation needs to be carried out further to opine on the impact of algae.

The energy and protein deficit was found in group I, II and III women at the time of registration. The increase in energy and protein was found in group I & II after NHE. There was still an energy deficit of 17 percent and 16 percent in group I and II women, respectively and an excess intake of 10 and 20 percent of protein was found in group I and II subjects, respectively after NHE. Though NHE was provided no much difference in goitrogen and iron rich food consumption pattern by group I and II subjects was observed but an improvement in consumption of vitamin-C rich foods was observed. After NHE, an improvement in consumption of salt, importance of iodine for pregnant women and how to recognize iodized salt was observed. This approach might have helped the population to sustain iodine levels in their circulation.

Thus, it can be concluded all pregnant women should be screened for iodine deficiency and/ or thyroid dysfunction as soon as the pregnancy is confirmed. Large number of subjects were found to be subclinically hypothyroid during pregnancy and they should be carefully monitored and if necessary should be put on levothyroxine. A global increase in thyroxine in early pregnancy is not appropriate. The hypothyroid women should get FT_4 and TSH tests done every month until the end of pregnancy to prevent risk of adverse maternal and neonatal outcomes. The trimester- specific- reference intervals for the Indian population using IA, Roche Elecsys 1010 analyzer method have been established. Seaweed such as *Caulerpa racemosa* can be used as an iodine supplement but prolonged period of supplementation needs to be carried out.

Introduction

1. INTRODUCTION

Thyroid disease is being increasingly diagnosed with greater awareness and is one of the chronic non-communicable disease affecting women more than the male population. In India thyroid disorders are in a transition zone from a predominant iodine deficient nation to now an iodine sufficient population. The global goiter prevalence is more than 2 billion with more than 40 million in India. The true prevalence and incidence in India of thyroid disorders is difficult to estimate, even conservative estimates put the geographical prevalence between 42 million including cases of iodine deficiency disorders. Functional studies of the goitrous subjects showed overall prevalence of 5.4 percent hypothyroidism and 1.9 percent hyperthyroidism. Prevalence of autoimmune thyroiditis demonstrable by fine needle aspiration biopsy among female goitrous students was 7.5 percent (Shah and Joshi 2011). On the basis of this countrywide study and other related studies, it can now be estimated that the total burden of significant thyroid disease in the country in the post salt-iodization phase is approximately 42 million. As India is now predominantly iodine sufficient we are nearing the peak prevalence of the autoimmune epidemic. It is estimated that about 7.1 crore Indians are suffering while 20 crore people are at the risk of iodine deficiency disorders (IDD) in our country. By 2012, the Union health ministry's target is to reduce IDD prevalence nationally to less than 10 percent and to 5 percent by end of 2017 (Sinha 2011).

Thyroid disease is common in younger women and may be a factor in reproductive dysfunction. Once adequately treated this disorder is associated with successful pregnancy outcome. The key is to recognize and to treat thyroid disorders in the reproductive-age woman before conception. Pregnancy is a euthyroid state that is normally maintained by complex changes in thyroid physiology.

Over the past several years it has been proved that maternal thyroid disorder influence the outcome of mother and fetus, during and also after pregnancy. 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. In pregnancy, overt hypothyroidism is seen in 0.2 percent cases (Casey and Levono 2006) and subclinical hypothyroidism in 2.3 percent cases (Biondi and Cooper

2008). Fetal loss, fetal growth restriction, pre-eclampsia and preterm delivery are the usual complications of overt hyperthyroidism seen in 2 of 1000 pregnancies whereas mild or sub clinical hyperthyroidism is seen in 1.7 percent of pregnancies and not associated with adverse outcomes (Casey, Dashe and Welle 2006). Autoimmune positive euthyroid pregnancy shows doubling of incidence of miscarriage and preterm delivery. Worldwide more than 20 million people develop neurological sequel due to intra uterine, iodine deprivation (Banerjee 2011). Other problems of thyroid disorders in pregnancy are post partum thyroiditis, thyroid nodules and cancer, hyper emesis gravidarum etc.

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

PHYSIOLOGY OF THE THYROID GLAND

The thyroid gland is the largest of the organs that function exclusively as an endocrine gland, weighing about 20g in an adult. The basic structure of the thyroid is unique for endocrine glands, consisting of follicles varying in size that contain colloid produced by follicular cells. Thyroid follicular cells are cuboidal to columnar, and their secretory polarity is directed toward the lumen of the follicles. Polarity of follicular cells is important for iodine uptake, but the follicle structure is required for the synthesis of thyroid hormones. The luminal surfaces of follicular cells protrude into the follicular lumen and have numerous microvillar projections that greatly increase the surface area in contact with colloid. An extensive network of interfollicular and intrafollicular capillaries provides the follicular cells with an abundant blood supply (Capen 2000).

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

THYROID HORMONE SYNTHESIS

Iodine is an essential nutrient primarily because of its role as an indispensable component of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). They are the only iodine-containing hormones in vertebrates. Without iodine there is no biosynthesis of thyroid hormones. Therefore, thyroid function ultimately depends on an adequate supply of iodine to the gland.

The individual steps in thyroid hormone formation and secretion may be characterized as follows:

1. Active uptake of iodide (I^-).
2. Iodination of tyrosyl residues of thyroglobulin (TG).
3. Coupling of iodotyrosine molecules within TG to form T_4 and T_3.
4. Proteolysis of TG, with release of free iodotyrosines and iodothyronines, and secretion of iodothyronines into the blood
5. Deiodination of iodotyrosines within the thyroid and reuse of the liberated iodide
6. Deiodination of T_4 to T_3 by type I and II deiodinases, which are present in the thyroid.

1. Active Uptake Of Iodide

The biosynthesis of thyroid hormones is also unique among endocrine glands because the final assembly of the hormones occurs extracellularly within the follicular lumen. Essential raw materials, such as iodide, are trapped efficiently at the basilar aspect of follicular cells from interfollicular capillaries, transported rapidly against a concentration gradient to the lumen, and oxidized by a thyroid peroxidase in microvillar membranes to reactive iodine (I_2). The ability of the thyroid follicular cells to concentrate I^- was first reported as early as 1915. The thyroid gland was found to be capable of concentrating I^-

by a factor of 20 to 40 fold with respect to its concentration in the plasma under physiologic conditions.

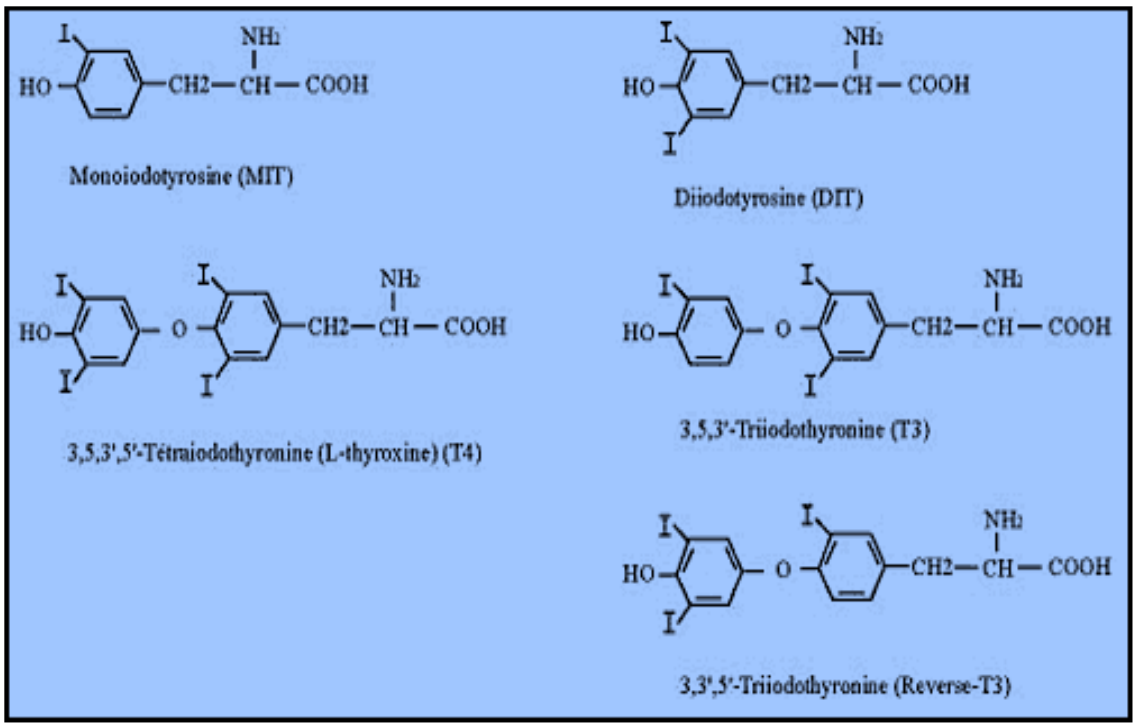
The mechanism of active transport of iodide has been shown to be associated with a sodium iodide (Na^+/I^-) symporter (NIS) present in the basolateral membrane of thyroid follicular cells and is a large protein containing 643 amino acids and 12 transmembrane domains. NIS mediates the first and key step in the process of supplying I^- to the gland for thyroid hormone biosynthesis (Carrasco 2000). Transport of iodide ion across the thyroid cell membrane is linked to the transport of Na^+. The ion gradient generated by Na^+-K^+-ATPase appears to be the driving force for the active co-transport of iodide. I^- is then passively translocated via a putative I^- channel across the apical membrane into the colloid, located in the follicular lumen.

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

2. Iodination of tyrosyl residues of thyroglobulin

The greatest part of iodine in normal thyroid gland exists in the form of TG, a large dimeric glycoprotein with a molecular weight of 6,60,000 and a sedimentation coefficient of 19S. TG does not have an unusual amino acid composition, but it is unique among body proteins in its content of iodinated amino acids. The structures of the iodoamino acids found in TG are shown in Fig. 1.1. Most of the TG in the normal gland is present in the follicular lumen and assumed to be in the soluble form. Normal human TG varies widely in iodine content; values as low as 0.1 percent and as high as 1.1 percent have been reported (Taurog 2000).

Thyroglobulin is a high molecular weight glycoprotein synthesized in successive sub-units on the ribosomes in follicular cells. The constituent amino acids (tyrosine and others) and carbohydrates come from the circulation. Human thyroglobulin contains complex carbohydrate units with up to four sulphate groups and units with both sulphate and sialic acid.



Source: thyroidmanager.org

Figure 1.1 Structure of major iodoamino acids found in thyroglobulin

The amino acid tyrosine is incorporated within the molecular structure of thyroglobulin. Recently synthesized thyroglobulin (17S) leaving the Golgi apparatus is packaged in apical vesicles and extruded into the follicular lumen.

Mechanism of iodination

Highly purified thyroperoxidase (TPO) catalyses both iodination and coupling. The mechanism of TPO-catalyzed iodination has been discussed by numerous investigators. Three proposed mechanisms are:

i. Free-radical mechanism
ii. Iodinium ion (I^+) as the iodinating intermediate
iii. Hypoiodite as the iodinating intermediate: This is the most acceptable observation. Following reactions were proposed

$$E + H_2O_2 \longrightarrow EO + H_2O$$

Enzyme Hydrogen peroxide Compound I

$$EO + I^- \longrightarrow [EOI]^-$$

Enzyme bound hypoiodite

$$[EOI]^- + H_2O_2 \longrightarrow O_2 + H_20 + I^- + E$$

TPO has no catalytic activity in the absence of H_2O_2, therefore H_2O_2 production plays an essential role in thyroid hormone formation (Taurog 2000). The H_2O_2 generating system is Ca^{2+} dependent and involves an NADPH oxidase.

3. Mechanism of the coupling reaction of iodotyrosine molecules within TG to form T_4 and T_3

Both iodination and coupling occur within the TG molecule and fig. 1.2 depicts how the originally postulated radical mechanism might occur within the matrix of the protein.

4. TG proteolysis and hormone release

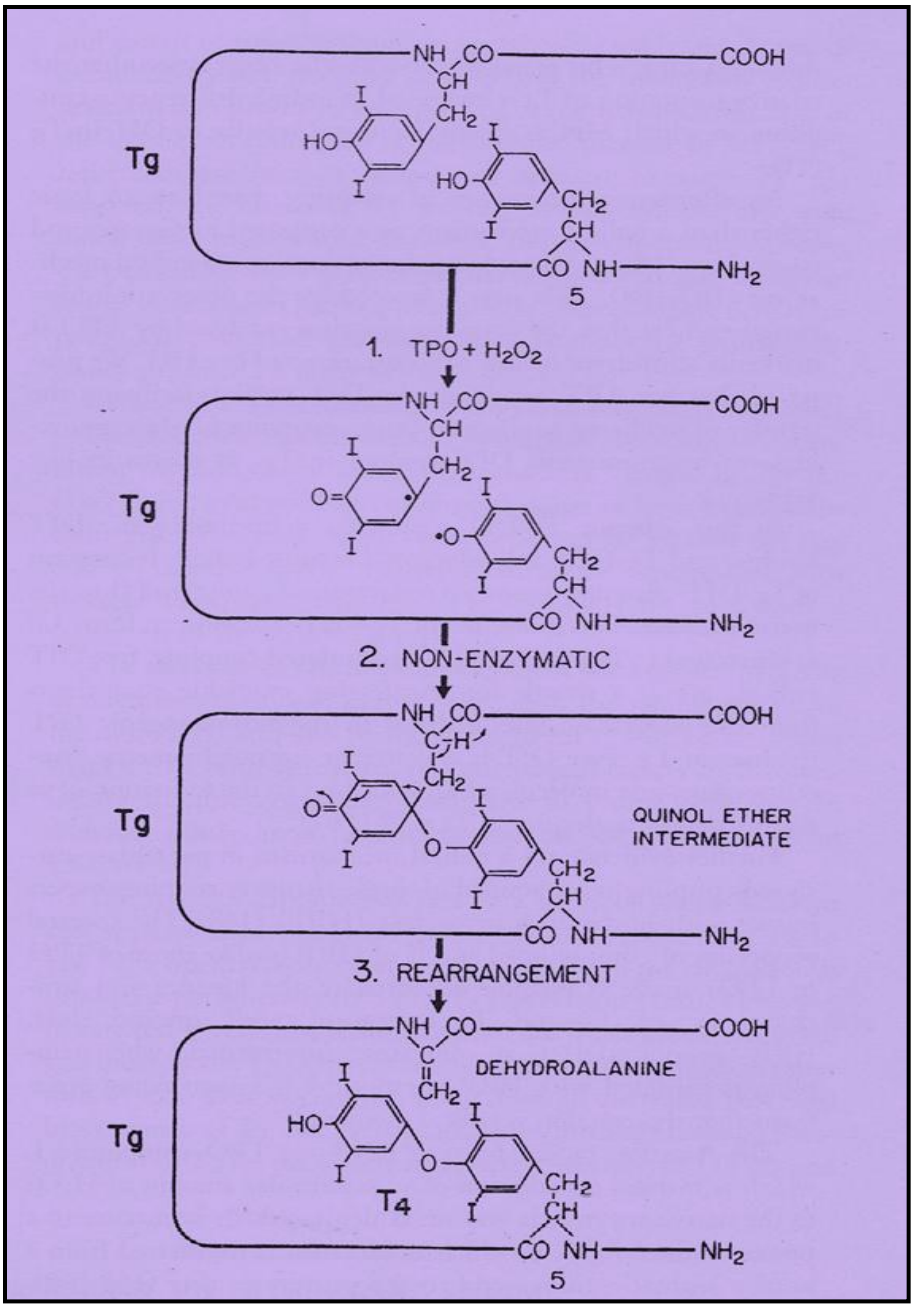
Internalized TG molecules that are conveyed to lysosome compartments are subjected to diverse hydrolytic reactions leading to the generation of free thyroid hormones and to complete degradation of the protein. Prior to their secretion from the thyroid, T_4 and T_3 must be released from peptide linkage within TG. The process is initiated by the retrieval of TG into the thyroid cell from the follicular lumen. The more common mode of TG retrieval under physiologic conditions in most mammalian species is by way of micropinocytosis. Some TG degradation and hormone release may precede entry into lysosomes.

5. Deiodination of iodotyrosines within the thyroid and reuse of the liberated iodide

Once released from TG, the thyroid hormones rapidly leave the cell and enter the circulation. Monoiodotyrosine (MIT) and diiodotyrosine (DIT), in the meantime, are deiodinated by an iodotyrosine-specific deiodinase. The iodide released by this action is partly reused for hormone synthesis and the remainder enters the circulation (Fig. 1.3).

6. Deiodination of T_4 to T_3 by type I and II deiodinases, which are present in the thyroid

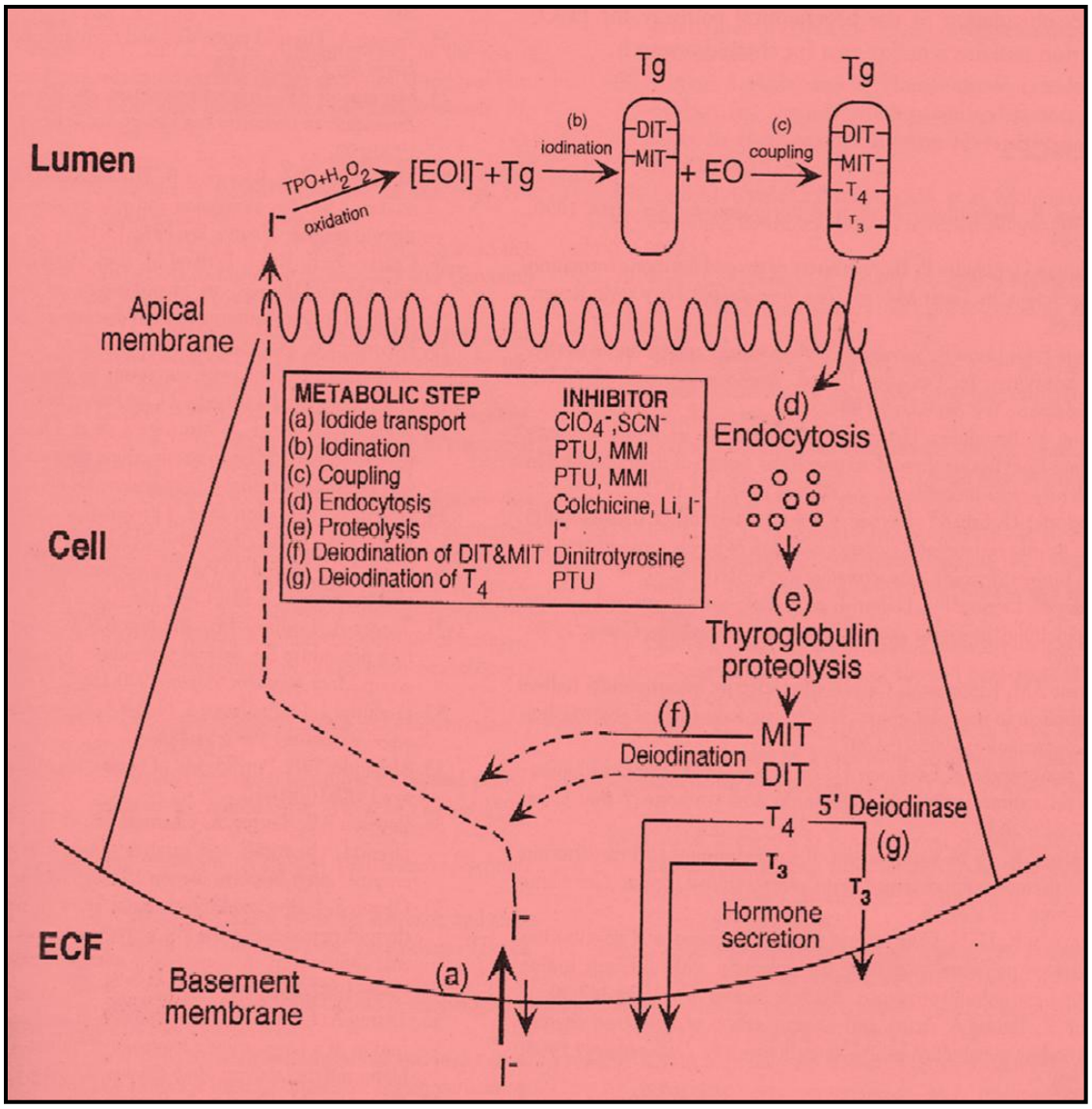
Some intrathyroidal conversion of T_4 to T_3 occurs. The thyroid gland contains an iodothyronine 5' deiodinase that resembles the type I enzyme found in peripheral tissue.



Source: Taurog 2000

Figure 1.2 Proposed coupling scheme for intramolecular formation of T_4 within the thyroglobulin molecule

Not all internalized TG reaches the lysosomes. Some is diverted into the bloodstream by transcytosis. In inside-out procrine follicles, 10% of endocytosed TG was transported by small vesicles from the apical to the basolateral cell surface and released. The process is stimulated by TSH and probably accounts for the appearance of TG in the serum.



Source: Taurog 2000

Figure 1.3 Summary diagram of major steps in thyroid hormone biosynthesis and secretion

PHYSIOLOGY OF HORMONE BINDING

The delivery system for the thyroid hormones in human includes a set of serum transport proteins that vary widely in concentration, affinity for thyroid hormone, and dissociation rate constants. The net result is that more than 99% of the hormone in serum is protein bound but can be liberated with great rapidity for entry into cells (Robbins 2000).

Thyroxine binding globulin (TBG), a minor component of α-globulin, carries about 70 percent of the serum T_4 and T_3 by virtue of its high affinity for the two hormones. T_3 binds to TBG 10 to 20 times less avidly than T_4, and dissociates still more rapidly (half life 4.2 seconds). The greater rate of dissociation is largely responsible for the lower affinity. In normal serum, about one-fourth of the TBG molecules contain a T_4 molecule.

Transthyretin (TTR), or T_4-binding prealbumin, binds only about 10% to 15% of the hormones but is responsible for much of the immediate delivery of T_4 and T_3 to cells because of its affinity for the hormones is lower and therefore they dissociate from it more rapidly. The affinity constant for the T_4-TTR interaction is intermediate between those of TBG and albumin. Only 1 in 300 TTR molecules contain T_4. The affinity constant for T_3 is about 10-fold lower. The dissociation rate constant for T_4-TTR is about fivefold greater than that of T_4-TBG, the contribution of T_4-TTR to the free hormone pool during capillary flow is about equal to that of T_4-TBG. TTR is the major thyroid-hormone binding protein in cerebrospinal fluid, where it may have a role in the distribution of T_4 and T_3 to the central nervous system.

Albumin, a protein that carries a multitude of small molecules, binds 15% to 20% of the serum T_4 and T_3. Its affinity for the hormones is even lower than that of TTR, and the hormone-albumin complexes dissociate rapidly.

The lipoproteins transport a minor fraction of serum T_4 and T_3 through specific interactions with various apolipoproteins. High density lipoprotein (HDL) carries about 3% of the T_4 and 6% of the T_3 in serum, mainly in very high density subfraction.

Physiologic importance of the transport proteins:

1. Extrathyroidal storage of hormone.
2. A buffering action by initiation of feedback control, so that the stored hormone is released on demand while the tissues are protected from excessive hormone.
3. A hormone-releasing function that allows the very small free hormone pool to be continuously replenished and made available to cells.

Thyroid homeostasis is characterized by the maintenance of a steady supply of hormones to tissues, resulting in steady hormone actions. Extrathyroidal storage contributes to this homeostasis. The ratio of the serum total T_4 concentration to the free T_4 concentration is an indicator of the total storage function of the transport proteins with respect to the major secretory product of the thyroid gland. When serum is depleted of TBG, the ratio falls to one-third of the normal as it is the major storage site for secreted hormone in the circulation.

THYROID HORMONE METABOLISM

Thyroid hormones synthesized and secreted by the thyroid gland is activated and inactivated primarily by a series of monodeiodination steps in the target tissues (Table 1.1). Sulfation is an additional method of thyroid hormone metabolism of particular importance in the fetus (Chopra and Sabatino 2000). The iodinated substances with demonstrable biological effects are:

Thyroxine (T_4): It is the most abundant iodothyronine in TG, being about 10 to 20 times more abundant than T_3, 20 to 100 times more abundant than reverse triiodothyronine (rT_3). Iodine constitutes about 65% by weight of the T_4 molecule. The extent of overall binding of T_4 is high (>99.9%), so that the serum free T_4 concentrations is less than 0.1% of the serum total T_4 concentrations.

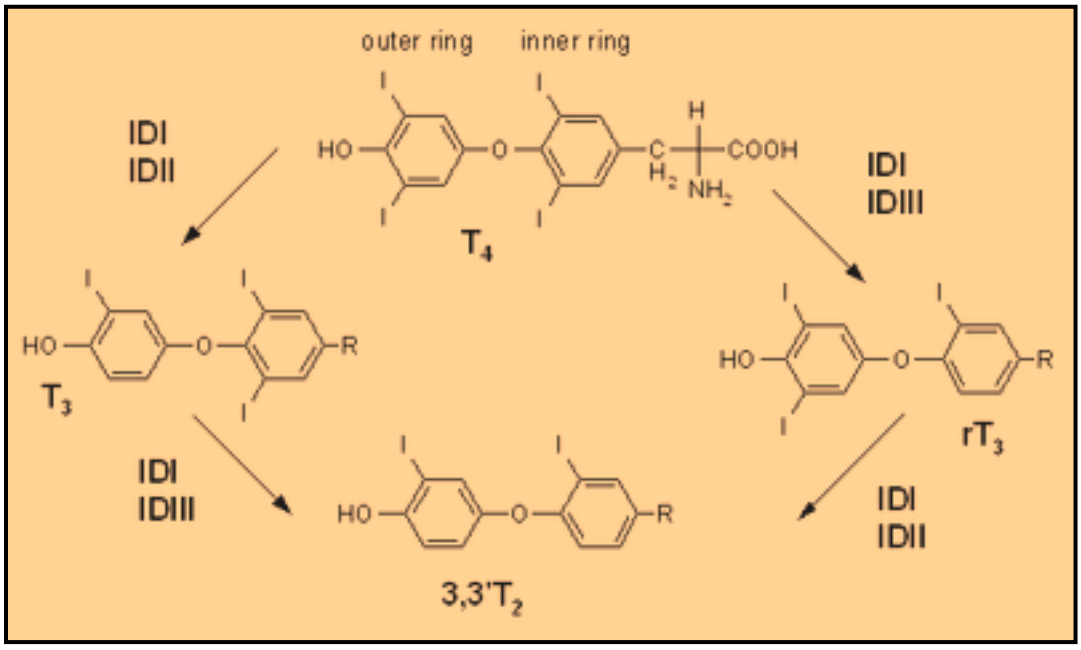
3, 5, 3'- Triiodothyronine: The majority (approx 80%) of the T_3 is produced by outer (phenolic) ring 5'-monodeiodination of T_4 in extrathyroidal tissues (Fig. 1.4). About 99.7% of T_3 is bound and 0.3% is free. It is three to four times more metabolically active than T_4.

Reverse triiodothyronine: It differs from T_3 in that iodine is missing from the inner or tyrosyl ring of T_4 rather than the outer or phenolic ring. It is produced by inner ring monodeiodination of T_4 (Fig. 1.4). This deiodination is catalyzed by a 5-deiodinase (5-D3). The tissue distribution of 5-D3 differs from that of 5'-D1 and 5'-D2, in that 5-D3 is present mainly in the central nervous system, skin and placenta. Nearly all rT_3 is derived from peripheral conversion and only 2% from the thyroid gland.

Table 1.1 Characteristic of the three types of iodothyronine deiodinases

ENZYMES	D1	D2	D3
Tissues	Liver, Kidney	Brain, Pitutary, Brown adipose tissue, Placenta	Fetal tissues and utero-placental unit
Function	• T_4 to T_3 • rT_3 to diiodothyronine • Inner ring deiodination of T_4 & T_3 sulfate conjugates	• T_4 to T_3 • Intracellular T_3 to tissues dependent on thyroid hormone	• T_4 to rT_3 • T_3 to Diiodothyronine
Substrate	$rT3>T_4>T_3$	$T_4>rT_3$	$T_3>T_4$
Activity	Major activating deiodinase in adults	Major activating deiodinase in fetus	Major deactivating enzyme
Hypothyroidism	Decrease	Increase	Decrease
Hyperthyroidism	Increase	Decrease	Increase

Source: www.thyroidmanager.org



Source: www.medscape.com

Figure 1.4 Conversion of T_4 to T_3 and reverse T_3 by mondeiodinases

MOLECULAR ACTIONS OF THYROID HORMONE

The thyroid hormones (TH) T_4 and T_3 have important effects on development, growth, and metabolism. Some of the most prominent effects of TH occur during fetal development and early childhood. In humans, the early developmental role of TH is illustrated by the distinctive clinical features of cretinism observed in iodine-deficient areas. In childhood, lack of TH can cause delayed growth and many of the effects of TH may be metabolic rather than developmental, as growth is restored rapidly after the institution of TH treatment. In adults, the primary effects of THs are manifested by alterations in metabolism. These effects include changes in oxygen consumption, protein, carbohydrate, lipid, and vitamin metabolism. The clinical features of hypothyroidism and hyperthyroidism emphasize the pleiotropic effects of these hormones on many different pathways and target organs.

Currently the predominant view is that the actions of thyroid hormone are initiated by an interaction of T_3 with specific nuclear receptors (Glinoer 1997). These receptors are members of a large superfamily that includes receptors for steroid hormones, vitamin D, retinoic acid, and peroxisomal proliferator activators. They function in concert with a broad range of other nuclear proteins in regulating the expression of target genes. Protein complexes formed in the nucleus serve to modify the effect of thyroid hormone on the expression of many target genes.

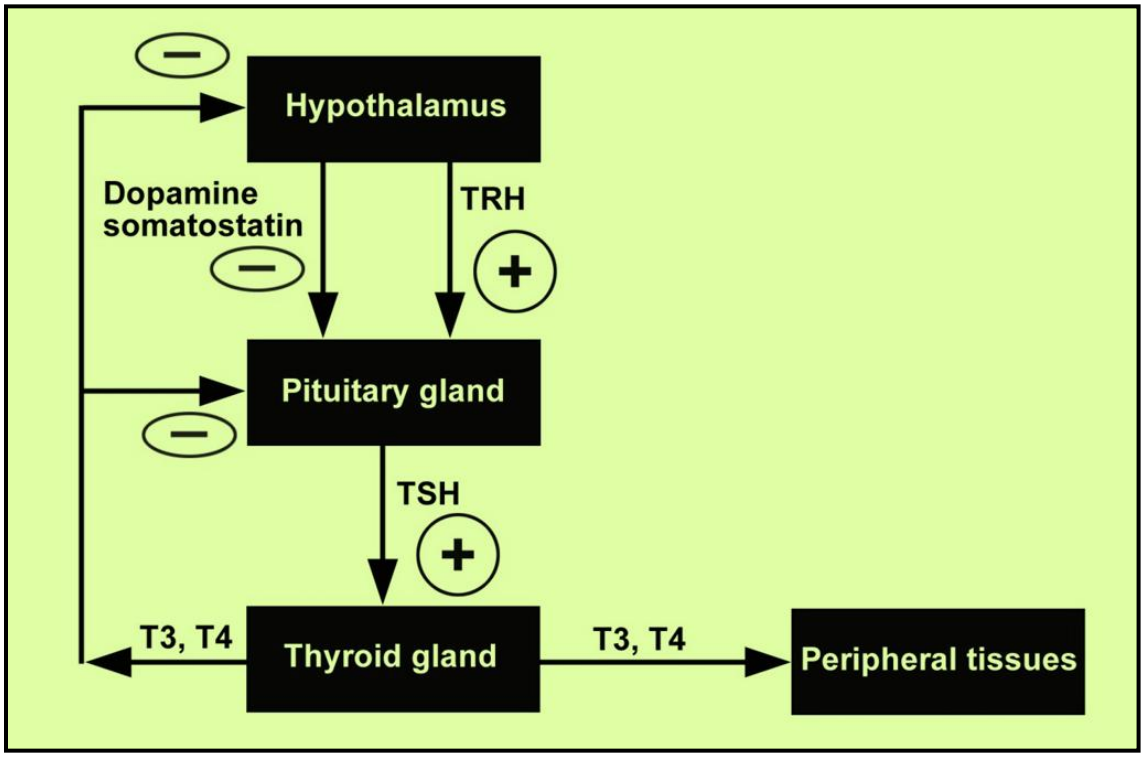
FACTORS THAT CONTROL THYROID FUNCTION

Pituitary thyrotropin or thyroid stimulating hormone (TSH) is synthesized and secreted by basophilic cells (thyrotrophs) in the anterior pituitary gland. Its secretion is controlled in part by thyrotropin-releasing hormone (TRH) and other molecules produced in the brain.

The hypothalamus regulates thyroid function by regulating TSH secretion because hypothyroidism occurs if the hypothalamus is damaged or if the pituitary stalk is transected. This hypothalamic stimulatory control is exerted primarily by TRH, a tripeptide produced by hypothalamic neurons and transported along their axons to specialized nerve terminals in the median eminence of the hypothalamus, from which it is released into the hypophyseal portal blood. Then it is carried directly to the anterior

pituitary gland. The other major regulators of TSH secretion are T_4 and T_3, which inhibit TSH secretion and to a lesser extent TRH secretion (Fig 1.5).

Other less important regulators of TSH secretion are somatostatin and dopamine, both of which inhibit the function of the thyrotrophs, and α-adrenergic agonists, which in general inhibit the thyrotrophs. Together, these substances, but mostly TRH and the thyroid hormones, maintain TSH and therefore thyroid secretion within narrow limits in normal subjects (Scanlon and Toft 2000).



Source: Scanlon and Toft 2000

Figure 1.5 Schematic representation of primary system for regulation of hypothalamic-pitutary-thyroid function

IODINE DEFICIENCY

Iodine is a trace element present in the human body in minute amounts (15-20 mg i.e., $0.02x10^{-3}$ % of body weight). It's only confirmed role is in the synthesis of thyroid hormones. Consequently, iodine deficiency if severe will impair thyroid hormonogenesis.

The dietary allowances of iodine recommended by the World Health Organization (WHO) are 150 µg/day for adolescents and adults, 250 µg/day for pregnant and lactating

women, 120 µg/day for children 6 to 12 years of age, and 90 µg/day for children 0 to 5 years of age (WHO/UNICEF 2007).

When the physiologic requirements of iodine are not met in a given population, a series of functional and developmental abnormalities occur, including thyroid function abnormalities and, when iodine deficiency is severe, endemic goiter and cretinism, decreased fertility rate, increased perinatal death, and infant mortality. These complications, which constitute a hindrance to the development of the affected populations, are grouped under the general heading of iodine deficiency disorders (IDD).

2 billion individuals worldwide have insufficient iodine intake, with those in South Asia and sub-Saharan Africa particularly affected (Zimmermann, Jooste and Pandav 2008). Although the disorders that result from iodine deficiency are preventable by appropriate iodine supplementation, they continue to occur because of various socioeconomic, cultural and political limitations to adequate iodine supplementation programs.

Adaptation of thyroid function to iodine deficiency

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

Increase in iodine trapping

The fundamental mechanism by which the thyroid gland adapts to an insufficient iodine supply is to increase the trapping of iodide. This result in the accumulation within the gland of a larger percentage of ingested iodide and a more efficient reuse of iodide directly released by the thyroid or generated by the degradation of thyroid hormones. The increased iodide trapping is the result of both TSH stimulation of the iodide pump and

perhaps TSH-independent augmentation of membrane iodide trapping involving the thyroid sodium iodide symporter (Spitzweg and Heufelder 1997).

For an adequate adjustment of iodide supply to the thyroid, iodide trapping must fulfill two conditions:

i. It must reduce the amount of iodine excreted in the urine to a level corresponding to the level of iodine intake, this condition being required to preserve preexisting iodine stores.

ii. It must ensure the accumulation in the thyroid of definite amounts of iodide per day (about 100 µg). This parameter is extremely important because it quantitatively controls all further steps of intrathyroidal iodine metabolism, including the secretion rate of thyroid hormones.

TSH stimulation and alterations in circulating thyroid hormones

The pattern of circulating thyroid hormones in clinically euthyroid adults in areas of severe iodine deficiency is characterized by low serum T_4, elevated TSH, and normal or supranormal T_3. The mechanism responsible for this pattern may include thyroidal secretion of T_4 and T_3 in the proportion in which they exist within the gland, preferential secretion of T_3, or increased peripheral conversion of T_4 to T_3. The shift to increased T_3 secretion and serum T_3:T_4 ratios play an important role in the adaptation to iodine deficiency because T_3 possesses about four times the metabolic potency of T_4 but requires only 75% as much iodine for synthesis (Greer, Grimm and Studer 1968). It is only under conditions of extreme thyroid failure, as are found in myxedematous endemic cretinism that both serum T_4 and T_3 are particularly low and serum TSH is dramatically elevated. In less severe goiter endemias, serum T_4 and T_3 levels are only slightly modified or remain normal.

HYPOTHYROIDISM

It is the most common clinical disorder of thyroid dysfunction. It is most often caused by some disorder of the thyroid gland that leads to a decrease in thyroidal production and secretion of T_4 and T_3, in which case it is referred to as primary or thyroidal hypothyroidism (Table 1.2). It is invariably accompanied by increased TSH secretion.

Much less often hypothyroidism is caused by decreased thyroidal stimulation by TSH, which is referred to as central, hypothyrotropic, or secondary hypothyroidism. Central hypothyroidism may be caused by either pituitary or hypothalamic disease, the latter causing deficiency of TRH (Braverman and Utiger 2000).

Table 1.2 Causes of Hypothyroidism

Type	Causes
Primary	Destruction of thyroid tissue • Chronic autoimmune thyroiditis- atrophic and goitrous forms • Radiation-^{131}I therapy for thyrotoxicosis, external radiotherapy to the head and neck for nonthyroid malignant disease • Subtotal and total thyroidectomy • Infiltrative diseases of the thyroid (amyloidosis, scleroderma) Defective thyroid hormone synthesis • Iodine deficiency • Drugs with antithyroid actions-lithium, iodine and iodine containing drugs and radiographic contrast agents
Central (Secondary)	Pituitary disease Hypothalamic disease
Transient	Silent thyroiditis including postpartum thyroiditis Subacute thyroiditis After withdrawal of thyroid hormone therapy in euthyroid patients

Source: Braverman and Utiger 2000

The clinical manifestations of hypothyroidism are largely independent of its cause. It affects persons of all ages and both sexes, although the majority of patients are women (Roberts and Ladenson 2004). It may be overt or subclinical. The former is defined as high serum TSH concentrations and low serum free T_4 concentrations, and the latter as high serum TSH and normal serum free T_4 concentrations.

Therapeutic approach to hypothyroidism

The goal of treatment of hypothyroidism is to normalize thyroid status in peripheral tissues, whatever the cause of hypothyroidism. The usual approach is to give sufficient T_4 to ameliorate all symptoms of hypothyroidism and, in patients with primary

hypothyroidism, to reduce serum TSH concentrations to within the normal range; doses effective in these regards raise serum total and free T_4 concentrations to well within their respective normal ranges or just above them, and serum total and free T_3 concentrations to within the normal range (Roberts and Ladenson 2004).

HYPERTHYROIDISM

The term hyperthyroidism is used to denote excess circulating concentrations of thyroid hormones- either T_4, T_3, or both irrespective of whether the derivation of the excess has been acute or chronic. The most common cause of this syndrome is Graves' disease, followed by toxic multinodular goiter, and solitary hyper functioning nodules. Autoimmune postpartum and subacute thyroiditis, tumors that secrete thyrotropin, and drug-induced thyroid dysfunction, are also important causes. Antithyroid drugs, radioactive iodine, and surgery are the traditional treatments for the three common forms of hyperthyroidism. β-adrenergic blocking agents are used in most patients for symptomatic relief, and might be the only treatment needed for thyroiditis, which is transient (Cooper 2003).

THYROID PHYSIOLOGY IN THE PERINATAL PERIOD

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

- Phase I development, during the first trimester, includes embryogenesis of the hypothalamus, the pituitary gland, and the thyroid gland.
- Phase II, during the second trimester, is a period of continuing fetal growth and relatively quiescent thyroid function.
- During the third trimester and the neonatal period, phase III maturation of hypothalamic-pitutary-thyroid interaction and control occurs and includes maturation of thyroid hormone metabolism and actions.

In the human infant, the period of thyroid-dependent brain development probably extends from midgestation to about 2-3 years of postnatal age, the most critical period of dependence being first 6-8 months postpartum.

THYROID SYSTEM ONTOGENESIS

Role of the placenta: Fetal development is dependent on the placenta, which regulates substrate supply, provides excretory functions, and synthesizes various polypeptide and steroid hormones that influence aspects of maternal and fetal metabolism. With regard to thyroid function, the placenta provides a relative barrier between the maternal and fetal systems. The mammalian placenta is impermeable to thyrotropin (TSH), freely permeable to iodide, and relatively impermeable to thyroid hormones. The last is due, in part, to the presence in the placenta of the type III iodothyronine inner-ring monodeiodinase (MDI) enzyme system that deiodinates T_4 to inactive rT_3 and T_3 to inactive diiodothyronine (T_2) (Burrow, Fisher and Larsen 1994).

The placenta also produces type II MDI, and the mixture of type II and type III activities converts T_4 to T_3 as well as T_4 to rT_3 and T_3 to T_2. Thus, the placenta functions to deiodinate and inactivate most of the T_4 and T_3 presented from the maternal or fetal circulation, and the iodide released serves to provide a continuing secondary source of iodide for fetal thyroid hormone synthesis.

Concentrations of T_4, T_3 and rT_3 have been measured in human coelomic and amniotic fluids, from 6 to 11 weeks gestation, before the onset of fetal thyroid hormone production (Morreale de Escobar G 2004). The T_4 concentrations in the coelomic fluid were positively correlated with the maternal circulating concentrations, but were <1% of the maternal values. T_3 was at least 10 fold lower than T_4, with rT_3 being higher than T_4 confirming high D3 activities of the placental barrier and fetal epithelia.

The placenta is permeable to TRH, but the maternal serum TRH concentration is low and most of the peptide transferred to the placenta is degraded. Thus, maternal serum contributes little, if any, TRH to the fetus. The placenta however is capable of TRH synthesis and combined placental and fetal extrahypothalamic TRH production, the last from fetal pancreas and gastrointestinal tissues, leads to high concentrations of TRH in fetal serum. The high fetal TRH concentration is maintained in part because of absent or low concentrations of TRH-degrading activity in fetal serum.

The placenta also produces large amounts of chorionic gonadotropin (hCG), which has inherent low level TSH like bioactivity.

Hypothalamic-Pitutary Development

The human fetal forebrain and hypothalamus begin to differentiate by 3 weeks of gestation under control of a series of homeodomain proteins or transcription factors.

Anatomically, the pituitary gland develops from two anlagen: an evagination of the floor of the primitive forebrain and a ventral pouch from the ectoderm of the primitive oral cavity. The latter, Rathke's pouch, is visible by 5 weeks evolving to a morphologically mature pituitary gland by 14 weeks. The pituitary-portal blood vessels have begun to develop by this time and maturation continues through 30-35 weeks of gestation. The hypothalamic nuclei, median eminence, and supraoptic tract are identifiable by 15 to 18 weeks, and significant concentrations of TRH are detectable at this time. The anterior pituitary hormones, including TSH can be identified by immunoassay between 10 to 17 weeks and concentrations increases progressively thereafter (Fisher and Brown 2000).

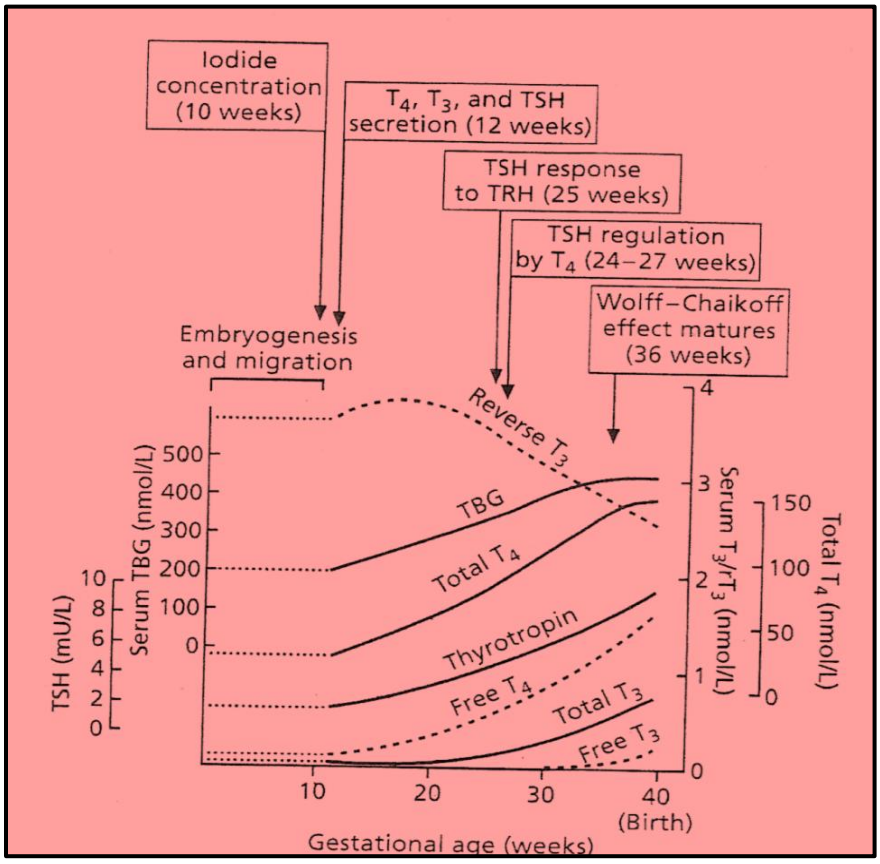
Thyroid Gland Development

Thyroid gland embryogenesis and descent are largely complete by 10 to 12 weeks of gestation. At this stage, tiny follicle precursors can be seen, iodine uptake can be identified, and thyroglobulin is present in the follicular spaces (Fig. 1.6). Thyroid hormonogenesis is detected by 11 to12 weeks, coincident with appearance of pituitary TSH in the fetal circulation (Fisher and Brown 2000).

MATURATION OF THYROID SYSTEM CONTROL

Maturation of control of thyroid hormone secretion is superimposed on a progressive increase in the fetal serum TBG concentration during the period of 10 to 35 weeks of gestation. The fetal serum TBG concentration increases because of the maturation of fetal hepatic TBG synthetic capacity. The secretion of TSH and of thyroid hormones is minimal until midgestation. At this time (18-20 weeks of gestation), fetal thyroid gland iodine uptake and serum T_4 concentrations begin to increase. The fetal serum TSH

concentration progressively increases from a low value at 16 to 18 weeks, associated with progressive increases in serum total and FT_4 concentrations between 20 weeks and term (Fig. 1.6).



Source: thyroidmanager.org

Figure 1.6 Maturation of thyroid gland development and function during gestation

Pituitary TSH responsiveness to exogenous TRH is present early in the third trimester and maturation of negative feedback control of pituitary TSH secretion develops progressively during the last half of gestation and the first 1 to 2 months of extrauterine life (Roti 1988). Although immature, the fetal hypothalamic-pitutary system is capable of responding to hypothyroxinemia; congenital hypothyroidism in the 24-week fetus is associated with a markedly increased serum TSH concentration.

During the neonatal period, the serum FT_4 concentration increases in response to an early neonatal TSH surge, and there is a marked increase in FT_4 concentration associated with a fall in TSH to the normal adult range by 1 to 2 months of age. The FT_4: TSH and FT_3:

TSH concentration ratios approximate adult values by 1 to 2 months of age (Fisher and Brown 2000).

Thus, the human fetus matures from a state of combined primary and hypothalamic-pituitary hypothyroidism during the first trimester, through a period of hypothalamic hypothyroidism during the last half of gestation, to a state of mature function by 1 to 2 months of postnatal life.

Development of thyroid gland maturation and autonomy

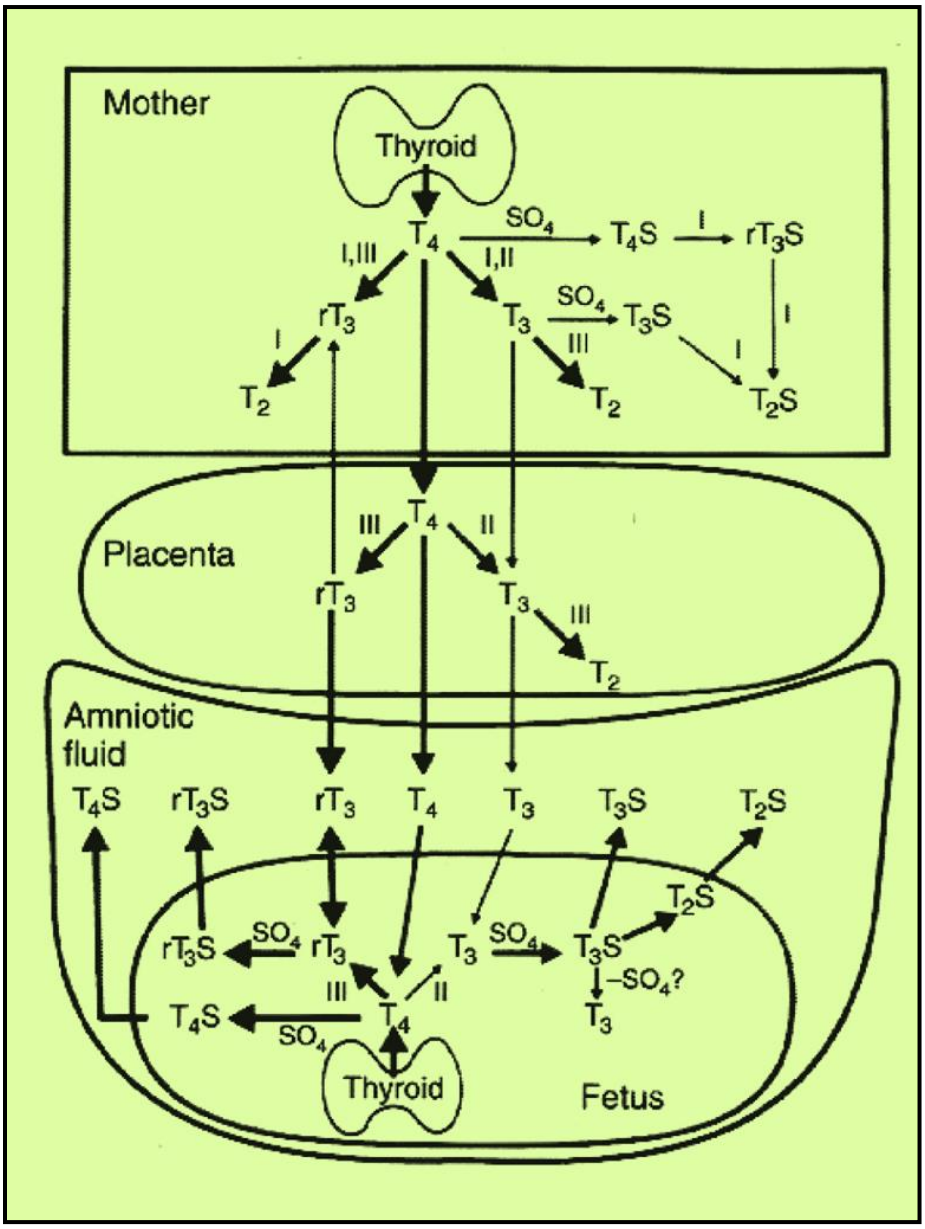
The progressive increase in fetal serum FT_4 concentrations during the last half of gestation appears to be due to both increasing fetal serum TSH and progressive maturation of the thyroid follicular cell responsiveness to TSH. Thyroid gland maturation also includes maturation of thyroid autoregulation of iodine transport. The ability of the thyroid gland to defend against the thyroid-blocking effect of excessive iodide does not develop until after 36 to 40 weeks of gestation in the human fetus (Fig. 1.6). This adaptation involves activity of the NIS regulating the capacity of thyroid follicular cells to decrease iodide transport and thus prevent the high intracellular iodide concentrations that cause blockade of hormone synthesis (Burrow, Fisher and Larsen 1994, Fisher and Brown 2000).

Maturation of thyroxine metabolism

Thyroid hormones undergo several types of biochemical transformation in tissues, including deiodination, side-chain metabolism, and conjugation (with sulfate or glucuronide). Sequential monodeiodination of iodothyronines is the most important pathway of thyroid hormone metabolism (Fig. 1.7).

The type II and III enzyme activities appear during the second trimester, whereas type I activity appears during the third trimester. The preterm increase in fetal serum T_3 concentrations is due to a progressive decrease in placental and fetal tissue type III deiodinase activity and increasing fetal type I activity. Serum concentrations of rT_3, T_4 sulfate, T_3 sulfate and rT_3 sulfate in the umbilical cord at this time are high (Burrow, Fisher and Larsen 1994). These analogues represent the predominant thyroid hormone metabolites in the fetus and, like rT_3, are not biologically active. Iodothyronine sulfation is the result of hepatic sulfotransferase enzyme activity. The sulfated analogues are

preferred substrates for hepatic MDI-I, but are not substrates for MDI-III. They accumulate through 30 weeks gestation because of the relatively low MDI-I activity.



Source: Burrow, Fisher, & Larsen, 1994

Figure 1.7 Differences in deiodinase activity in the maternal, placental and fetal compartments

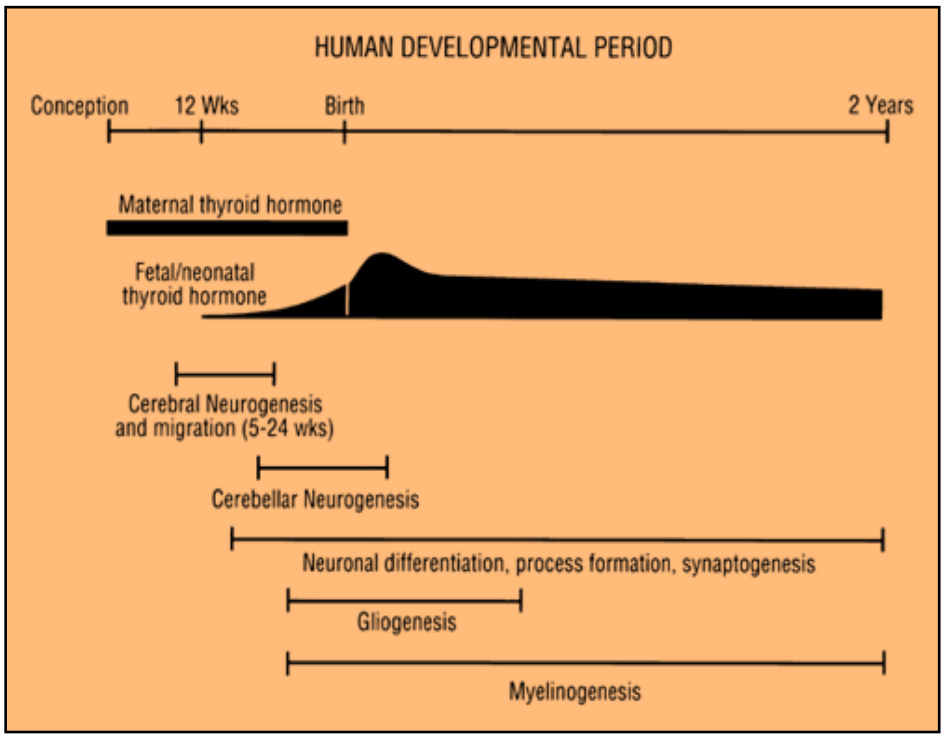
In fetus with hypothyroidism, type II deiodinase activity increases. These change favor shunting of T_4 to brain tissues; so even limited placental transfer of maternal T_4 may protect the fetal brain from thyroid deficiency.

THYROID HORMONE ACTIONS

Studies of the timing of thyroid hormone effects on thermogenesis, hepatic enzyme activities, skin and brain maturation, growth hormone metabolism and growth factor metabolism (insulin like growth factors, nerve growth factor, epidermal growth factor) indicate that most of these thyroid effects appear during first 4 weeks postnatally.

In human fetal brain, low levels of nuclear T_3 binding have been detected at 10 weeks gestation, with higher levels at 16 to 18 weeks. Liver, heart and lung receptor binding also have been identified at 16 to 18 weeks (Fisher and Brown 2000).

Human fetal growth is the net result of a complex interplay of genetic, hormonal and growth factor effects, which are thyroid hormone dependent. Bone maturation of the hypothyroid infant, however, is delayed in 50% to 60% and fontanelle closure often is delayed.

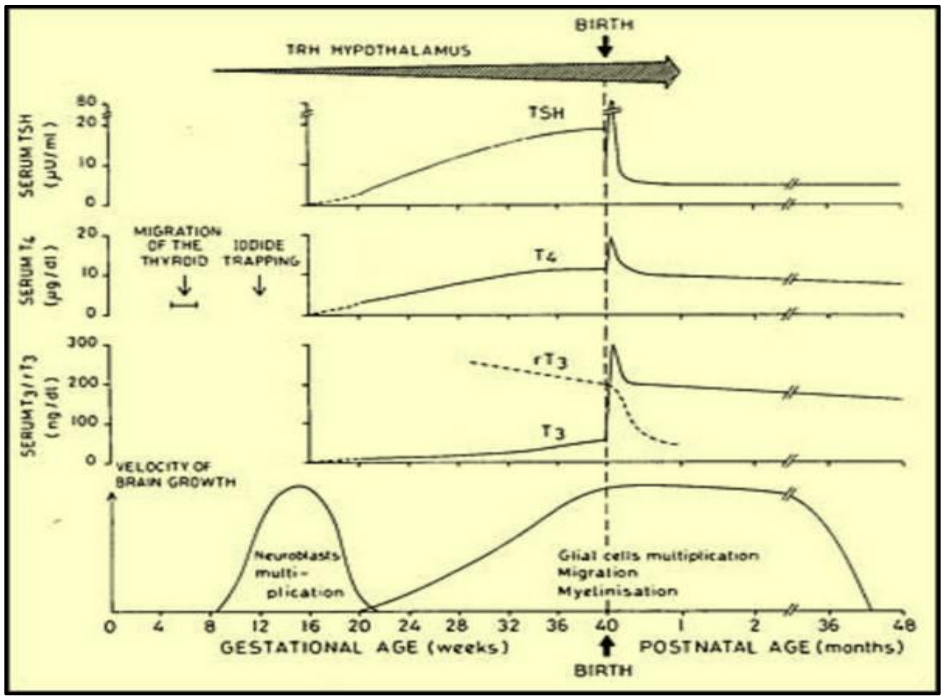


Source: Porterfield 1994

Figure 1.8 Potential availability of thyroid hormones during fetal/neonatal brain development relative to brain developmental stages

Thyroid Hormone and Central Nervous System Development

Thyroid hormone is essential for normal central nervous system (CNS), maturation, regulating a diverse array of processes, including neurogenesis and neural cell migration, neuronal differentiation, dendritic and axonal growth, synaptogenesis, gliogenesis, myelination, and neurotransmitter enzyme synthesis (Fig. 1.8). These processes lead to the establishment of neural circuits essential for normal brain development and follow a precise developmental program. The absence of thyroid hormone appears to delay rather than eliminate the timing of critical morphologic events or gene products, resulting in disorganization of intracellular communication (Fig.1.9).



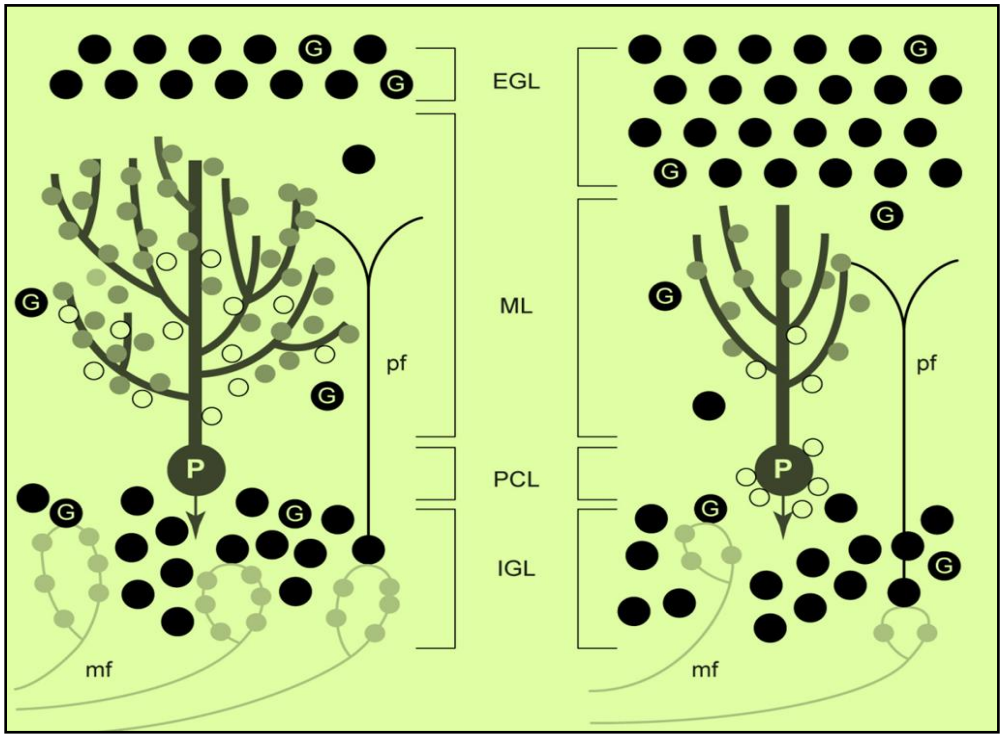
Source: thyroidmanager.org

Figure 1.9 Ontogenesis of thyroid function and regulation in humans during fetal and early postnatal life in relation to the velocity of brain growth

The presence of thyroid hormone receptors (TRs) early in the development of the human fetal brain shows that developmental events sensitive to thyroid hormones occur before mid-gestation (Morreale de Escobar, Obregon and Escobar del Rey 2007). Iodine is required for the synthesis of thyroid hormones, which exert action through binding of T_3 to nuclear receptors. Nuclear T_3 receptors can be identified in the brain of 10 weeks old human fetuses; they increase by more than six-folds by 12 weeks and 10-fold by 16

weeks, long before fetal thyroid fully functions. This time course corresponds to the maximum growth velocity of brain structures regarding neuronal multiplication, migration and organization that occur early in second trimester. T_3 deficiency causes striking abnormalities in neuronal migration, neuronal differentiation, outgrowth of neuronal processes, synaptogenesis, myelinization, glial proliferation and neuronal death (Poterfield and Hendrich 1993, Akinci and Karakas 2009).

In the cerebellum, various anatomical alterations are induced by perinatal hypothyroidism. These include: reduction of growth and branching of dendritic arborization of Purkinje cells, reduction of synaptogenesis between Purkinje cells and granule cell axons, delayed proliferation and migration of granule cells, delayed myelination and changes in synaptic connection among cerebellar neurons and afferent neuronal fibers (Fig. 1.10). Under such conditions, neurons become hypoplastic, and have reduced axonal count, dendritic branching, synaptic spikes and interneuronal connections. These abnormalities cannot be avoided unless TH is replaced within two weeks of birth (Koibuchi and Chin 2000).



Source: Koibuchi and Chin 2000

Figure 1.10 Schematic diagram showing the effect of perinatal hypothyroidism on neurogenesis and differentiation in the cerebellar cortex

Studies show that iodine deficiency and maternal fetal hypothyroxinemia have negative effects on fetal neural maturation, dendrite arborization and synaptic formation. They delay the myelinization process and gliogenesis, which start in second half of gestation and continues in postnatal life (Akinci and Karakas 2009). Iodine deficiency during the fetal period leads to impairment of mitosis and differentiation of neural cells of the fetus. The insult involves impairment in the development of dendritic spines, their density and distribution along the apical shafts of deep pyramidal neurons of the most superficial layers of cerebral cortex. This effect leads to a deficit in the general synaptology along apical shafts especially in the visual and auditory areas. Iodine induced hypothyroidism during the fetal period also leads to a decrease in the proportion and density of radial glial cells fibers of the hippocampal- formation of the brain (Sethi and Kapil 2004).

In addition to the actions of thyroid hormone on cerebral and cerebellar development, thyroid hormones have important effects on development of the visual and auditory cortex, hippocampus, and basal ganglia over a critical period. The proliferation of neurons occurs during the second trimester; cell differentiation and neurite outgrowth associated with increased brain weight and protein content occur during the third trimester and the postnatal period (Fig. 1.8).

These findings have important applications to human infants exposed to prenatal or postnatal hypothyroidism as well as to the critical period before or after which correction of the hypothyroidism cannot normalize brain maturation/function. Thus, a characteristic syndrome has been described in patients with endemic cretinism resulting from severe iodine deficiency involving both fetal and maternal hypothyroidism (Morreale de Escobar, Obregon and Escobar del Rey 2007). This syndrome in its most severe form includes mental retardation, deaf-mutism, pyramidal tract disturbance, and extrapyramidal dysfunction with a spastic diplegia or quadriplegia and a characteristic gait disturbance; microcephaly. The former abnormalities result from prenatal damage to the cerebral cortex, cochlea, and basal ganglia during the second trimester of pregnancy (complicated by the adverse effects of untreated postnatal hypothyroidism), whereas the microcephaly is a consequence of hypothyroidism during the third trimester. Iodine

replacement before the third trimester of pregnancy results in a significantly reduced incidence of microcephaly and neurologic abnormalities compared with treatment after this time (Fisher and Brown 2000, Sethi and Kapil 2004, Morreale de Escobar, Obregon and Escobar del Rey 2007).

Maturation of Brown-Fat Thermogenesis

The transition from fetal to neonatal life involves profound metabolic changes (Table 1.3). One of the most important changes is the development of nonshivering thermogenesis. The ability of human infants and selected other homeothermic newborn mammals to maintain body temperature in the immediate extrauterine environment depends on the presence and function of brown adipose tissue (BAT), the cells of which are characterized by high concentrations of mitochondria. BAT mitochondria contains a unique 32 kDa molecular weight protein (uncoupling protein or thermogenin) that sits on the inner membrane and uncouples phosphorylation by dissipating the proton gradient created by the respiratory chain.

Heat production by BAT is stimulated by catecholamines by way of β-adrenergic receptors and is thyroid hormone dependent. BAT T_3 is generated locally by deiodination of circulating T_4. Human BAT contains predominantly type II deiodinase. T_3 influences BAT thermogenesis by modulation of the deiodinase activity and stimulation of transcription of thermogenin (Fisher and Brown 2000).

The volume and functional activity of BAT, including deiodinase activity and thermogenin levels, increase progressively with fetal age so that BAT thermogenic activity is maximal in the perinatal period.

REGULATION OF THYROID FUNCTION IN NORMAL PREGNANCY

Thyroidal economy in normal pregnancy

Pregnancy has significant effects on thyroid function and on the course of thyroid diseases. Because autoimmune thyroid disease is common in women during the childbearing period, it is important to understand both the expected changes in thyroid

function tests in normal pregnancy and how pregnancy may affect preexisting thyroiditis, hypothyroidism, and Graves' disease in these women.

Table 1.3 Changes in thyroid function associated with fetal transition to extrauterine life at term

TRANSIENT CHANGES	• Neonatal TSH surge • Increased thyroid T_4 and T_3 secretion • Stimulation of BAT thermogenesis
PERMANENT CHANGES	• Elimination of placental T_4 and T_3 degradation • Decreased tissue type III monodeiodinase activity • Increased tissue type I monodeiodinase activity • Increased serum T_3 concentration • Decreased production of inactive thyroid hormone analogues (rT_3, T_4S, T_3S, rT_3S, T_2S) • Decreased extrahypothalamic TRH production • Decreased serum TRH concentration • Decreased serum TSH concentration • Resetting of the hypothalamic-pitutary free T_4 set point for control of TSH secretion.

Early in pregnancy, renal blood flow and glomerular filtration increase, leading to an increase in iodide clearance from the plasma, and resulting in a decreased plasma iodide and an increased requirement for iodide in the diet (Fantz, *et al.* 1999). In addition, there is further increment in iodine requirements as a result of the transplacental transport of iodide, which is required for iodothyronine synthesis by the fetal thyroid gland.

Current evidence indicates that human chorionic gonadotropin (hCG) is a thyroid stimulator during early pregnancy. A small, transient, but definite increase in free thyroxine occurs during the first trimester of normal pregnancy (Kol, Karnieli and Kraiem 1996). There is an inverse relationship between serum hCG and TSH concentrations: at the end of the first trimester, there is a mirror image between serum TSH (*nadir*) and hCG (*peak*) levels.

The increased plasma concentration of TBG, together with the increased plasma volume, results in several fold increase in the total thyroxine pool during pregnancy. The changes in TBG are most dramatic during the first trimester, but the increase in plasma volume continues until delivery. Thus, if the FT_4 concentration is to remain unaltered, the T_4 production rate must increase (or its degradation rate decrease) to allow the additional T_4 to accumulate. The increase in total serum T_4 and T_3 that occurs during pregnancy is due to an increase in serum TBG. This change appears early, and TBG concentrations are doubled by 16 to 20 weeks gestation (Glinoer 2001). Two factors are responsible for this increase:

1. Stimulatory effects of estrogen on TBG synthesis.
2. Reduced clearance of the more highly sialylated forms of protein.

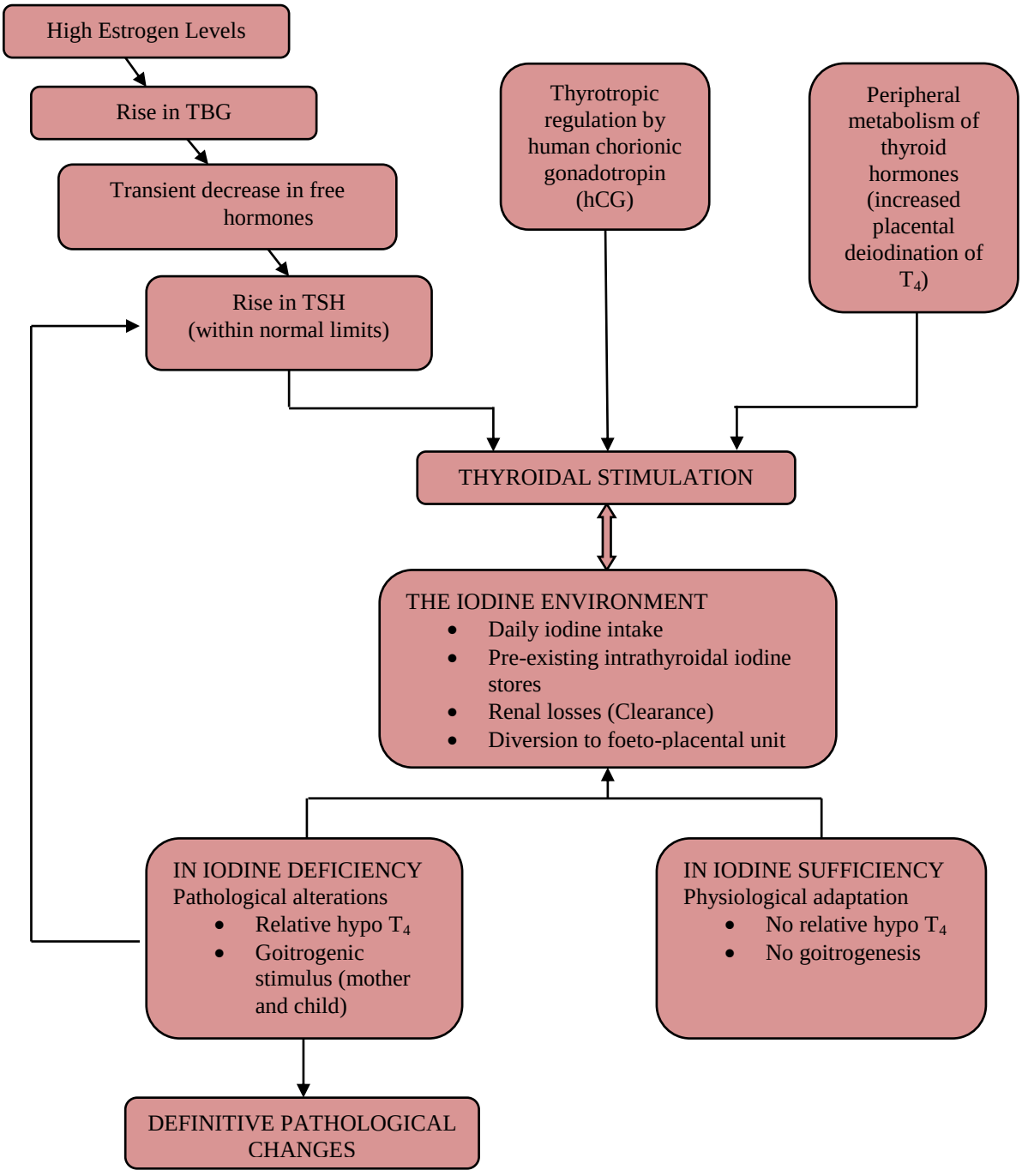
In addition to the twofold to threefold increase in serum TBG, modest decreases in both serum transthyretin (TTR) and albumin are commonly found in pregnancy. There is about a 30% to 50% increases in T_4 production during gestation.

THYROID FUNCTION IN HEALTHY PREGNANT WOMEN WITH IODINE DEFICIENCY (OR RESTRICTION)

The most adverse effect of iodine deficiency is damage to the fetus. Maternal thyroxine crosses the placenta before onset of fetal thyroid function at 10-12 weeks of gestation and represents up to 20 to 40% of T_4 measured in cord blood at birth (Sack 2003).

Reduced iodine intake during pregnancy leads to chronically enhanced thyroidal stimulation through the pituitary-thyroid feedback mechanism and is frequently accompanied by thyroidal alterations, mainly relative hypothyroxinemia and goitrogenesis (Glinoer 2001)(Fig 1.11). Also, a goiter formed during gestation may regress only partially after parturition; therefore, pregnancy may represent one of the environmental factors that may help to explain the higher prevalence of goiter and thyroid disorders in women compared to men. When iodine supplementation is given during early pregnancy and is maintained throughout gestation, it allows for the

correction and almost complete prevention of goitrogenesis. Goiter formation during pregnancy affects the mother and progeny (Glinoer 2001).



Source: Glinoer 1997

Figure 1.11 From physiologic adaptation to pathological alterations of the thyroidal economy during pregnancy

Normal amounts of thyroid hormones are needed for neuronal migration and myelination of the fetal brain, and insufficient iodine irreversibly impairs development of the brain. Severe iodine deficiency during pregnancy increases risk of stillbirths, abortions, and congenital abnormalities (Morreale de Escobar G 2004). Infant survival is improved in infants born to women whose iodine deficiency is corrected before or during pregnancy. Iodine treatment of pregnant women in regions of severe deficiency reduces fetal and perinatal mortality and improves motor and cognitive performance of the offspring (Zimmermann, Jooste and Pandav 2008, Zimmermann 2009).

AUTOIMMUNE THYROID DISEASE AND PREGNANCY

Several reports showed that euthyroid women who are thyroid antibody positive in the first trimester miscarry twice as often as women who are thyroid antibody negative. These findings raise the possibility that the presence of autoantibodies may be a marker for less readily recognized, but more generalized, autoimmune dysfunction. Hypothyroidism or hyperthyroidism probably will be found to be common in antibody-positive women when systemic follow-up studies of thyroid function are performed during the post-partum period.

In the early stages of gestation, antibody-positive women usually are able to maintain normal thyroid function as a result of sustained thyrotropic stimulation. At delivery, however, a marked reduction in FT_4 is often observed, with almost half of the antibody-positive women having FT_4 values in the hypothyroid range, thus confirming that such women have a reduced functional thyroid reserve and are therefore at risk of developing subclinical hypothyroidism during pregnancy (Glinoer, Rihai and Grun 1994).

EFFECT OF HYPOTHYROIDISM ON PREGNANCY OUTCOME

In general, infants of hypothyroid mothers appear healthy and without evidence of thyroid dysfunction, provided no iodine deficiency has been present in-utero. Severe maternal hypothyroidism during pregnancy raises concern about the potential long lasting psychoneurologic consequences for the progeny resulting from insufficient transplacental transfer of maternal thyroid hormones to the developing fetus during the first half of gestation, before the fetal thyroid gland becomes functional. In severe iodine deficiency, when both the maternal and fetal thyroid systems are unable to produce sufficient thyroid hormone, the damage to central nervous system development of fetus will be greater due to the absence of placentally transported thyroxine.

EFFECT OF HYPERTHYROIDISM ON PREGNANCY OUTCOME

Hyperthyroidism occurring during pregnancy is relatively uncommon. It has been estimated indirectly that approximately one or two of 1,000 pregnancies will be complicated by hyperthyroidism (Rinaldi and Stagnaro-Green 2007). The major cause of thyrotoxicosis in women of childbearing age is Graves' disease. Another cause has been characterized, resulting from direct stimulation of the thyroid gland by hCG, which can induce a transient form of thyrotoxicosis during first half of gestation known as gestational transient thyrotoxicosis (GTT).

Patient should be treated with antithyroid drug (ATD) to achieve euthyroid state, using the smallest possible dosage of these drugs. All ATD crosses the placenta and therefore may affect fetal thyroid function. Propylthiouracil (PTU) is more water soluble and is therefore less well transferred from the maternal to fetal circulation as well as from the maternal circulation into breast milk (Mestman 1998). This finding led to the recommendation that PTU should be used in preference to methimazole (MMI) or carbimazole (CMI) during pregnancy, unless specific therapy is directed to suppress thyroid function in the fetus.

CONGENITAL HYPOTHYROIDISM

The most common cause of congenital hypothyroidism worldwide is iodine deficiency, which can be eradicated by iodine supplementation. In iodine sufficient regions, the most common cause is thyroid dysgenesis which accounts for about 80% of cases of congenital hypothyroidism. The early recognition and treatment of congenital hypothyroidism results in normal intelligence quotient (IQ) score of patients compared with siblings and matched and unmatched normal children. Neurodevelopmental evaluations, however, may show subtle residual deficits in neuromotor, language and cognitive function in children whose hypothyroidism was quite severe or in whom the diagnosis was delayed or treatment was inadequate or excessive for prolonged periods. A delay in the diagnosis and institution of T_4 therapy beyond 6 to 8 weeks of age in infants with low serum T_4 concentrations since birth is likely to be associated with some impairment of intellectual performance. Infants with CH lose 3 to 5 points in IQ score each month that diagnosis and adequate T_4 therapy are delayed (Viley 2001).

SEAWEED: NATURAL APPROACH TO COMBAT THYROID DYSFUNCTION

Seaweeds are good sources to meet dietary requirements of iodine. Goiter precipitation caused by iodine deficiency is less prevalent in countries where marine algae form a part of the diet. The Japanese are extraordinarily free from goiter, apparently due to high iodine content of their diet of which seaweed forms a constant ingredient.

Seaweed has such a large proportion of iodine compared to dietary minimum requirements, that it is primarily known as a source of this nutrient. The highest iodine content is found in brown algae, with dry kelp ranging from 1500-8000 ppm (parts per million) and dry rockweed (Fucus) from 500-1000 ppm. In most instances, red and green algae have lower contents, about 100-300 ppm in dried seaweeds, but remain high in comparison to any land plants. Daily adult requirements, currently recommended at 150 µg/day, could be covered by very small quantities of seaweed. Just one gram of dried brown algae provides from 500-8,000 µg of iodine and even the green and red algae (such as the purple *nori* that is used in Japanese cuisine) provides 100-300 µg in a single gram.

Benthic marine macroalgae, commonly known as seaweeds, are increasingly viewed as potential sources of bioactive compounds with immense pharmaceutical, biomedical and nutraceutical importance. Many macroalgal species have been used as ingredients in both medicinal and food preparations, traditionally, in different regions across the world (Cardozo, Guaratini and Barros 2007, Chandini, Ganesan and Suresh 2008). There are 250 macroalgal species which have been listed as commercially utilized worldwide, among which 150 are consumed as human food (Barrow 2007). They are also considered as low calorie foods with high contents of minerals, vitamins, proteins and carbohydrates. Being rich in minerals, vitamins, trace elements and bioactive substances, seaweeds are called medical food of the 21st century (Khan and Satam 2003). Species of *Ulva, Porphyra, Gracilaria, Suhria, Caulerpa, Laminaria, Sargassum and Codium* are utilized in Japan, China and other countries. These algae are consumed by people as salads, puddings, jellies, soup etc.

Although the main source of iodine is *Chilie Salt Petre*, it is also being produced at present in smaller quantities from marine algae in France, Norway, Java and Japan from brown seaweeds and in Russia from red seaweeds. Certain seaweeds have the capacity to accumulate iodine in their tissues and therefore some of them can become natural source

of iodine. *Ascophyllum* can concentrate iodine up to 220 times that of seawater (0.01-0.007 ppm) while *Asparagopsis taxiformis* can accumulate up to 0.3% (Thomas, *et al.* 1987).

The edible seaweeds present in Indian coastal waters are species belonging to *Ulva, Enteromorpha, Chaetomorpha, Caulerpa, Codium, Hydroclathrus, Dictyota, Padina, Colpomenia, Rosenvingea, Chnoospora, Sargassum, Turbinaria, Porphyra, Halymenia, Grateloupia, Centroceras, Gracilaria, Hypnea, Rodymenia, Acanthophora and Laurencia* (Kathiresan 1990)**.**

Hence it was worth wile to observe our population to understand whether there is a need of screening these mothers for an early prevention. Keeping all these aspects in mind, a study was designed with the following broad objective:

Screening of pregnant women for thyroid disorders so as to prevent brain damage of fetus

Specific Objectives:

- **i.** Early prevention of thyroid disorders by protecting the mother from thyroid dysfunction.
- **ii.** To see the trimester specific changes in maternal thyroid hormones.
- **iii.** Neonatal screening (cord blood or within 3-7 days).
- **iv.** Comparison between normal pregnancies and thyroid insufficient pregnancies.
- **v.** To assess their nutritional status through anthropometry.
- **vi.** To assess their knowledge, attitude and practices (KAP) and food consumption pattern.
- **vii.** To provide nutrition health education to the all the women with low urinary iodine concentration and Hemoglobin.
- **viii.** To create awareness among mothers and family members regarding the use of iodized salt.
- **ix.** To suggest a referral to mothers in case of newly diagnosed hypothyroidism.
- **x.** To identify iodine rich seaweeds available in Gujarat coast.
- **xi.** Supplementation of iodine rich seaweeds to iodine deficient or thyroid insufficient pregnancies.

Review of Literature

2. REVIEW OF LITERATURE

The review of literature is broadly explained into following headings & subheadings

2.1 Pregnancy and maternal fetal interactions

2.2 Pregnancy and associated thyroid disorders

- 2.2.1 Hypothyroidism
  - 2.2.1.1 Fetal Hypothyroidism
  - 2.2.1.2 Maternal hypothyroidism
- 2.2.2 Hyperthyroidism
- 2.2.3 Antibodies and fertility, pregnancy and post partum thyroiditis

2.3 Iodine deficiency and damaged reproduction

- 2.3.1 Iodine intake and pregnancy
- 2.3.2 Iodine and the fetus & newborn
- 2.3.3 Iodine intake and thyroid autoimmunity

2.4 Iodine status in different countries

- 2.4.1 USA, Europe, Australia and Middle East
- 2.4.2 South East Asia- Japan, Korea, Thailand
- 2.4.3 India

2.5 Methodology of assessment of iodine deficiency

- 2.5.1 Urinary iodine excretion test
- 2.5.2 Thyroid stimulating hormone (TSH)

2.6 Drug therapy for thyroid disorders

2.7 Screening for thyroid dysfunction during pregnancy

2.8 Seaweed and iodine

2.1 PREGNANCY AND MATERNAL FETAL INTERACTIONS

Pregnancy affects virtually all aspects of thyroid economy (Burrow 1990).Hormonal changes and metabolic demands during pregnancy result in profound alterations in the biochemical parameters of thyroid function. Pregnancy can be viewed as a prolonged physiological condition in which a combination of events concur to modify the thyroidal economy. Such events may act independently, synergistically, or even antagonistically to produce subtle or major thyroidal effects. Furthermore these events take place at different time points during gestation, resulting in complex effects that may be seen only transiently or, by contrast, that persist until term.

There are two principal requisites for the maternal thyroid to be able to meet the burden imposed by the conceptus, namely

(i) The thyroid tissue is not functionally impaired, and
(ii) The supply of iodine for the synthesis of sufficient T_4 is almost double.

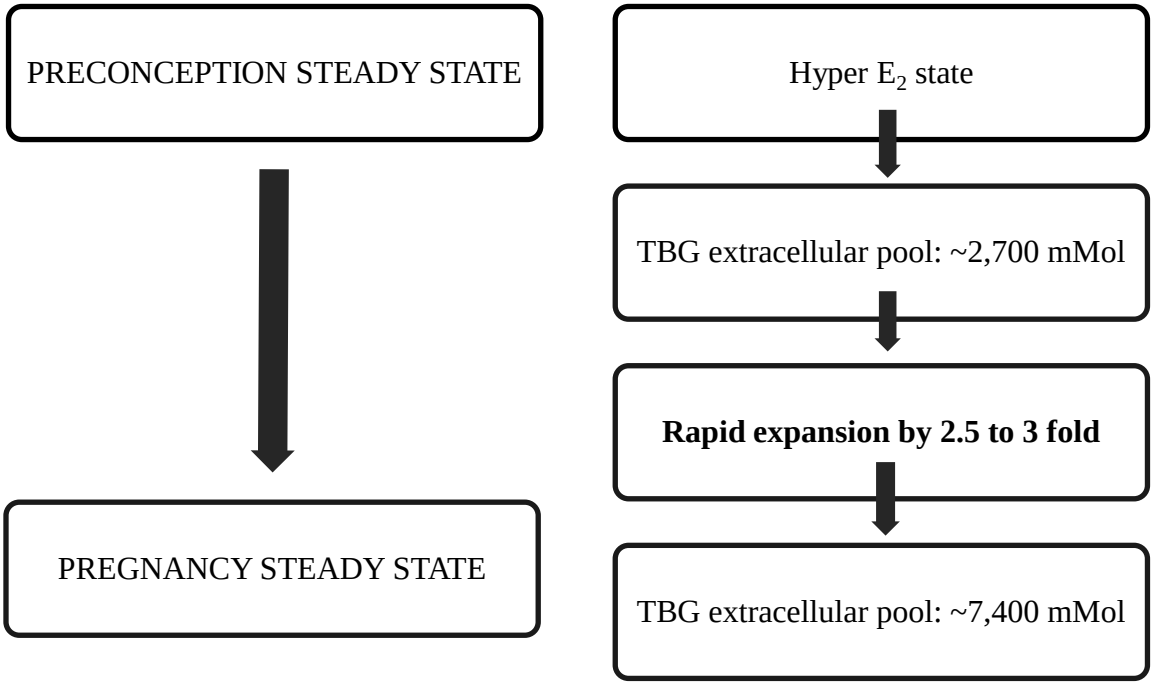
The thyroid hormone transport proteins: Thyroid hormones (TH) are transported in serum, noncovalently bound to three proteins: T_4 binding globulin (TBG), albumin and transthyretin. The relative distribution of TH among the binding proteins is directly related to both their affinities and concentrations. In steady state conditions the bound hormone fraction is in equilibrium with a free unbound fraction, which represents a minute amount of the total circulating TH: 0.04% for T_4 and 0.5% for T_3. Despite the fact that TBG in serum is by far the least abundant of the three transport proteins, about two thirds of the T_4 in serum of normal subjects is carried by TBG, owing to its extremely high affinity for the hormone.

The exact mechanisms by which this pregnancy-induced increase in TBG occurs is still unknown, but likely involve a combination of factors, including,

- increased estrogen/ estradiol (E_2), a primary female sex hormone, due to placental secretion
- increased TBG production by the liver
- prolonged TBG half-life because of increased sialyation as increased sialic acid content inhibits the uptake of protein by specific asialylo-glycoprotein receptors on hepatocytes

- increased TBG molecular stabilization because more T_4 is proportionately bound to it (Glinoer 1997).

Compared with preconception concentrations (average 15-16 mg/liter), serum TBG begins to increase in pregnancy after a few weeks and reaches a plateau around midgestation, 2.5 fold higher than the initial value (between 30-40 mg/liter). Thereafter, the TBG concentration remains practically unchanged until term (Fig. 2.1).



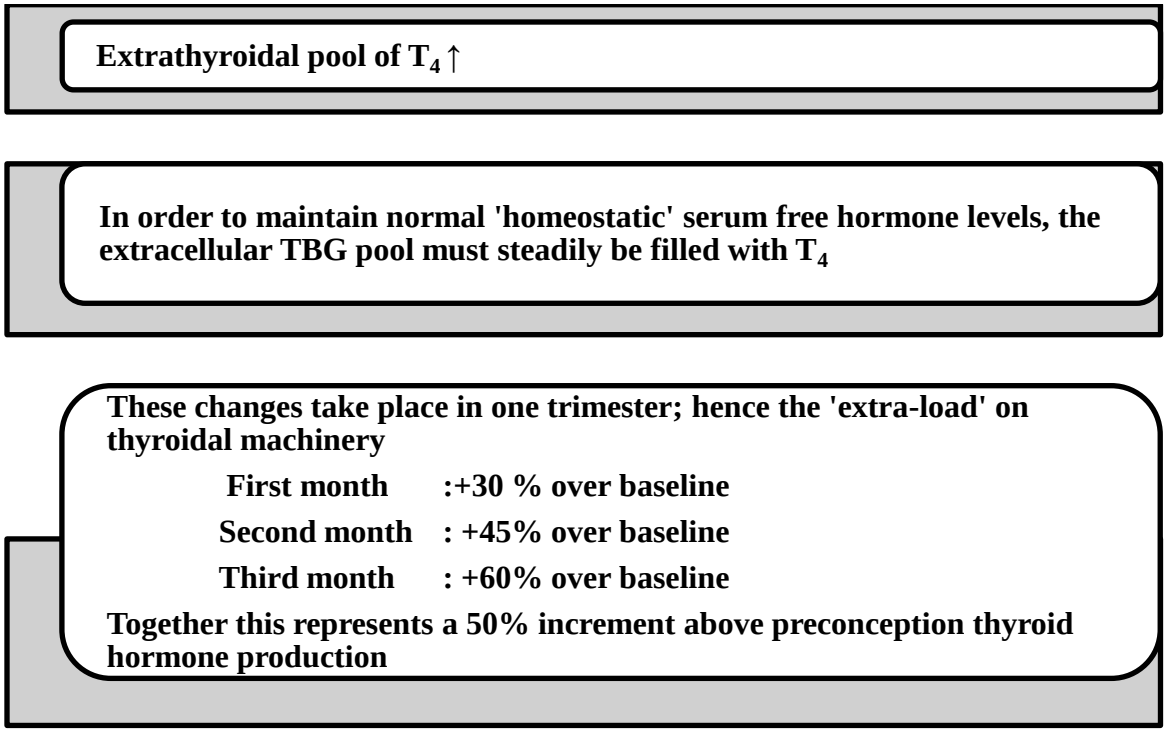
Source: www.thyroidmanager.org

Figure 2.1 Rapid changes that occur in serum total binding capacity of TBG during the first half of gestation under the influence of elevated estrogen levels

Total thyroid hormones: In pregnancy, the alterations in total TH levels are the direct consequence of the marked increase in serum TBG: total T_4 and T_3 levels increase significantly during the first half of gestation. Levels of serum T_4 rise sharply between 6 and 12 weeks, progress more slowly thereafter, and stabilize around midgestation. Total T_4 concentrations reach a peak at the 20^{th} gestational week and high levels of total T_4 are maintained through the second and third trimesters. (Hotelling and Sherwood 1971). Because of the 20-fold greater affinity of TBG for T_4 compared with T_3, changes in T_4 levels follow the changes in TBG more closely. The concentration of TBG doubles during early pregnancy and reaches a plateau at approximately 20^{th} gestational week. The

rise in total T_3 is more progressive throughout pregnancy. It can be therefore expected that the T_3/T_4 molar ratio should remain essentially unaltered during pregnancy (Glinoer, De Nayer, *et al.* 1990, Glinoer 1997).

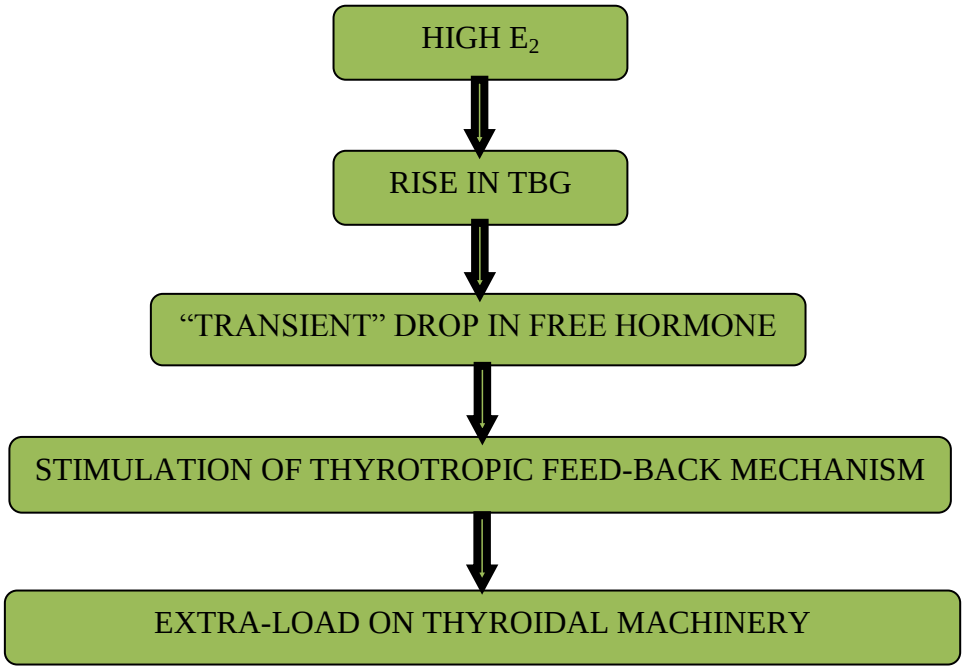
These modifications represent the necessary adjustment from the "old" (preconception) steady state equilibrium to the "new" (gestational) equilibrium of the thyroidal economy. The changes are initiated by the progressive expansion of the TBG extracellular pool, which increases from ~2,700 to 7,400 nmol over a trimester, accompanied by a major increase in hormone-binding capacity of the serum. In the nonpregnant woman, approximately one third of circulating TBG carries a T_4 molecule i.e. the molar T_4/TBG ratio is 0.35-0.40. To ensure homeostasis of the free hormone concentrations during pregnancy, the extrathyroidal T_4 pool must increase in parallel (Fig. 2.2). The thyroidal adjustment thus implies that, in the early stages of pregnancy, a transient period takes place, during which T_4 and TBG concentrations are constantly changing (Glinoer 1991).



Source: www.thyroidmanager.org

Figure 2.2 To maintain unaltered free T_4 levels, the markedly increased TBG extra-cellular pool must steadily be filled with increasing amounts of T_4, until a new equilibrium is reached

Because the rapid rise in the serum hormone-binding capacity due to increased serum TBG levels tends to induce a trend toward slightly decreased free hormone concentrations, the thyroidal adjustment is regulated primarily through the normal pitutary-thyroid feedback mechanism, i.e. by TSH stimulation of the thyroid gland (Fig. 2.3). In healthy pregnant women, the "extra load" on thyroidal machinery is relatively minor, and these physiological changes are unnoticeable: an increase in serum TSH is not commonly observed. On the contrary, in women with iodine deficiency or autoimmune thyroiditis and subclinical hypothyroidism, the TSH surge is amplified, and increases in serum TSH is demonstrated, revealing the underlying mechanism of adaptation (Glinoer 1997).



Source**:** Glinoer 1997

Figure 2.3 Adjustment of thyroidal economy in relation with elevated estrogen levels

Free thyroid hormones: Free T_4 and T_3 levels also increase slightly during the first trimester following stimulation of thyroid gland by hCG, but return to normal by about 20 wk of gestation and remain so until delivery. Reports to how this increase is vary markedly in different studies, since changes in serum protein fractions have an effect on

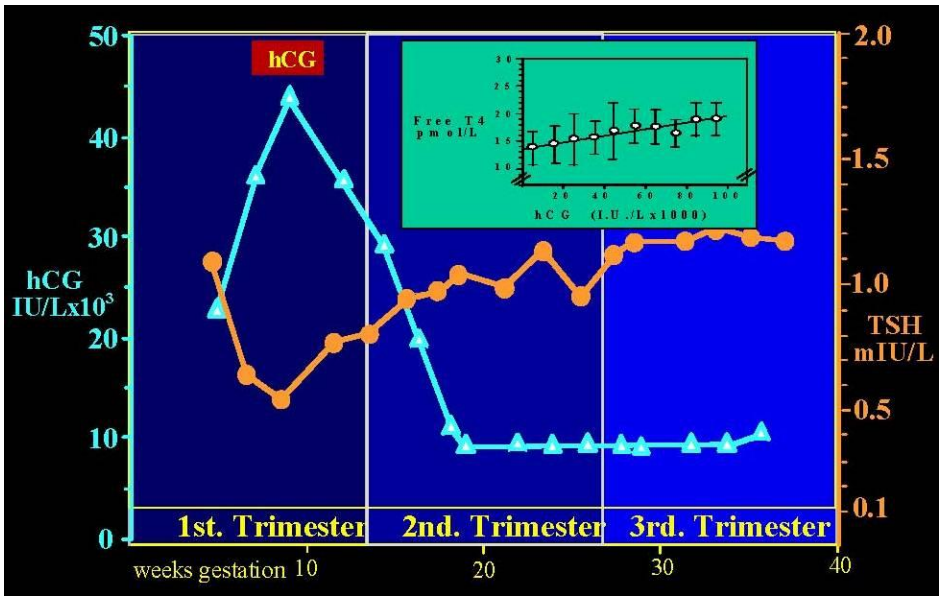
assays of free thyroid hormones, especially as regards FT_4 immunoassays (Roti, *et al.* 1991, Lee, *et al.* 2009). However, serum FT_4 levels are on an average higher in the first trimester of pregnancy than in nonpregnant women (Glinoer, De Nayer, *et al.* 1990, Soldin, Tractenberg, *et al.* 2004, Kahric-Janicic, *et al.* 2007), although in some studies this has not been observed (Lee, *et al.* 2009, Anckaert, *et al.* 2010). In the second and third trimester, serum FT_4 levels are 20-30% decreased compared with first trimester concentrations, a fact observed using all current methods (Glinoer, De Nayer, *et al.* 1990, Kahric-Janicic, *et al.* 2007, Lee, *et al.* 2009, Anckaert, *et al.* 2010). Longitudinal studies based on reliable methodology (i.e. methods that are not influenced by changes in serum TBG and albumin levels) in large numbers of pregnant women without iodine deficiency have confirmed that serum free T_4 levels are lower by an average of 10-15% at delivery, in comparison with reference range of nonpregnant female subjects (Glinoer 1997). Changes in free T_3 levels follow a parallel pattern. In most pregnant women, however, free hormone levels are maintained within the nonpregnant reference range (Burrow 1993).

The serum levels of thyroglobulin (TG): Even though the TG molecule has no peripheral hormonal action, the serum levels of TG represent a sensitive, albeit nonspecific, indicator of the activity or stimulation state of the thyroid gland. Several studies have indicated that TG is frequently elevated during pregnancy: the increase in TG can be observed as early as first trimester, but by later stages of gestation and particularly near term is significantly more pronounced (Rasmussen, *et al.* 1989).

Iodine metabolism: In pregnancy, the renal clearance of iodide increases significantly because of an increased glomerular filtration rate. Renal hyperfiltration and increased clearance, observed for iodide begins in the early weeks of gestation and persists until term, thereby constituting an obligatory renal iodine “leakage” (Dafnis and Sabatini 1992, Glinoer 1993) . The iodide loss tends to lower the circulating levels of inorganic iodide and induces a compensatory increase in thyroidal iodide clearance, which reaches 60 ml/min and is accompanied by an absolute elevation of iodide entry into the gland.

A second mechanism of iodine deprivation in the mother occurs later in gestation, from the passage of a part of the available iodine from the maternal circulation to the fetal-placental unit. At midgestation, the fetal thyroid gland starts to produce thyroid hormones that are indispensable for adequate development of the fetus (Glinoer 1997).

Hypothalamic pitutary control of thyroid function and the role of human chorionic gonadotropin (hCG): In prospective studies on maternal thyroid function in pregnancy, the regulatory role of hCG was first investigated in a cohort of several hundred women in whom TSH and hCG levels were systematically determined between 8-14 weeks of gestation (Glinoer, De Nayer, *et al.* 1990) (Fig. 2.4). The results showed that a lowering in serum TSH was coincident with the peak hCG levels. The profiles of changes in serum TSH and hCG were clear mirror images, and there was a significant reciprocal correlation between TSH and hCG in individual samples. Mean serum TSH levels increase as levels of hCG decrease after the 10th gestational week, and mean second and third trimester TSH levels are higher than those in the first trimester (Glinoer 1997). The changes observed in the hypothalamic-pitutary-thyroid axis during pregnancy are summarized in figure 2.5.



Source: Glinoer, De Nayer, *et al.* 1990

Figure 2.4 The pattern of serum TSH and hCG changes as a function of gestation age in 606 healthy pregnant women

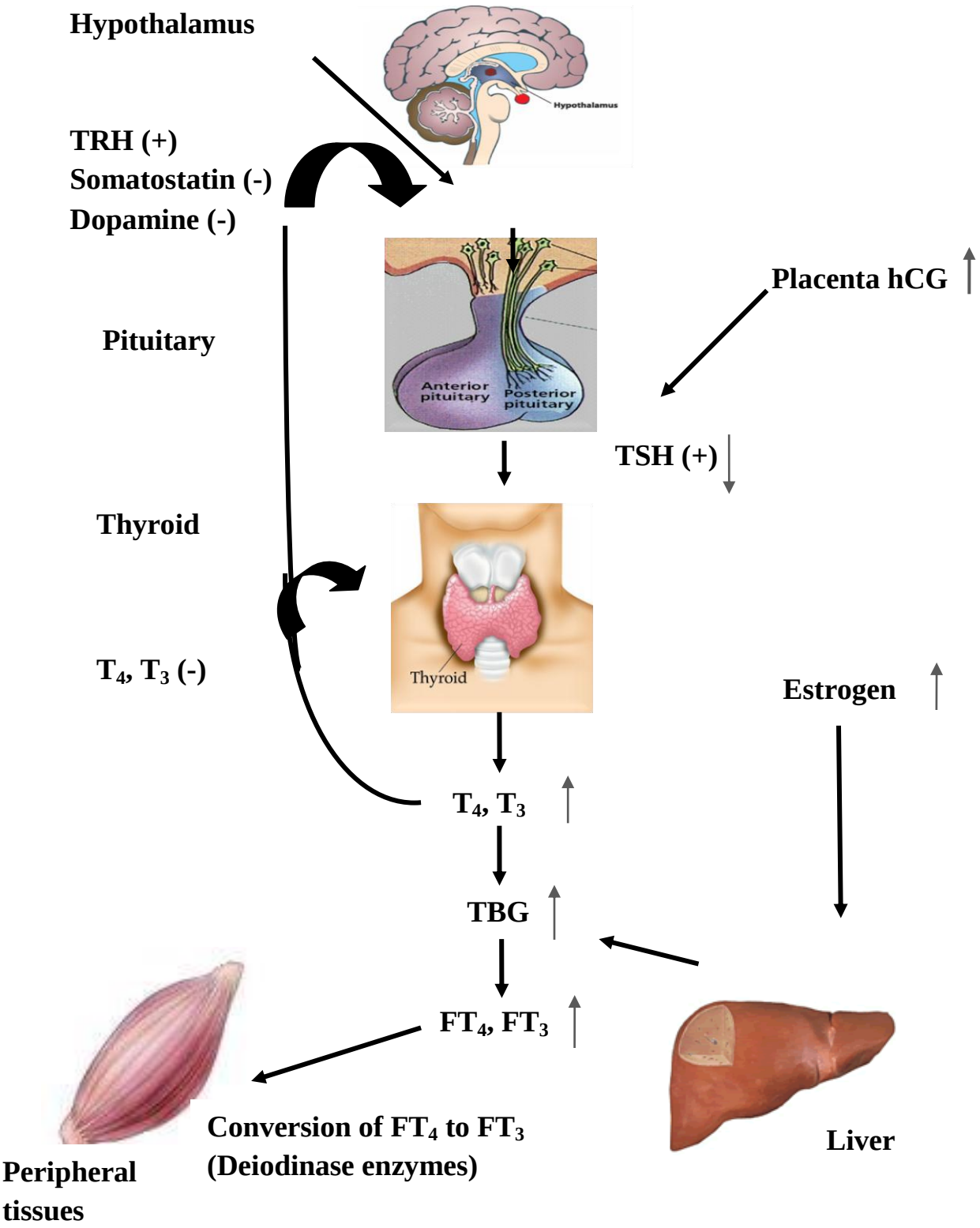


Figure 2.5 Changes in the hypothalamus-pitutary-thyroid axis during pregnancy

The result also indicated a linear relationship between hCG and free T_4 concentrations during early gestation. Thus, the lowering of TSH corresponds to a transient and partial blunting of the pitutary-thyroid axis associated with an increased hormonal output by the thyroid gland. These results suggested that hCG is a thyroid regulator in normal pregnancy. Ballabio, Posyachinda and Ekins 1991 also proposed that hCG can be considered as "a putative physiological regulator" of maternal thyroid function in normal pregnancy.

This pattern coincides with that of hCG levels, which is the probable cause of the increase in free hormone levels in the first trimester (Ballabio, Posyachinda and Ekins 1991). A slight decrease in serum TSH during the first trimester indicates that the free T_4 and T_3 changes are not dependent on the hypothalamic-pituitary axis (Yoshikawa, Nishikawa and Horimoto 1989). Owing to the increase in the glomerular filtration rate (GFR), iodide clearance increases during gestation, leading to increased dietary requirements. If these are not met, serum T_4 falls, TSH increases, and goiter ensues. There is no clinically significant change in the size of the thyroid during pregnancy in normal women receiving adequate quantities of iodide.

Serum TSH is suppressed, in keeping with the no pituitary source of the thyroid stimulation. The hyperthyroidism of molar pregnancy is usually not severe and is relieved by evacuation of the molar tissue. In early pregnancy, hCG may also play an important role in hyperemesis gravidarum, in which may occur an elevated free T_4 and, at times, free T_3 levels and suppressed TSH during the acute phase (Norman, Green-Thompson and Jilal 1981, Goodwin, Montoro and Mestman 1992).

The basal metabolic rate (BMR) increases during the second trimester owing to the increase in the total mass of body tissue consequent to the pregnancy. The changes of pregnancy, together with the decreased peripheral vascular resistance, vasodilation, and modest tachycardia, may suggest thyrotoxicosis. It is important to appreciate that these are normal physiological changes of pregnancy, especially when one treats hyperthyroidism in the pregnant patient.

Throughout gestation, maternal thyroid hormone concentrations are crucial to normal fetal development. The release of fetal T_4 into the circulation only starts at approximately 20 weeks of gestation, and even during the second half of gestation normal fetal thyroid hormone levels are partly maintained through maternal thyroid hormone supply (Morreale de Escobar, Obregon and Escobar del Ray 2004).

The peripheral metabolism of thyroid hormones is also altered during normal pregnancy. D1 is thought to function as in nonpregnant subjects. D2 and D3 are present in the placental tissue, the former preferring T_4 and rT_3 as substrates and the latter converts T_4 and T_3 to their inactive forms. D2 is present in the chorionic and decidual membranes of placenta and D3 in the trophoblasts. The activity of D2 increases when the availability of T_4 decreases, and therefore the placental tissues are able to maintain T_3 production in the placenta even when T_4 values are reduced (Glinoer 1997).

Fetal thyroid ontogeny begins at 10-12 weeks gestation and is not complete until delivery; T_4 is not secreted until 18-20 weeks (Glinoer and Delange 2000). T_4 is critical for many aspects of brain development including neurogenesis, neuronal migration, axon and dendrite formation, myelination, synaptogenesis and neurotransmitter regulation. Although these requirements evolve over months, an especially critical time is the second trimester (Morreale de Escobar, Obregon and Escobar del Rey 2000). During later phases of fetal brain development, from third trimester onwards, the supply of thyroid hormones to the fetus is essentially of fetal origin. Therefore, while severe maternal hypothyroidism during the second trimester will result in irreversible neurological deficits, maternal hypothyroxinemia occurring at later stages will result in less severe , and also partially reversible, fetal brain damage (Smallridge and Ladenson 2001). It is considered that 30% of serum T_4 levels measured at birth in cord blood is of maternal origin.

An approximately 50% increase in levothyroxine dose is required during gestation for women with primary hypothyroidism (Kaplan 1992). One possible explanation for this phenomenon is an increased rate of T_4 deiodination or conjugation by the placental and/or fetal T_3. However, no changes in the metabolic clearance of T_4 were found during

pregnancy in one study. Absorption of levothyroxine may be reduced during pregnancy, or the sensitivity of the hypothalamic-pituitary axis may be altered.

2.2 PREGNANCY AND ASSOCIATED THYROID DISORDERS

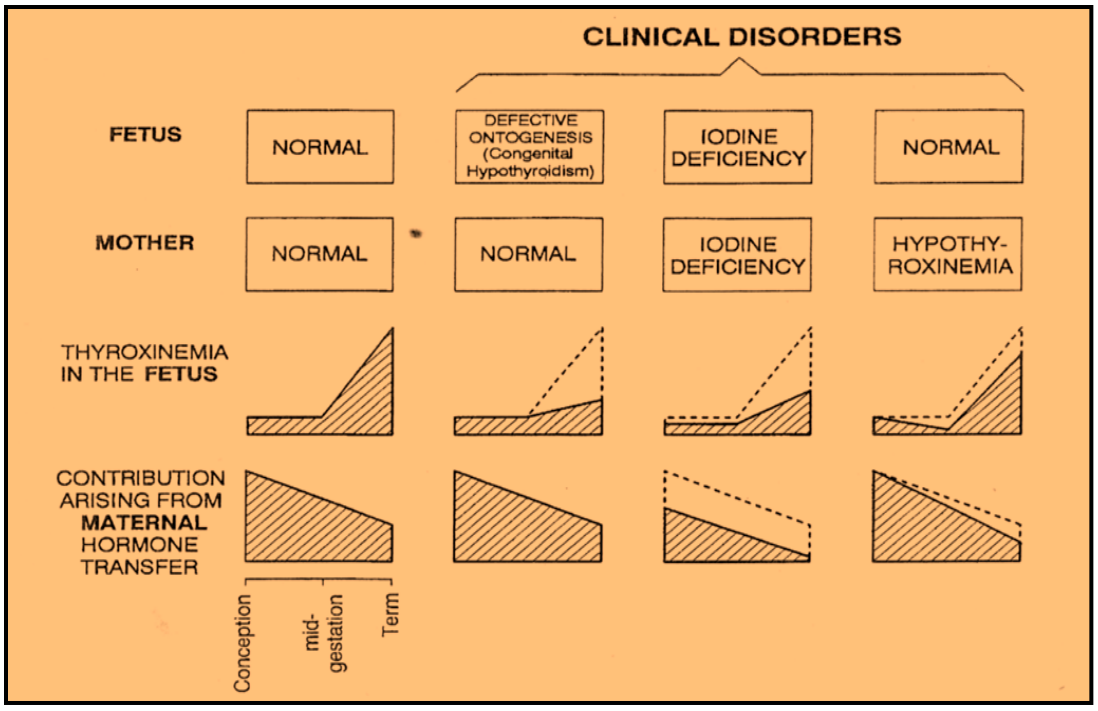
2.2.1 Hypothyroidism

Cause of thyroid dysfunction

Abnormal thyroid gland function may be restricted to the fetus, the expectant mother or both. Fetal hypothyroidism can be permanent or transient. When transient it results from transplacental passage of autoantibodies or drugs, or to immaturity of the HPT axis in premature infants. Combined maternal and fetal hypothyroidism is almost always due to iodine deficiency (Glinoer 1997, Glinoer and Delange 2000). Severe maternal hypothyroidism is not common, but mild thyroid failure in which the serum TSH is elevated with a normal FT_4 level has been reported in 2.5% of pregnancies in the United States (Klein, *et al.* 1991).

In thyroid disease, three sets of clinical disorders ought to be considered and are schematically illustrated in fig. 2.6. For infants with a defect of thyroid gland ontogeny leading to congenital hypothyroidism, the participation of maternal thyroid hormones to the circulating fetal thyroxine environment is unaffected due to sufficiency from mother. As a result of this the risk of brain damage results exclusively from insufficient fetal thyroid hormone production. In contrast, when the maternal thyroid is deficient (i.e. in women with thyroid autoimmunity), it is both the severity and temporal occurrence of maternal hypothyroxinemia that drive the resulting consequences for fetal neuronal development. Finally, in iodine deficiency, both the maternal and fetal thyroid functions are affected, and it is the degree and precocity of iodine deficiency that drive the potential repercussions for fetal neurologic development.

It is now believed that even mild maternal hypothyroidism (from mild iodine deficiency, thyroid autoimmunity, or thyroid under-replacement) may affect fetal brain development. The implications of this finding are yet to be clearly defined, but have raised many questions that need resolution (Smallridge and Ladenson 2001).



Source: Glinoer and Delange 2000

Figure 2.6 Schematic representation of three sets of clinical conditions that may affect thyroid function in the mother alone, fetus alone or the fetomaternal unit, that may eventually lead to alterations in fetal thyroxinemia

2.2.1.1 Fetal hypothyroidism

Before the 1970s, 40% of children with congenital hypothyroidism (CH) required special education. Neonatal screening programs are now implemented throughout the developed world and have reduced the need for special education to only 10% of CH children (Van Vilet 1999). The severity and duration of fetal hypothyroidism reflects the level of intellectual impairment, and can be assessed by serum T_4 and skeletal maturation measured by knee bone area at birth. Those newborns with T_4 less than 20 µg/L and knee bone surface area less than 0.05 cm^2 have IQs in childhood that are 12-16 points lower than those with mild CH.

Congenital Hypothyroidism (CH) is one of the most common preventable causes of mental retardation. The worldwide incidence is 1:4000 live births. Worldwide, neonatal screening program for CH have significantly reduced the intellectual deficits in the hypothyroid children treated early. Newborn screening and thyroid therapy started within

2 weeks of age can normalize cognitive development (Foley T 2006). Growth rate and adult height are normal in children with CH in whom thyroxine therapy is consistently maintained. There are only minor differences in intelligence, school achievement, and neuropsychological tests in adults with CH that was treated early with thyroxine compared with control groups of classmates and siblings.

Studies from India, though limited, show a high incidence of CH. The initial reports came from screening of over 22,000 newborns from different parts of the country with and without iodine deficiency to determine the incidence of CH. The data showed that the incidence of CH was about a hundred-fold more in seriously iodine deficient endemic districts. National neonatal screening programs are in place in developed countries to prevent early diagnosis and treatment to prevent irreversible sequelae, that is, short stature and mental retardation. Unfortunately there is no such program of congenital hypothyroidism in India.

The exact incidence of congenital hypothyroidism in India is unknown. Study by Desai 1997 based on a neonatal screening program in Mumbai places the incidence at 1: 2500 – 1: 2800. Higher incidence was described from Hyderabad, and preliminary data from Kerala (Devi and Naushad 2004, Mathew, Jain and John 2007).

Although the American Association of Pediatrics (AAP) recommends a heel prick sample after 48 hours of life, umbilical cord sampling is a practical and effective way to diagnose CH and has been recommended by the Indian Academy of Pediatrics (IAP) as an alternative (Manglik, Chatterjee and Ghosh 2005).

2.2.1.2 Maternal Hypothyroidism

The frequency of mild and overt hypothyroidism among pregnant women in the United States has been reported (Klein, *et al.* 1991). From this report, they found a serum TSH level greater than 6 mIU/L in 2.5% (49 of 2000) of women at 15-18 weeks gestation. Overt hypothyroidism was present in 0.3% of women. Untreated hypothyroidism is associated with several complications; most notably pre-eclampsia and low birth weight, but also placental abruption and increased risk of spontaneous miscarriage and perinatal mortality (Smallridge and Ladenson 2001).

Several studies across the world have evaluated the incidence of hypothyroidism during pregnancy (Table 2.1). (Casey, Dashe and Well, *et al.* 2005) screened pregnant women before 20 week gestation and reported doubling of the rate of preterm delivery in those with subclinical hypothyroidism (SCH).

A study on 960 healthy Dutch women with term gestation and cephalic fetal presentation, thyroid parameters were assessed at 36 weeks of gestation and related to fetal head position (anterior cephalic vs. abnormal cephalic). It concluded that lower the maternal FT_4 concentration at 36 weeks of gestation, the higher the risk of abnormal presentation at birth (Wijnen, *et al.* 2009).

In another study Kuppens, *et al.* prospectively followed 1058 Dutch Caucasian healthy pregnant women from 12 weeks of gestation until term (≥37 weeks) delivery. Compared with women with fetuses in the cephalic position, those women who presented in breech at term had significantly higher TSH concentrations, but only at 36 weeks gestation ($p=0.007$). No between group differences were obtained for FT_4 level at any assessment. Breech position was significantly and independently related to high maternal TSH concentration (≥2.5 mIU/L) at 36 weeks gestation (O.R. 2.23, 95% CI: 1.14-4.39), but not at 12 and 24 weeks gestation. The same group in 2004 had found that breech presentation at term is related to FT_4 at 12 weeks gestation using a highly selected sample of women (i.e. those with FT_4 levels below the 10^{th} percentile matched to those with an FT_4 between the 50^{th} and 90^{th} percentile)(Pop, Brouwers and Wijnen 2004).

In 1999, another research study by Pop, Kuijpens, *et al.* 1999 tested mental and psychomotor development in 220 ten-month-old infants living in the Netherlands, an iodine-sufficient country. They found that if the mother's FT_4 was in the lowest 10^{th} percentile at 12 weeks gestation, the infants had increased risk of delayed psychomotor development.

Intellectual and motor development of children at 25 to 30 months of age is separately associated with abnormalities of maternal thyroid at 16-20 weeks gestation. Maternal subclinical hypothyroidism, hypothyroxinemia or euthyroidism with elevated TPO-Ab titers were all statistically significant predictors of lower motor and intellectual development at 25-30 months (Li, *et al.* 2010).

Table 2.1 Studies that evaluated the incidence of hypothyroidism during pregnancy

First Author	Year	N	Country	Gestational week	TSH	T_4	Hypothyroidism	
							Subclinical	Overt
Mannisto	2009	5,805	Finland	12th week	>95th percentile	<5th percentile	3.9%	0.9%
Benhadi	2009	2,497	Netherlands	Mean 13th week	>5.6 mU/L	<7.5 pmol/L	0.5%	
Sahu	2009	633	India	13-26 week	>5.5mU/L	NR	6.5%	4.6%
Cleary-Goldman	2008	10,990	USA	1st trimester	>97.5th percentile	<2.5th percentile	2.2%	0.3%
Aoki	2007	209	USA		>5.0 mU/L		6.9%	
Vaidya	2007	1560	UK	Median 9th week	>4.3µIU/ml	<12.0 pmol/L	1.6%	1.0%
Casey	2005	17,298	USA	<20 weeks	>97.5th percentile	<0.680 ng/dl	2.3%	0.2%
Allan	2000	9403	USA	15-18 weeks	>6 mU/L	NR	2.2%	
Klein	1991	2000	USA	15-18 weeks	>6 mU/L	<2 SD	2.2%	0.3%

NR= Not reported; SD= standard deviation; Gestational week= the gestational week during pregnancy that the sample was obtained

There is growing recognition of the importance of diagnosing thyroid insufficiency in early pregnancy. Mothers with subclinical hypothyroidism are more prone to experience fetal death than euthyroid controls (Allan, Haddow and Palomaki 2000).

In a study (Abalovich, Gutierrez, *et al.* 2002), the authors showed that when hypothyroid patients were not rendered euthyroid, their pregnancy either ended in spontaneous abortion (in over 60% of cases) or led to an increased prevalence of preterm deliveries. Conversely, in pregnant hypothyroid women with adequate treatment, the frequency of abortions were minimal and, in general, the pregnancies were carried to term without complications. Some studies have evaluated the relationship between hypothyroidism and fetal demise which is presented in table 2.2.

In addition, recent reports suggesting that maternal subclinical (mild) hypothyroidism during early pregnancy may compromise the IQ of the child, has prompted recommendations that all the pregnant women should have TSH and TPO-Ab levels measured in the first trimester of their pregnancy (Haddow, *et al.* 1999). In this study the severity of hypothyroidism varied from overt hypothyroidism (OH) to probable SCH among the women whose children were investigated at school age. It was concluded that children born to untreated hypothyroid women had, on the average, an IQ score that was fully 7 points below the mean IQ of children born to healthy women and thyroxine-treated women. Furthermore, there were three times as many children with IQs that were 2 SD scores below the mean IQ of the controls in the children born to untreated hypothyroid women. It indicated that undisclosed and untreated hypothyroidism (and probable SCH) during pregnancy was associated with a risk of poorer outcome in the progeny and a 3-fold increased predisposition for having learning disabilities.

The physiological log/linear TSH/FT_4 relationship dictates that a serum TSH abnormality is the earliest indicator of developing primary thyroid dysfunction. The accuracy of serum TSH reference limits critically impacts the designation of what constitutes an "abnormal" serum TSH. Improvements in the sensitivity of TSH methods over the last 3 decades have now established the lower TSH limit as 0.3 – 0.4 μIU/ml and are responsible for a contraction of TSH upper reference limit from 10 to 4 μIU/ml. The current TSH reference range (0.4 to 4 μIU/ml) does not confirm to a normal Gaussian

Table 2.2 Studies that evaluated the relationship between hypothyroidism and fetal demise

First Author	Year	Country	Number		Thyroid		Miscarriage		Fetal death		Gesta-tional age	P
			Total	Hypo-thyroid			Hypo-thyroid	Control	Hypo-thyroid	Control		
Sahu	2009	India	633	41- SCH 29- OH	SCH TSH >5.5 µIU/ml Normal FT_4	OH TSH >5.5 µIU/ml Low FT_4	NR	NR	25%- SCH 13%- OH	1.4%	13-26 Wks	NS
Mannisto	2009	Finland	5,805	224- SCH 54- OH	SCH TSH >95th Normal FT_4	OH TSH >95th FT_4 <5th	16.1%- SCH 22.2%- OH	19.5%	1.8%- SCH 1.9%- OH	0.8%	12th Wk	NS
Hallengren	2009	Sweden	63	19- Hypo 12- Hyper	Normal TSH 0.4 – 4.0 µIU/ml		NR		Euthyroid- 6% Hypo/Hyper- 29%		1st trim	<0.05
Cleary-Goldman	2008	USA	10,990	240- SCH 243- Hypo	SCH TSH >97.5th Normal FT_4	Hypo Normal TSH FT_4 <2.5th	0.4%- SCH 0.0%- Hypo	0.6%	0.0%- SCH 0.4%- Hypo	0.3%	1st trim	NS
Casey	2007	USA	17,298	233	Isolated hypothyroxinemia TSH 2.5th – 97.5th T_4 <0.680 ng/dl		NR	NR	0%	0.5%	<20 Wks	NS
Matalon	2006	Israel	139,168	1,102					1.4%	1.3%		NS
Casey	2005	USA	17,298	404	SCH TSH >97.5th percentile T_4 <0.680 ng/dl		NR	NR	0.5%	0.5%	<20 Wks	NS
Abalovich	2002	Argentina	150	35-SCH 16- OH	Adequate R_x TSH 4mIU/L		60%- OH 71%- SCH	0%- OH 0%- SCH	NR	NR	NR	<0.006
Allan	2000	USA	9,403	209	Hypo definition TSH 6 µIU/ml		3.8%	0.9%	NR	NR	15-18 Wks	<0.05

NR= Not reported, Wks= Weeks, Hypo= Hypothyroidism, Hyper= Hyperthyroidism, SCH= Subclinical hypothyroidism & OH= Overt hypothyroidism

distribution and exhibits a skewed upper limit (Spencer, Takeuchi and Kazarosyan 1996). Whereas there is now broad agreement that the lower TSH reference limit approximates 0.3 – 0.4 μIU/ml, the accuracy of the current upper limit (reported as 4.0 μIU/ml by most laboratories) is uncertain. Since most rigorously selected normal euthyroid subjects have serum TSH values below the Gaussian limit of 2.5 μIU/ml, it is likely that the TSH upper limit is skewed by the inclusions of individuals with occult, early autoimmune thyroid disease (AITD), misclassified as "normal euthyroid subjects" (Hollowell, Staehling and Flanders 2002).

Benhadi, *et al.* 2009 examined the relationship between maternal TSH and FT_4 concentrations in early pregnancy and the risk of miscarriage, fetal or neonatal death in a cohort of 2497 Dutch women without overt thyroid dysfunction. It was reported that the incidence of child loss increased by 60% for every doubling in TSH concentration. Maternal FT_4 levels and child loss were not associated.

Prevalence of subclinical hypothyroidism among pregnant women is fairly high among Indians and have high rates of TPO antibody positivity (Gayathri, Lavanya and Raghavan 2009). Five hundred pregnant women attending two government Obstetrics and Gynecology hospitals in Chennai during a period of 5 months in 2007, were studied. Excluding subjects with known thyroid diseases, 495 subjects were examined. Subclinical hypothyroidism was detected in 2.8%, among them TPO antibodies positivity was seen in 57.1% whereas euthyroid women had significantly lower positivity (7%). No association was seen between hypothyroidism or TPO antibody positivity with gestational age or parity. Hypothyroidism diagnosed by elevated TSH value (> or = 5.0 mg/l) was significantly associated with increasing gestational age (Trend $chi^2 = 6.02$, $p = 0.014$).

A study by Rao *et al.*, 2008 demonstrated that hypothyroidism has a statistically significant relationship with recurrent pregnancy loss (RPL) in the first trimester and suggests that diagnosis of hypothyroidism could help couples with recurrent pregnancy loss to have a successful outcome in subsequent pregnancies. The study included 163 non-pregnant women with recurrent pregnancy loss in a gestational age up to ≤ 12 weeks verified by a pregnancy test or ultrasonography, and a total of 170 age matched women

with at least one successful pregnancy and no history of miscarriages were selected as controls. Hypothyroidism was found in seven (4.12%) women with RPL and one in control group. The differences in the levels of serum T_3, T_4 and TSH between euthyroid and hypothyroid women were found significant in women with RPL in first trimester.

Sahu, *et al.* 2010 studied 633 pregnant women to find prevalence of thyroid dysfunction in pregnancy and its impact on obstetrical outcome in Indian population. Patients were enrolled in second trimester and followed till delivery. Prevalence of thyroid dysfunction was high in this study, with subclinical hypothyroidism in 6.47% and overt hypothyroidism in 4.58% women. Overt hypothyroids were prone to have pregnancy-induced hypertension (P=0.04), intrauterine growth restriction (P=0.01) and intrauterine demise (P=0.0004) as compared to control. Cesarean section rate for fetal distress was significantly higher among pregnant subclinical hypothyroid women (P=0.04). Neonatal complications and gestational diabetes were significantly more in overt hyperthyroidism group (P=0.03 and P=0.04, respectively). Significant adverse effects on maternal and fetal outcome were seen emphasizing the importance of routine antenatal thyroid screening.

Maternal and fetal hypothyroidism

Combined maternal and fetal hypothyroidism occurs mostly in regions with dietary iodine deficiency. The most severely affected infants have neurological cretinism, manifested by mental retardation and impaired gait and motor function (Delange 2000). The abnormalities have been associated with maternal T_4, but not T_3, levels during pregnancy (Pharoah, *et al.* 1984).

2.2.2 Hyperthyroidism

Clinical hyperthyroidism is not uncommon in pregnancy, with a reported prevalence of 0.1% to 0.4%, and it is caused most frequently by Graves' disease (Mestman 2004). Overt hyperthyroidism complicates approximately 0.05-0.2% of pregnancies (Burrow 1993). Subclinical hyperthyroidism has a prevalence of approximately 1.7% in pregnant women (Casey, Dashe and Wells, *et al.* 2006). Number of other studies have also demonstrated the incidence of hyperthyroidism during pregnancy (Table 2.3).

Table 2.3 Studies that evaluated the incidence of hyperthyroidism during pregnancy

First Author	Year	N	Country	Gestational week	TSH	T_4 pmol/L	Hyperthyroidism	
							Subclinical	Overt
Mannisto	2009	5805	Finland	12th week	<95th percentile	>5th percentile	3.5%	1.3%
Benhadi	2009	2,497	Netherlands	Mean 13th week	<0.34 µIU/ml	>21.1	5.0%	0%
Sahu	2009	633	India	Mean 13th week	<0.5 mU/L	NR	0.9%	0.8%
Lazarus	2007	1497	UK	9-15 weeks	<0.02 µIU/ml	>23.1	1.3%	0.17%
Aoki	2007	209	USA		<0.1 µIU/ml		2.9%	
Vaidya	2007	1560	UK	Median 9th week	<0.03 µIU/ml	>23	1.2%	0.7%
Casey	2006	25,765	USA	<20 weeks	<2.5th percentile	>1.75 (ng/dl)	1.7%	0.4%
Yeo	2001	184	Singapore	8-14 weeks	<0.36 mU/L	>19.1	22%	12%
Glinoer	1997	760	Belgium	8-14 weeks	<0.02 µIU/ml	>26		2.4%

NR= Not reported

Prompt diagnosis and treatment of hyperthyroidism are of paramount importance in preventing maternal and fetal morbidity and mortality (Table 2.4). Hyperthyroidism is associated with ovulatory dysfunction, miscarriages and difficulties conceiving (Anselmo, *et al.* 2004).

Maternal and fetal complications are significantly increased in patients remaining hyperthyroid in the second half of pregnancy (Mestman 2004). In one study 88% of the untreated, compared with 25% of the partially treated and 8% of the adequately treated mothers, had a medically indicated preterm delivery (Millar, *et al.* 1994).

Table 2.4 Potential maternal and fetal complications in uncontrolled hyperthyroidism

MATERNAL	FETAL
Pregnancy-induced hypertension	Hyperthyroidism
Preterm delivery	Neonatal hyperthyroidism
Congestive heart failure	Intrauterine growth retardation
Thyroid storm	Small-for-gestational age
Miscarriage	Prematurity
Placental abruption	Stillbirth
Infection	

TSH is suppressed in hyperthyroidism during pregnancy, just as it is non- pregnant individuals. Transient hyperthyroidism in the first half of pregnancy caused by the inappropriate secretion of hCG is now the most frequent cause of hyperthyroidism in pregnancy. It is also known as gestational transient thyrotoxicosis (GTT). Other causes accounts for less than 10% of all hyperthyroidism cases. There is sometimes a modest suppression of TSH (between 0.1 and 0.5 μIU/ml) during the 8^{th} to 14^{th} weeks of normal pregnancy because of stimulation of the thyroid by hCG during this interval, which may cause difficulty in interpretation. A TSH level of 0.1mU/L and elevated FT_4/FT_3 strongly suggest coexistent hyperthyroidism (Benhadi, *et al.* 2009).

Difficulty in conception and fetal wastage are increased in women with Graves' disease, but occasional patients become pregnant despite antecedent untreated hyperthyroidism. More commonly, a woman under treatment becomes pregnant or hyperthyroidism develops after pregnancy is underway. Whatever the sequence, pregnancy complicates the diagnosis and treatment of hyperthyroidism and influence its severity and course (Davis, Lucas and Hankins 1989).

The natural cause of Graves' hyperthyroidism in pregnancy is characterized by aggravation of symptoms in the first half of pregnancy, with amelioration in the second

half and recurrence in the postpartum period. It is advisable for hyperthyroid women desiring to conceive to achieve euthyroidism before conception (Mestman 2004).

Three reasons have been given to explain the spontaneous improvement associated with pregnancy. First, there is partial immune-suppression, with a significant decrease in autoantibody titers, and hence TSH-R titers. Second, the markedly increased serum hormone binding capacity (related to rise in serum TBG levels) tends to reduce the free T_4 and T_3 fractions. Third, the obligatory iodine losses specific to the pregnant state tend to reduce iodine availability for the thyroid gland: relative iodine deficiency may be advantageous for pregnant patients with Graves' disease (Amino, *et al.* 1982).

GTT is defined as non –autoimmune hyperthyroidism of variable severity that occurs in women who have a normal pregnancy, typically in association with hyperemesis (Glinoer 1997). GTT differs from Graves' disease in that it occurs in women who have no history of thyrotoxicosis and occurs in the absence of detectable TSH-R. Its etiology is directly related to the thyrotropic stimulation of the thyroid gland associated with hCG (Kimura, *et al.* 1993).

The precise pathogenic mechanism underlying GTT still are not fully understood. It remains possible that abnormal molecular variant of hCG are produced in these situations, with a prolonged half-life explaining the sustained high levels or hCG variants with a more potent thyrotropic activity (Yoshimura and Hershman 1995).

GTT is frequently associated with morning sickness, increased vomiting and hyperemesis gravidarum. There is no indication of increased vomiting among pregnant women with Graves' disease; hyperemesis in pregnancy appears to be significantly associated with hCG-induced thyrotoxicosis. The most likely explanation is that elevated and sustained hCG levels in the circulation promote estradiol production in these women: the combination of high hCG, estradiol, and free T_4 concentrations, transiently promotes emesis near the period of peak hCG (Yoshimura and Hershman 1995, Goodwin and Hershman 1997).

2.2.3 Antibodies and fertility, pregnancy and post partum thyroiditis

Thyroperoxidase antibody (TPO-Ab) positivity is increasingly recognized as a risk factor for reproductive problems such as infertility, miscarriage and unsuccessful in-vitro fertilization.

One IVF study reported that both the presence and magnitude of TPO-Ab elevation lowered the clinical pregnancy rate (26% versus 39%, TPO-Ab positive versus TPO-Ab negative, respectively) and increased the miscarriage rate (40% versus 11%, respectively). Many studies now report a strong association between the presence and degree of elevation of TPO-Ab positive patients. Since fetal wastage is seen with non-thyroid autoimmune conditions, it could reflect underlying abnormal stimulation, of the immune system leading to early rejection of the fetal allograft (Abrmson and Stagnaro-Green 2001).

Several studies have been carried out since 1990 to find out the relationship between thyroid autoimmunity (TAI) and pregnancy loss (Table 2.5). In 1990, (Stagnaro, *et al.*) were the first to report a doubling of the miscarriage rate in unselected euthyroid pregnant women with positive thyroid antibodies.

In 1997, the results of comprehensive screening for seven autoantibodies in 1197 Japanese healthy women during the first trimester of pregnancy were reported. In this prospective study, positive thyroperoxidase antibodies were detected in 10.6% of the cohort. Women with TAI miscarried twice as frequently as TAI-negative women. The study was of particular interest because among all the autoantibodies measured, only the thyroid and antinuclear antibodies were significantly associated-albeit independently of one another-with an increased rate of pregnancy loss (Iijima, *et al.* 1997).

(Dendrinos, *et al.* 2000) reported that TAI was seen three fold more frequently among the women with recurrent spontaneous abortions, compared with controls.

In 2001, Bagis and colleagues (Bagis, Gokcel and Saygili 2001) investigated 876 consecutive pregnancies, among which 12.3% tested positive for thyroid antibodies. In

the TAI positive group, 50% had at least one spontaneous abortion, while in TAI negative control women only 14% had experiences of pregnancy loss.

Table 2.5 Large studies in which the effects of thyroid dysfunction/ thyroid autoantibodies during pregnancy have been evaluated

First Author	Year	Setting and population	n	Definition of thyroid dysfunction	Adverse outcome
Haddow	2011	Prospective-population based cohort	10062	TPO-Ab-positive	Placental abruption OR1.83 (0.99-3.37) and OR 2.2 (1.21-3.99) in first and second trimester
Ashoor	2011	Case- control study on hospital patients	4420	TPO-Ab or Tg-Ab-positive	Similar rates of positive TPO-Ab and/or Tg-Ab in term and preterm births
Abbassi-Ghanavati	2010	Excess serum of population from hospital serving medically indigent	17298	TPO-Ab-positive	Placental abruption OR 3.4 (1.7-6.7)
Ashoor	2010	Case- control study	4520		Cases with miscarriage had higher TSH and lower FT_4
Ashoor	2010	Case- control study	4420		Cases with late onset preeclampsia had higher TSH and lower FT_4
Haddow	2010	Prospective population-based cohort	10062	TPO-Ab and/or Tg-Ab-positive	Preterm premature rupture of membranes OR 1.67 (1.05-2.44)

Kuppens	2010	Prospective population-based cohort	1058	Subclinical hypothyroidism: TSH > 2.5 mU/L (in 3rd trimester)	Breech presentation OR 2.23 (1.14-4.39)
Negro	2010	Cohort of ambulatory patients from community hospitals	4125	Subclinical hypothyroidism: TSH 2.5-5 mU/L, TPO-Ab-negative	Pregnancy loss 6.1 *vs.* 3.6% in subclinical hypothyroid *vs.* euthyroid mothers
Negro	2010	Randomized study: screening and treatment of ambulatory patients from community hospitals	4562	Subclinical hypothyroidism: TSH > 2.5 mU/L, TPO-Ab positive	Less adverse outcomes if treated
Hamm	2009	Population-based cohort	879	Hypothyroxinemia: FT_4 < 8.5 pmol/L	None

In a review of 14 articles for a total of 14,148 pregnant women, the prevalence of anti-TPO antibodies and/or TGAb in pregnancy was found to be 10.8% and there was a strong association with hypothyroidism (Smallridge, Glinoer, *et al.* 2005). Therefore, untreated or inadequately treated chronic autoimmune thyroiditis is the most common cause of thyroid hormone deficiency in pregnancy.

A significant correlation between antithyroid antibodies and the increased risk of spontaneous abortions by pregnant women with a normal thyroid gland function was reported by Todorova *et al.* (Todorova, Genova and Konova 2008).

Alternatively, the presence of TPO-Ab could be a reflection of occult mild thyroid insufficiency that becomes exacerbated by the increased thyroxine concentrations in the face of increased TBG. This latter explanation is in accord with the study of Vaquero et al who reported that levothyroxine treatment reduced pregnancy loss and increased the percentage of live births in TPO-Ab positive recurrent aborters compared with intravenous immunoglobulin treated patients (Vaquero, Lazzarin and de Carolis 2000). The presence of TPO-Ab not only increases risk for fetal loss but has also been reported to increase the risk of pregnancy related complications such as pre eclampsia. In one such

study 33.3% of patients with PET had thyroid antibodies detected compared with 14.5% in the control group.

The detection of TPO-Ab in the first trimester is a strong risk factor (specificity 95% / sensitivity 50%) for post partum thyroiditis (PPT) that affects 5-9% of new mothers in the first year after delivery (Stagnaro-Green 2002). Although detection of TPO-Ab in early (<12 wks) pregnancy has only a 50% predictive value for PPT, the magnitude of TPO-Ab elevation increases PPT risk and mothers with a history of PPT have an increased risk after subsequent pregnancies. If a pregnant woman is positive for TPO antibodies early in pregnancy, her chances of developing postpartum thyroiditis is 30–52% (Lazarus 1998).

PPT is the occurrence of hyperthyroidism, hypothyroidism, and/or hyperthyroidism followed by hypothyroidism in the first year postpartum in women without overt thyroid disease before pregnancy.

PPT is typically characterized by a transient spectrum of thyroid dysfunction ranging from hyperthyroidism to hypothyroidism. Although most patients return to euthyroidism within a few months, a significant number of PPT patients (25%), especially those with very high TPO-Ab, become permanently hypothyroid (Lazarus 1998).

Roberton first described post-partum thyroiditis in 1948. During the last twenty years the prevalence of postpartum thyroiditis in different parts of the world has been reported to be from 1.9% - 16.7 % with a mean prevalence rate of 7.2 %. Zargar *et al* reported the prevalence of 7 % in Kashmir Valley of Indian subcontinent (Zargar 2002).

Postpartum Thyroid Dysfunction is a well recognized cause of morbidity in the period following childbirth. Raised levels of circulating TPO-Ab are detected in 10% of pregnant women at 16 weeks gestation, of which 50% develop a destructive thyroiditis, characterized by transient hyperthyroidism and/or hypothyroidism during the first 6 months of the postpartum period. Permanent hypothyroidism is reported in as many as 30% of these cases after 3 years, and in 50% at 7-10 years (Premawardhana, *et al.* 2000).

“Postpartum” thyroiditis may also occur after loss of pregnancy at 5–20 wk gestation. The recurrence rate in postpartum thyroiditis is high. Seventy percent of women with a prior episode of postpartum thyroiditis develop a recurrence in the subsequent pregnancy.

Antithyroid peroxidase-positive women, who did not develop postpartum thyroiditis during an initial pregnancy, have a 25% chance of having postpartum thyroiditis after the next pregnancy. In contrast, women who had neither antithyroid peroxidase nor postpartum thyroiditis during an initial pregnancy do not develop postpartum thyroiditis in future pregnancies (Lazarus, Ammari, *et al.* 1997)

2.3 IODINE DEFICIENCY AND DAMAGED REPRODUCTION

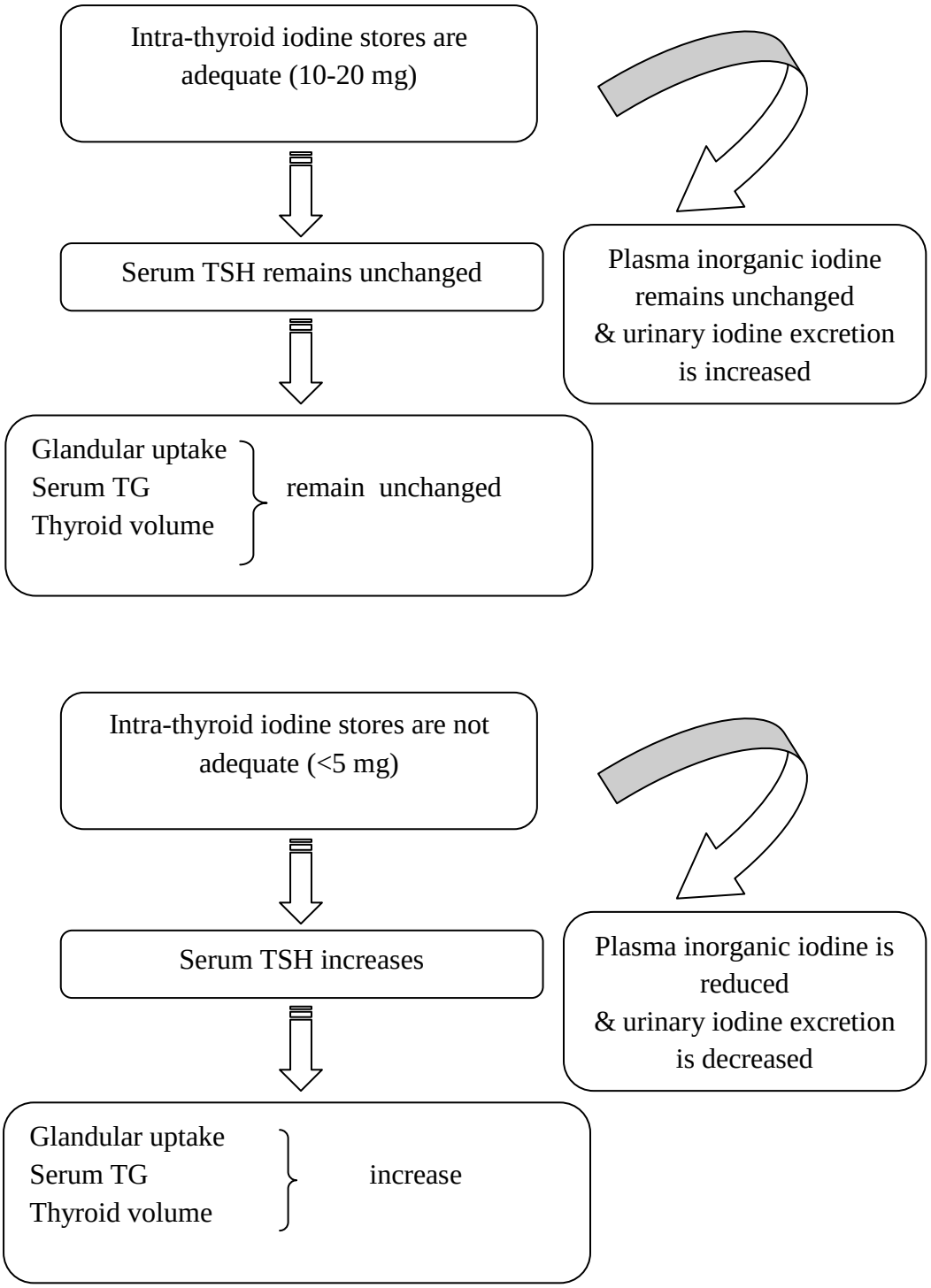
Iodine deficiency results in a wide spectrum of adverse consequences throughout the lifecycle. Of greatest concern is the effect of iodine deficiency on the developing brain. The most serious effect of iodine deficiency is cretinism, which occurs in women who are severely iodine deficient during pregnancy.

2.3.1 Iodine intake and pregnancy

In 1998, over one third of the world's population lived in areas of iodine deficiency. Iodine is an essential component of thyroid hormones, and its importance stems from that role. In areas of iodine sufficiency, healthy women maintain iodine stores of 15-20 mg in the thyroid. During pregnancy, to meet the approximately 50% increase in maternal iodine requirements; women may draw on these significant iodine stores (WHO/ UNICEF/ ICCIDD 2007). However, 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 occur that adversely affect maternal and fetal health.

The most obvious consequence of iodine deficiency is goiter. This adaptive response, mediated principally by TSH, attempts to cope with a shortage of the raw material (iodine) needed for hormone synthesis (Fig. 2.7). The term "iodine deficiency disorders" serves to emphasize the many other consequences of iodine deficiency (Hetzel 1983). Of these, damage to reproductive function and to the developing fetus and infant is the most severe.

Pregnant women need more than recommended daily intake of 150 µg iodine required for non-pregnant adults, to cover the iodine needs of the developing fetus and to compensate for increased renal iodine losses. Renal clearance of iodine increases during pregnancy;



Source: Glinoer 2007

Figure 2.7 A conceptual model of iodine nutrition and thyroid function when iodine stores are adequate (upper diagram) or not adequate (lower diagram)

in one study, the concentration of iodine in urine was 60% higher during pregnancy in women from a mildly iodine deficient area (Smyth, *et al.* 1997). WHO recommends a daily intake of 250 µg/day for pregnant women, a value approximately 10 % higher than the RDA (WHO/ UNICEF/ ICCIDD 2007)**.**

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

The median urinary iodine is recommended by WHO/ UNICEF/ ICCIDD 2007 for assessing iodine nutrition in pregnant women. A recent WHO expert group recommended the median UI that indicates adequate iodine intake during pregnancy to be 150-249 µg/L.

Using a median cut-off of 150 µg/L, several recent studies have found marginal or deficient iodine status in pregnant women from areas with only partial household coverage with iodized salt, including Italy, India, Thailand and the United States (Ategbo, *et al.* 2008, Moleti 2008, Caldwell, *et al.* 2008, Gowachirapant, *et al.* 2009).

For the mother, although the iodine requirement is high (200-290 µg/day), after accounting for iodine loss in breast milk, the median UI in lactating women that indicates adequate iodine nutrition is the same as that of non-pregnant, nonlactating women (WHO/ UNICEF/ ICCIDD 2007).

Pregnancy and lactation increase the demand for iodine in the mother (Burrow, Fisher and Larsen 1994, Glinoer 1997). It is natural to anticipate that the risk for iodine

deficiency disorders is increased during pregnancy and lactation in an area of mild to moderate iodine deficiency. In Jutland, Denmark, it was initially observed that the serum level of thyroglobulin was considerably higher in pregnant than in control women (Perdersen *et al*, 1998) and these researchers hypothesized that this was caused by worsening of iodine deficiency during pregnancy (Pedersen, Borlum, *et al.* 1990).

2.3.2 Iodine and the fetus & newborn

An adequate supply of thyroid hormones is necessary for normal development of the human brain both in utero and during the first years after delivery. A lack of iodine in the diet may result in the mother becoming iodine deficient, and subsequently the fetus. The mother and the fetus, however, respond differently to this situation, with the mother remaining euthyroid and fetus becoming hypothyroid (Morreale de Escobar, Obregon and Escobar del Ray 2007). Iodine deficient pregnant women remain euthyroid for two reasons. Firstly, the increase in maternal FT_4 that occurs at the end of the first trimester in response to hCG depresses the concentration of maternal TSH. Secondly, the maternal thyroid gland responds to a state of relative iodine deficiency by invoking the same responses in the thyroid gland as would occur in the non-pregnant state, such as increased iodine trapping, preferential synthesis of T_3 over T_4, hyperplasia, and eventually goiter. Thus, the woman will appear to be euthyroid as both her TSH and T_3 concentration will fall within the normal reference range. In such situations, localized hypothyroxinemia occurring in specific parts of the developing fetal brain is believed to be responsible for the neurodevelopmental damage seen in iodine deficiency (Morreale de Escobar, Obregon and Escobar del Ray 2004). The autoregulatory mechanisms available to the mother do not take place in the fetus because the fetal gland has not fully matured (Fig. 2.8). Consequently, in the fetus there is a decreased synthesis and secretion of T_4 and T_3, and an increase in the concentration of TSH, resulting in fetal hypothyroidism (Morreale de Escobar, Obregon and Escobar del Ray 2007). This explains why neonatal TSH is used as an index of iodine deficiency in a population.

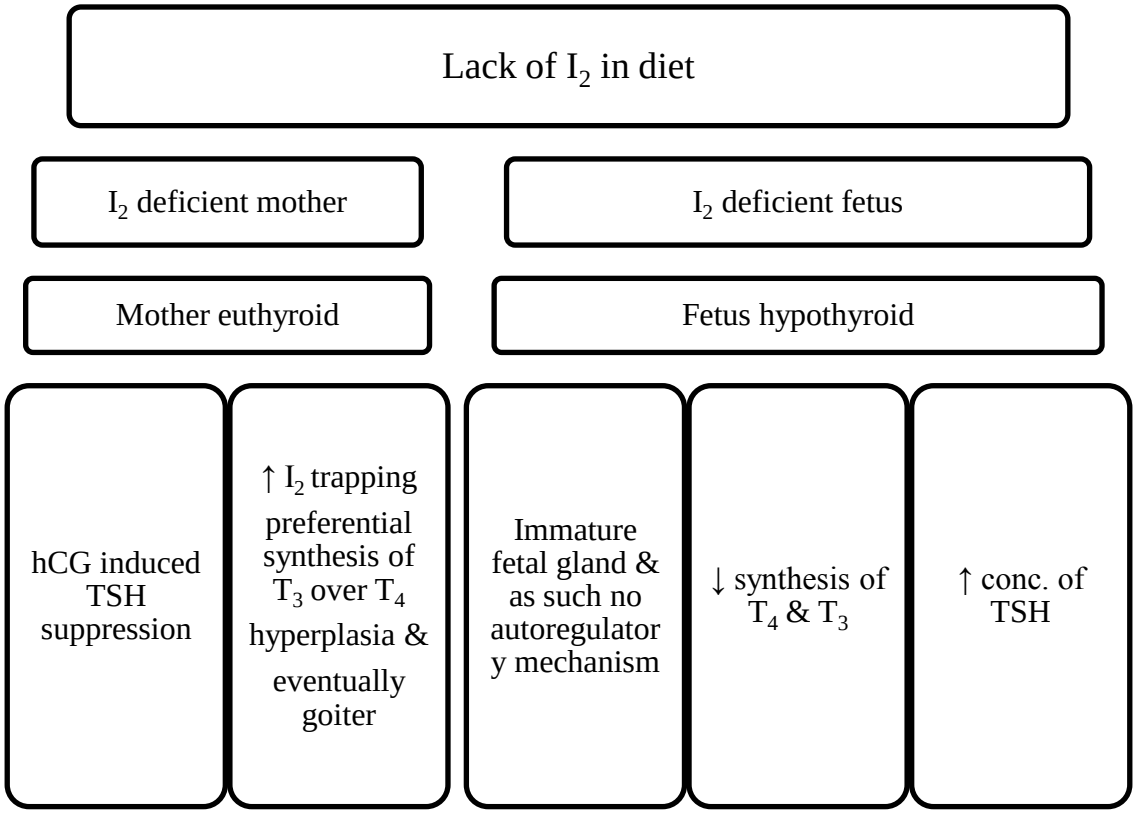


Figure 2.8 Flowchart depicting effect of iodine deficiency on mother and fetus

In children born without thyroids but in whom adequate thyroid hormone substitution therapy is initiated shortly after birth, there seems to be no irreversible brain damage (Delange 1997). This is caused by the small amounts of thyroid hormones crossing the placenta from the mother (Vulsma, Gons and De Vijlder 1989) and probably by adaptation of brain iodothyroxine deiodinase with a high production of T_3 from T_4 in the brain (Escobar del Rey, *et al.* 1987). The hypothyroidism continues during the first years of life, when brain development is highly dependent on thyroid hormone. Laurberg, Nohr and Pedersen, *et al.* 2000 found a negative correlation between TSH and thyroglobulin in cord blood. As discussed, high thyroglobulin is a marker of low iodine supply in these neonates.

Iodine deficiency increases neonatal mortality. It may impair the immune response and, thus, lower the child's defense against infection. The most vulnerable target for iodine deficiency is the developing brain. Iodine is critical to maturation of the central nervous system, particularly its myelination. DeLong, Xue-Yi and Xin-Min 1998 have carefully

examined the effects at different stages of pregnancy. Correction of iodine deficiency during the second trimester reduced neurological abnormalities, increased head growth, and improved the development quotient in a severely iodine-deficient area of western China. Correction at a later period did not improve neurological development, although there was a trend toward slightly larger mean head circumference and higher development quotients than in untreated individuals. The principal effects of T_4 are on somatogenesis, neuronal differentiation, and formation of neural processes, particularly active for the cerebral cortex, cochlea, and basal ganglia during the second trimester; brain growth and differentiation are more active in the third trimester (Koibuchi and Chin 2000). In iodine deficiency, maternal T_4, which must cover fetal needs during the first trimester before the fetal thyroid makes its own, is low; hence the fetus is exposed to inadequate T_4 throughout gestation.

Many studies have compared performance of iodine-deficient children with that of iodine sufficient peers on standardized intelligence tests. A metaanalysis of 18 such studies, comprising 2214 subjects, concluded that iodine deficiency lowered a mean intelligence quotient by 13.5 points (Bleichrodt and Born 1994).

Severe iodine deficiency exists when more than 30% of children have goiter, the population has a MUIC < 20 μg/L, and pregnant women living in the area give birth to cretins. Cretinism is always associated with a significant impairment in mental function and/or defects in hearing, speech, stance, gait, hypothyroidism and growth (Chen and Hetzel 2010). Normal IQ in a population is 100, while the IQ of cretins has been reported to be around 30 (Rajatanavin 2007). In areas with endemic cretinism, around 5–15% of non-cretinous children will have impaired mental function with an IQ of 50–69; these children are sometimes referred to as "sub-cretins" (Chen and Hetzel 2010). Although factors such as the presence of goitrogens in the diet, thyroid autoimmunity, and interactions with other trace elements such as selenium have been postulated to have a role in the development and type of cretinism, the overarching reason for cretinism is severe iodine deficiency in the mother (Zimmermann 2009). The importance of adequate dietary iodine in preventing cretinism was highlighted in the 1960s in a large trial of 165,000 people living in a part of Papua New Guinea with severe iodine deficiency and endemic cretinism (Pharoah, Buttfield and Hetzel 1971). In this study, it was found that

an injection of iodized oil before conception or in early pregnancy reduced the incidence of cretinism and improved the motor and cognitive functions of children compared with those women who received the placebo. A meta-analysis of Chinese studies reported a decrease of 8.7 IQ points in children born to mothers living in severely iodine deficient areas untreated in pregnancy compared to supplemented mothers also living in severely iodine deficient areas (Qian, *et al.* 2005).

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

A number of clinical trials published between 1991 and 2002 in pregnant women living in regions with moderate deficiency are summarized in table 2.6. Daily iodine supplements ranging from 100 to 300 μg of iodine were taken by women in their first or second trimester of pregnancy. The majority of the studies showed that iodine supplements were effective in alleviating aspects of iodine deficiency in supplemented women compared to those taking the placebo. In 2009, two randomized trials were published investigating the effect of iodine supplementation in moderately iodine deficient pregnant women on neurodevelopment in their children.

Berbel *et al.* recruited three groups of pregnant women living in Spain at different phases of gestation; the first group of women had T_4 concentrations >20th percentile at recruitment (*i.e.*, >0.92 ng/dL at 4–6 weeks gestation), while the second and third groups of women had T_4 concentrations <10th percentile (*i.e.*, <0.83 ng/dL) at 12–14 weeks gestation and near term, respectively. All three groups of women were supplemented with 200 μg of iodine until the end of lactation. When the children were 18 months old, the development quotient of children in mothers supplemented in the first group (*i.e.*, 4–6 weeks) was significantly higher than that of children whose mothers received supplements from 12–14 weeks gestation and near term. A limitation of this study was the small numbers of children tested, with less than 20 children in each of the three groups.

Table 2.6 Studies investigating the effect of iodine supplementation in pregnancy

Author (Year	Country	*n*	Methods	Baseline UIC µg/L	Effect on child neurodevelopment
Romano, Jannini and Pepe 1991	Italy	35	Women in first trimester randomized to 0 or iodine supplement (120–180 µg I/day).	37	Not assessed.
Pedersen, *et al.* 1993	Denmark	54	Women at 17–18 weeks gestation randomized to 0 or 200 µg I/day.	55	Not assessed
Glinoer, Nayer and Delange 1995	Belgium	120	Euthyroid women with signs of excessive thyroid stimulation randomized to 0 or 100 µg I/day.	36	Not assessed
Liesenkotter, *et al.* 1996	Germany	118	Women at 10–12 weeks gestation given 300 µg I/day *vs*. untreated women.	53	Not assessed
Nohr and Laurberg 2000	Denmark	144	Retrospective allocation at term based on self-reported intake of supplements containing 150 µg I/day.	Not given	Not assessed
Antonangeli, *et al.* 2002	Italy	86	Healthy women 10–16 weeks gestation randomized to 50 or 200 µg I/day.	74	Not assessed
Berbel, *et al.* 2009	Spain	96	Women 4–6 weeks gestation with FT_4 > 20th percentile (Group1) *vs*. women with FT_4 < 10th percentile at 12–14 weeks (Group 2) or at 37–40 weeks (Group3) given 200 µg I/day until end of lactation.	75	Brunet-Lezine developmental quotient of children at 18 months was: 101.8 in Group 1 *vs*. 92.2 in Group 2 ($p < 0.05$) or 87.5 in Group 3 ($p < 0.001$)
Velasco, *et al.* 2009	Spain	191	Women <10 weeks gestation (Group 1) *vs*. last month of pregnancy (Group 2) given 300 µg I/day until end of lactation.	87	Bayley Psychomotor Development Index of children was: 108.74 in Group 1 *vs*. 102.65 in Group 2 ($p = 0.02$)

Furthermore, the women supplemented later in pregnancy or at term were specifically selected because they had low FT_4 (*i.e.*, <10th percentile) in pregnancy, while the women supplemented earlier in pregnancy had a higher FT_4 (*i.e.*, >20th percentile), thus a difference in FT_4 rather than the iodine supplementation may account for the findings (Berbel, *et al.* 2009).

A second Spanish study conducted in an area of moderate iodine deficiency (*i.e.*, UIC of pregnant women in this area was <100 μg/L) by Velasco, *et al.* 2009 found that children of mothers supplemented with 300 μg of iodine in the first trimester had higher psychomotor development scores than children from mothers who did not start supplementation until the last month of pregnancy. A limitation of this study was that children were tested at different ages in this study (5.5 months *vs*. 12.4 months).

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

2.3.3 Iodine intake and thyroid autoimmunity

The high incidence and prevalence of hypothyroidism caused by autoimmunity in high iodine intake areas and the much earlier onset of *Graves' Disease* in high iodine-intake area both support such a mechanism. Laurberg *et al.* found that circulating thyroid antibodies were more prevalent in the low iodine-intake area despite a lower frequency of thyroid dysfunction caused by autoimmunity in this population (Laurberg, Pedersen, *et al.* 1998).

Not only impaired thyroid function of the child but also even slight maternal hypothyroidism alone during pregnancy may lead to reduced intellectual function of the child (Haddow, *et al.* 1999). A reasonable safety margin for preventing iodine deficiency is advisable. Consequently, an iodine supplementation programme to correct mild-to-moderate iodine deficiency is advisable. The iodine intake level should be increased with caution in order to prevent potential hypothyroidism.

Overall, the available evidence supports the conclusion that the iodine intake of a population should be brought to the level at which iodine deficiency disorders are avoided but not higher (Laurberg 1994) and that surveillance of population iodine intake and supplementation are important. Optimally, iodine supplement should be constructed to minimize the risk for subgroups of the population to have deficient or excessive iodine intake level. The exact level of intake that produces the most favorable (lowest) prevalence of thyroid disorders in a population remains to be settled, and other environmental factors may be of importance. The iodine supplementation is guided by a survey of iodine intake and of the prevalence and incidence of thyroid abnormalities as recommended by ICCIDD/WHO/UNICEF (Laurberg, Nohr and Pedersen, *et al.* 2000).

2.4 IODINE STATUS IN DIFFERENT COUNTRIES

Iodine deficiency is the world's single greatest cause of preventable mental retardation. It is especially damaging during the early stages of pregnancy and in early childhood. There are almost no countries in the world where iodine deficiency was not a public health problem. About thirty eight million newborns in developing countries every year remain unprotected from the lifelong consequences of brain damage associated with iodine deficiency disorders. This shortcoming affects a child's ability to learn, and later in life, to earn, therefore, preventing children, communities and nations from fulfilling their potential.

International support for the elimination of iodine deficiency dates from the World Summit for children in 1990 (Table 2.7) (UNICEF 2007).

Table 2.7 Major United Nations milestones for elimination of iodine deficiency

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

Elimination of iodine deficiency also contributes to six of the eight Millenium Development Goals agreed to by UN Member States in 2000. Meeting these goals would transform the lives of millions of children during the next 10 years (Table 2.8). Although programs to control iodine deficiency, such as salt iodization, have been effective for decades, iodine deficiency remains a major threat to the health and development of populations around the world, particularly among preschool children and pregnant women in low-income countries.

Table 2.8 IDD and the Millenium Development Goals (UNICEF 2007)

Goal 1 – *Eradicate extreme poverty and hunger*	Eliminating IDD increases learning ability and intellectual potential, leading to better educated citizens earning higher wages.
Goal 2 – *Achieve universal primary education*	Improved cognitive development and learning potential leads to improved school performance and reduced drop-out rates.
Goal 3 – *Promote gender equality and empower women*	Eliminating IDD in children reduces women's childcare burdens, frees up household resources and allows women more time for income generating work.
Goal 4 – *Reduce child mortality*	Reducing iodine deficiency lowers rates of miscarriage, stillbirth and other pregnancy complications, and neonatal deaths.
Goal 5 – *Improve maternal health*	Lower rates of thyroid disease and other clinical results of iodine deficiency improve the health of women of reproductive age.
Goal 8 – *Develop a global partnership for development*	Programmes for sustainable elimination of iodine deficiency strengthen partnerships at global, regional and country levels. They also leverage resources and commitments through alliances of public organizations, civil society and the private sector.

The overall global status of iodine deficiency has improved since 2003, reflecting the fact that current strategy of salt iodization is effective (Table 2.9). There are now fewer countries where iodine deficiency is considered to be a public health problem in 2007 than there were in 2003. However, 47 countries continue to have problem with iodine deficiency (de Benoist, *et al.* 2008).

Table 2.9 Global Scorecard 2010

Country	% of households consuming iodized salt (2003-2008)	Median UIE (µg/L)	Prevalence of low UIE (< 100 µg/L)	Total goiter rate (%)	Iodine deficiency protected population (thousands)	Iodine deficiency unprotected population (thousands)	Iodine deficiency protected infants (thousands)	Iodine deficiency unprotected infants (thousands)
India	51	#	#	#	602,520	578,892	13,726	13,187
Nepal	63	188	27.4	40	18,150	10,660	461	271
United States	-	249	15.9	-	-	-	-	-
France	-	#	#	#	-	-	-	-
Australia	-	96	46.3	#	-	-	-	-
UAE	-	162	21	40.4	-	-	-	-
Japan	-	#	#	-	-	-	-	-
Thailand	47	#	#	2.2	31,671	35,715	459	518
Iran (Islamic Republic)	99	165	19.7	#	72,579	733	1,374	14
Iraq	28	-	-	-	8,427	21,669	264	680

Source: www.iodinenetwork.net

Data exist but are not nationally representative and/or for school age children

- Data does not exist

2.4.1 USA, Europe, Australia and Middle East

In the USA, iodine began to be added to the diet in the 1920s. The total diet study of the Food and Drug Administration (FDA), a yearly program that measures the amounts of minerals in foods and estimates their intake in representative diets of specific age groups, indicated that the average intake of iodine in the US was adequate (Pennington 1996). An excessive iodine intake with a median UI concentration of 320 µg/L was documented by the first National Health and Nutrition Examination Survey (NHANES I) in the 1970s. The NHANES III survey, conducted between 1988 and 1994, reported that the US population is iodine sufficient according to WHO criteria (J. G. Hollowell, W. H. Staehling, *et al.* 1998). NHANES III indicated that median UI concentrations had decreased to 145 µg/L, while 14.9% of women aged 15-44 years and 6.9% of pregnant women had a UI concentration 50 µg/L. The concentrations of serum T_4 and TSH of women with a low UI concentration did not, however, indicated an iodine deficiency (Hollowell and Haddow 2007).

Generally, the United States population have iodine levels within acceptable limits, but particular groups, namely women aged 40 - 49 and 50 - 59 yr and other women of child-bearing age, may be at risk of iodine deficiency. In the newborn, serum thyroglobulin concentrations and thyroid volume are also decreased (J. G. Hollowell, W. H. Staehling, *et al.* 1998). This finding emphasizes the importance of adequate iodine nutrition. Also, from their study, they found the iodine intake has decreased over the past 20 years. They consider a need to monitor the food supply, especially the intake of iodine in women of childbearing age to prevent the possibility of the re-emergence of iodine deficiency in the United States.

The 2001-2006 NHANES data from urine iodine spot tests for pregnant (n=326), lactating (n=53), and nonpregnant, nonlactating (n=1437) women of reproductive age were analyzed and WHO, 2007 criteria was used to define iodine sufficiency. The iodine status of pregnant women was found to be just sufficient (median UIC 153 µg/L) while lactating (115 µg/L) and nonpregnant, nonlactating (130 µg/L) women were iodine sufficient. Fifty two percent of the pregnant women never or rarely consumed table salt, 81% were not consuming a supplement that contained iodine, and 14% consumed no

dairy products in the previous 24 h. Dairy product consumption was an important contributor to iodine status among both pregnant and nonpregnant, nonlactating women, and those who do not consume dairy products may be at risk of iodine deficiency (ICCIDD 2010).

In Europe, less than 50% pregnant women receive iodine-containing supplements, and for most women in this life stage, dietary iodized salt is the major source of iodine. (Moleti 2008) prospectively evaluated thyroid function in 100 consecutive thyroid antibody-negative pregnant women from a mildly iodine deficient area. Women were divided into 2 groups as long term users (using iodized salt for at least 2 yr prior to becoming pregnant) and short-term users (started consuming iodized salt upon becoming pregnant). They found marked differences in thyroid function between the two groups. The prevalence of thyroid dysfunction was almost 6-fold higher in short term users (36.8%) than in long term users (6.4%). The data suggested that long term iodine prophylaxis using iodized salt reduced the risk of maternal thyroid dysfunction by 82.5%.

In 2002, a review by (Delange 2002) of the iodine deficiency in Europe found a limited number of countries had re-evaluated IDD on a national basis. A key point arising from his review is that, with the exception of the Netherlands, none of these countries has yet reached unquestionable sufficiency. Thyroid function is usually normal in adults in Europe. In contrast, it is frequently altered in pregnant women. The effects of marginal iodine deficiency during pregnancy in Belgium on the thyroid function of neonates include even more elevated serum levels of TSH and thyroglobulin (TG) in cord blood than in the mothers and slight enlargement of the thyroid gland (Glinoer 1997). The role played by iodine deficiency in the changes in mothers and neonates is demonstrated by the fact that they are prevented by iodine supplementation in the mothers during pregnancy and they do not occur in iodine replete areas in Europe such as the Netherlands (Berghout, *et al.* 1994).

Iodine deficiency is still prevalent in Europe. The most probable cause of the phenomenon has been the insufficient awareness, until recently, of the problem of IDD by the health authorities, including the medical and paramedical world, and by the public (Delange 2002).

In Switzerland, one study reported that mean UI was found to be < 100 μg/L, which indicated mild IDD, despite long-term national efforts to control and eradicate IDD with iodized salt (Als, *et al.* 2004). During the first few weeks of pregnancy and before its configuration, the probability of IDD is high (Glinoer, Nayer and Delange 1995), and as the aim of therapeutic intervention is to avoid hypothyroxinemia and goitrogenesis in both mothers and newborns, iodine supplementation should be initiated.

Australia is an example of a country that was iodine sufficient for decades until changes in dairy industry practices inadvertently reduced the iodine content of the dairy products, contributing to iodine deficiency among the population (M. Li 2001).

One study (McElduff, *et al.* 2002) investigated neonatal TSH concentrations in northern Australia. Their results support the suggestion that northern Sydney is an area of mild iodine deficiency. In their opinion, as iodine requirements are increased in pregnancy, more demanding criteria and a higher urine iodine standard should be applied, and a number of women in their study should be considered as having borderline mild iodine deficiency. There was a positive correlation between natural log of neonatal whole blood TSH concentration and natural log of maternal UI concentration.

Another study's findings (Gunton, *et al.* 1999) suggested that Australia should no longer be automatically considered an iodine-replete country. They found that postpartum women had a median iodine concentration, which was lower than the WHO recommendation. It is not clear whether the apparently higher levels in pregnant women were pregnancy related or, in fact, masked iodine deficiency in pregnancy. Thus, the high frequency of iodine deficiency found in their participants suggests that dietary sources of iodine in Australia may no longer be sufficient. However, further population studies are required.

The survey for iodine deficiency disorders in Saudi Arabia has shown a mild degree of iodine deficiency in southern provinces. It suggested the government to launch a control program to ensure the exclusive availability of iodized salt in Saudi Arabia (Al-Nuim, *et al.* 1997).

A cross-sectional study was conducted in four cities of Islam Republic of Iran among pregnant and lactating women. It was found that in one city 84% of the pregnant women

had a UIC of ≥200 µg/L, while in the other cities this percentage ranged from 45 to 55%. The mean UIC in lactating women was 250 µg/L, and 16% of women had UIC <100 µg/L. Grade I goiter was present in 8% of lactating women, and another 8% had grade 2 goiter. This calls for further attention to iodine intake during pregnancy and lactation (Azizi 2007).

2.4.2 South East Asia- Japan, Korea, Thailand, Hong Kong

Recent reports have shown that various degrees of dietary deficiency exist in South-East Asia (Heywood and Marks 1993). To eliminate IDD, the most common method for the majority of countries is using iodized salt as the dietary vehicle for intake.

Japan

In Japan, it is a different story. Although Japan has a very high iodine intake, hypothyroidism is very common (Okamura, *et al.* 1989). The main source of excess iodine intake in Japan is seaweed. One study has revealed a significant correlation of high UI with hypothyroidism but not with hyperthyroidism (Konno, *et al.* 1993). This correlation was observed only in thyroid autoantibody (TAA) negative group. The mechanism of the development of hypothyroidism in the absence of TAA may be an unmasking of some preexisting subclinical abnormality by excess iodine intake, especially in some aged subjects.

Korea

Korea's study indicates that the urinary iodine excretion of Koreans depends on the amount of seaweed consumption, as it is with iodine intake (Kim, *et al.* 1998). They evaluated the iodine nutrition for physically active person working in hot and humid environments and concluded that iodine loss due to sweat must be taken into consideration.

Thailand

One study (Pongpaew, *et al.* 2002) from Thailand, found that fortified fish sauce might be the best and most feasible method of goiter prevention in their study area, given the fact that the villagers use fish sauce instead of salt more often in their cooking and that the supply of iodized salt is often irregular and more expensive than non-iodized salt. In

control of IDD in Thailand, continuous efforts have been made to improve logistics, monitoring and evaluation. Otherwise, a decrease in IDD once achieved cannot be maintained and the problem might recur and remain a significant public health problem. They also considered that health education is important.

The UIC of mothers in rural Thailand is adequate, with a median of 103 µg/L. However, in 2000; the median UIC of mothers in Bangkok was only 85 µg/L. It was also concluded that neonatal TSH screening using whole blood collected from a heel prick at 3 days of age is not sensitive enough to assess the iodine nutrition of neonates and neonatal screening using cord sera can be used to assess iodine nutrition in neonates (Rajatanavin 2007).

Hong Kong

Hong Kong, a coastal city in southern China, is not an endemic goitrous area, and IDD is not known to be prevalent. However, a recent survey conducted by Hong Kong Consumer Council of local brands of salt sold in supermarkets and grocery stores, showed either no iodization or low levels of iodization. It is generally believed that iodine deficiency, as a cause of clinical thyroid problems, does not exist in Hong Kong, because of its plentiful supply of seafood. There is circumstantial contradictory evidence refuting this belief (J. W. Kung, *et al.* 2001).

In 1996, a research study by Kung *et al* tested the urinary iodine levels and showed that 45.3% of children, 51.7% of adults, and 55.3% of the elderly had urine iodine concentration below the cutoff value for fasting urinary iodine concentration of 100 µg/L (Kung, Chan, *et al.* 1996).

In the same year, they evaluated the problem of dietary insufficiency during pregnancy; a cross-sectional study of 253 healthy, southern Chinese pregnant women and their neonates was conducted in Hong Kong (A. W. Kung, T. T. Lao and L. C. Low, *et al.* 1997). The results demonstrated the existence of borderline iodine intake; reflecting the fact that 35.8% of pregnant women had a urinary iodine concentration below 100 µg/L. This borderline iodine intake in pregnant women had a significant effect on both maternal and fetal thyroid function as evidenced by the following:

- A negative correlation between maternal serum TSH concentration and urinary iodine concentration
- Higher cord blood TSH levels in infants whose mothers had a low urinary iodine concentration compared with those whose mothers had normal urine iodine concentrations
- Women who had given birth to an infant with a cord blood TSH level ≥ 16 mIU/L had lower urinary iodine concentrations and serum FT_4 levels compared with those who had given birth to infants with normal TSH levels, and their infants also had higher cord blood thyroglobulin (TG) levels.

Their study indicated that the dietary iodine intake of the Hong Kong population is borderline sufficient, but is inadequate to meet extra requirements at times of thyroidal stress, such as during pregnancy, neonatal, and pubertal growth. They recognized the existence of mild iodine deficiency during pregnancy. They considered that iodine supplementation should be initiated during the second trimester to improve brain growth and development.

Cross-sectional and prospective studies of pregnant women in various trimesters of pregnancy in Hong Kong have revealed an increase in the UIC as pregnancy advances. With a median UI of approximately 100-120 μg/L during the various stages of pregnancy, a significant percentage of women had a sub-normal serum TH concentration at full term: 63% had a low FT_3 concentration, 53% had a low thyroxine concentration and 5% had a low FT_4 index (A. W. Kung, T. T. Lao and M. T. Chau, *et al.* 2000).

It has been shown that even a mild degree of maternal iodine deficiency and maternal hypothyroidism during pregnancy can result in poorer psychological and neurological performance in the child, as well as neonatal hypothyroidism (Pharoah, *et al.* 1984, Haddow, *et al.* 1999). Besides, early iodine supplementation can prevent goiter development in both the pregnant mother and the fetus (Glinoer, Nayer and Delange 1995).

Kung *et al.* 1997 found that iodine supplementation during pregnancy is essential in Hong Kong because seafood is not as commonly consumed as thought. According to

dietary survey, 50 to 80% of subjects never consume high iodine containing foods, such as seaweed, kelp, or laver and seafood intake was low in those subjects with low iodine status.

It seems that there are only a few places in the world that can ensure adequate dietary iodine intake through natural food sources. From their study, Kung *et al* reported that milder forms of iodine insufficiency can and do exist in non-goitrous areas. Overall, based on Kung's study, the iodine intake of the Hong Kong population should be brought up to a level at which IDD can be avoided but not higher. Health care policies are urgently needed to establish intervention programmes aimed at a relatively uniform iodine intake, avoiding deficient or excessive iodine intake in subpopulations. In the interim period, at-risk subpopulations including pregnant and lactating women, and young children of up to 3 years should receive physiological doses of iodide supplementation (J. W. Kung, *et al.* 2001).

Singapore

From ICCIDD documents, it was reported that IDD is not considered a problem in Singapore, but no data is available of median urinary iodine, household iodized salt use, and status. ICCIDD considered it as sufficient, but not established. There is no salt iodization legislation, IDD program, supplementation, and monitoring system in Singapore. But ICCIDD has reported, iodized salt is available at some retail outlets, and is required to meet iodization levels specified by the Food Regulations of Singapore. However, so far no such regulations actually exist in Singapore. There appears to be no data on consumption of iodized salt and ICCIDD considers it is probably low. Moreover, the food preservation methods, Sumar and Ismail 1997 reported, such as deep freezing and freeze-drying may reduce iodine concentration of food as much as 20 to 25%. The high temperature processing methods of food, for example, grilling and frying also can reduce iodine content. Even boiling can cause a loss up to 60%.

In 1976, one study by Yeo *et al.* 1976 showed T_3 toxicosis and its relation to the iodide status in Singapore. They considered the pattern of dietary intake of iodine remains to be established, pending analysis of local foods, as at the moment there is a remarkable paucity of food tables as regard iodine content in South-East Asia. However, they

thought, in Singapore island where sea foods are in abundance, the iodine intake exceeds the average intake of between 100 to 150 μg/L each day. They came to the conclusion that in Singapore where the iodine status is comparable to the goiter-free regions of the world and T_3 toxicosis is rare.

After that, no further research related to the IDD in Singapore. ICCIDD homepages do not provide any information of IDD of Singapore. Most countries report details about their iodine nutrition status there, and enact policy to administrate the problem in order to control the iodine status to meet the recommendations of WHO.

2.4.3 India

India is poised to become one of the world's leading economies along with the United States of America and China. However, India's quest to become a superpower and the effort to keep its prestigious role in shaping the global economy tried its best to defeat chronic iodine deficiency in the past 50 years respite all efforts. As per the latest projections of the Ministry for the Development of the North Eastern region India loses 4% of its GDP due to malnutrition. India's economic power is largely dependent on high-end services like Information Technology (IT) and outsourcing of knowledge to high-end technology services, which draws on intellectual capital of its young people. Iodine Deficiency Disorders (IDD), an easily preventable disease has been successfully eliminated in many countries, by establishing regular consumption habits of adequately iodized salt. Surprisingly, in India, only 51% of the population consumes adequately iodized salt. This shows that India till date has had limited success to protect its citizens from the risk of impaired intellectual development. Yearly 13.8 million newborns in India are at risk of lowered intellectual capacity. At the time when India needs its youth more than ever to propel it into the league of developed countries, this weakness will prove detrimental in its quest to become a developed economy (Pandav, *et al.* 2009).

ICCIDD in collaboration with various stakeholders carried out state level IDD survey in seven states of India from 1999 to 2006. The surveyed states were Jharkhand, Bihar, Orissa, Goa, Tamil Nadu, Kerala and Rajasthan. None of the seven surveyed states met all the three criterions for IDD elimination. All state except Goa reported less than 90 percent adequately iodized salt consumption at household level. Only three out of seven

states reported median urinary iodine levels greater than cut-off value of 100 µg/L (Table 2.10). Five out of seven states reported goiter prevalence of greater than 5%. The results of the survey clearly showed that IDD continues to be a significant public health problem in India.

A study was carried out to explore the relationships between the use of iodized salt in Rajasthan and the iodine status of children and pregnant women living in the area. The median UIC of children and pregnant women was 139 µg/L and 127 µg/L, respectively. Salt iodine content was 15 mg/kg in 41.9% of households and 23% used non-iodized salt. In households, using non iodized salt the median UIC's were 96 µg/L and 100 µg/L in children and women, respectively. It was observed that iodine status of both children and pregnant women attained the optimal range only when salt iodine content was close to 30 mg/kg (Ategbo, *et al.* 2008).

A study carried out by Kapil *et al.* in 1999 amongst pregnant women of second and third trimester attending antenatal clinic, Rural Health Training Centre, Najafgarh, New Delhi, showed prevalence of anemia, IDD and Vitamin A Deficiency was 78.8%, 22.9% and4.8%, respectively. They concluded that micronutrient deficiencies amongst pregnant women of urban slum are high.

A hospital based, non-interventional, cross-sectional study was undertaken to assess the iodine status of pregnant women attending the antenatal clinic at a medical college in Kolkata, India, during the different trimesters of pregnancy and to compare their iodine status with those of age-matched non-pregnant control women. UIE and FT_4 were significantly lower and TSH was significantly higher in pregnant women than in non-pregnant controls. However, no significant difference in median values for FT_3 concentration between the groups was seen ($p = 0.4$). Only 4 cases out of 200 pregnant women had an UIE of less than the lower cut-off value for UIE recommended by the WHO corresponding to optimal iodine intake. The results indicate most pregnant subjects attending the antenatal clinic at Medical College Kolkata, India, a tertiary care institution, did not suffer from significant iodine depletion. This may be ascribed to increased awareness of this condition and the accessibility of iodized salt among the study population (Chakraborty, Mazumdar, *et al.* 2010).

Table 2.10 Results of IDD survey in seven states

No.	Variables	Jharkhand	Bihar	Orissa	Goa	Tamil Nadu	Kerala	Rajasthan	Goal
1.	Proportion of households consuming adequately iodized salt % (≥ 15 PPM by titration)	64.2	40.1	45.0	91.9	18.2	48.9	42.1	> 90%
2.	Median urinary iodine µg/L	173.2	85.6	85.4	76	89.5	123.3	138.7	> 100
3.	Urinary iodine excretion (UIE) < 100 µg/L (%)	26.4	55.3	60.3	58.9	56	32.5	36.7	< 50%
4.	Urinary iodine excretion (UIE) < 50 µg/L (%)	10	31.5	32.2	36.2	22	8.2	16.9	< 20%
5.	Goiter prevalence (%)	0.9	5.2	8.0	17.5	13.5	16.6	3.3	< 5%

Source: IQ plus Jagriti 2009

Iodine status of women in reproductive age group in urban slums of Cuttack city revealed that 62.5% had iodine deficiency i.e. UIE <100 µg/L and 74.3% had moderate to severe iodine deficiency. The median UIE of the study population was 64.5 µg/L (Panigrahi, Mishra and Mohapatra 2009).

Using the three stage sampling technique, a study was conducted in twenty-eight villages of Jodhpur district to assess the magnitude of three micronutrient deficiency disorders (iron, vitamin A and iodine). It was found that majority of the women were anemic. Anemia was higher among pregnant and lactating women (80.7%). Vitamin A deficiency was observed to be higher among pregnant women (8.8%). A high proportion of women (80.8%) consumed salt, having inadequate iodine content. Median UIE values were less in lactating women (85 µg/L) and pregnant women (117.5 µg/L) as per WHO, 2007 cut-off points. Median UIE was below the optimal levels in 58.8% of pregnant women and 55.6% of lactating women, indicating an unsatisfactory situation. Average intake of nutrients showed deficiency of protein and energy, iron and folic acid and vitamin A deficiency (Singh, Fotedar and Lakshminarayana 2009).

Iodine deficiency was found to be leading cause of hypothyroidism in women of reproductive age group (n=101) residing in the sub-Himalayan plain areas of Darjeeling district of West Bengal. Results revealed that among 37.62% (n=38) of biochemically established hypothyroid women, 76.32% (n=29) suffered from iodine deficiency and the rest had hypothyroidism due to other causes. Moreover, iodine deficiency persisted in 57.42% (n=58) of the women in the study. Iodine deficiency disorders are still a major problem in this region and lacunae in iodine supplementation process needs to be reviewed (Ray, *et al.* 2009).

A hospital-based, cross-sectional, non-interventional study among 267 full term pregnant mothers, and the neonates born to them was carried out in a rural hospital of West Bengal. The overall iodine status of the pregnant women was estimated by measuring the UIE and the serum TSH levels. The neonatal thyroid function was estimated by measuring the TSH levels in the cord blood. A total of 78.4 percent pregnant women showed UIE > 10 mµg/dl with 7 percent having a UIE < 5 mµg/dl. The median UIE and the serum TSH values in the pregnant women were found to be 14.4 µg/dl and 4.1

mIU/L, respectively. Only 2.9 percent of the neonates showed a cord blood TSH value > 5 mIU/L which is just below the recommended criteria for mild endemicity for IDD in the study population. Pregnant women of the study area were iodine repleted. The neonatal thyroid function was also within normal range. The findings of the study indicated that the iodine supplementation of the salt should be maintained in the area with periodical surveillance (Chakraborty, Chatterjee, *et al.* 2006).

2.5 METHODOLOGY OF ASSESSMENT OF IODINE DEFICIENCY

The most important information in the determination of the status of iodine nutrition of a given population comes from the measurement of the urinary excretion of iodine and from the measurement of blood TSH in neonates or pregnant women (Table 2.11). The results of these two determinations indicate the severity of the problem, and can also be used to assess the effectiveness of remedial measures (Rendl, *et al.* 1998).

Table 2.11 Indicators of impact at population level (WHO/ UNICEF/ ICCIDD 2007)

Monitoring indicator (Units)	**Age group for assessment**	**Advantages**	**Disadvantages**
Median Urinary Iodine Concentration (µg/L)	School-age children and pregnant women	Spot urine samples are easy to obtain	Assess iodine intake only over the past few days
		The most practical biochemical marker for iodine nutrition, when carried out with appropriate technology and sampling	Meticulous laboratory practice is required to avoid contamination with iodine
		Feasible to process large numbers of samples at cost	A sufficient large number of samples must be collected to allow for various degrees of subject hydration and other biological variations among individuals
		Cut-off points proposed for classifying iodine nutrition into different degrees of public health significance are well established	
		External quality control program in place	

TSH (mIU/L)	Newborns	Measures thyroid function at a vulnerable age when iodine deficiency directly affects the developing brain	Not recommended to be setup solely to assess community iodine deficiency due to expense
		If screening programs to detect congenital hypothyroidism is in place then only additional cost will be for data analysis	Cannot be used when antiseptics containing iodine are used during delivery
		Collection by heel stick and storage on filter paper is simple	Requires use of a standardized, sensitive assay
		Blood spots can be stored for several weeks at cool, dry room temperatures	Should be taken either from the cord at delivery or by heel prick at least 48 hours after birth to avoid physiological newborn surge

2.5.1 Urinary iodine excretion test

Most iodine absorbed in the body eventually appears in the urine. Therefore, urinary iodine excretion is a good marker of very recent dietary iodine intake. In individuals, urinary iodine excretion can vary somewhat from day to day and even within a given day. However, this variation tends to even out among populations (WHO/ UNICEF/ ICCIDD 2007). Urinary iodine analysis is the most common biochemical method used for assessing the iodine status of populations and therefore plays an important role in public health surveillance in many countries. Urinary iodine analysis was recommended as the screening method by the World Health Organization in 1994. There is a great diversity in available urinary iodine methods with respect to cost, technical sophistication, sample processing capacity, and performance.

Daily iodine intake can be estimated by measuring daily excretion, or by random spot urine sampling calculated either in relation to urinary creatinine excretion or as UI concentration per liter. The choice among methods depends on the intended application, the number of samples, technical capability and cost.

Studies have convincingly demonstrated that a profile of iodine concentrations in morning or other casual urine specimens (child or adult) provides an adequate assessment

of a population's iodine nutrition, provided a sufficient number of specimens are collected. Round the clock urine samples are difficult to obtain and are not necessary. Relating urinary iodine to creatinine, as has been done in the past, is cumbersome, expensive, and unnecessary. Indeed, urinary iodine/ creatinine ratios are unreliable, particularly when protein intake – and consequently creatinine excretion – is low.

Iodine nutritional status is most closely estimated by the amount of iodine excreted in the urine over 24 hours. Often the 24 hour urine samples for UI determination are impractical to obtain, and can be unreliable because of incorrect or incomplete collection (WHO/ UNICEF/ ICCIDD 1994, J. G. Hollowell, W. H. Staehling, *et al.* 1998). Spot urine iodine concentrations collected from a population are currently the internationally accepted criteria for accessing and monitoring the iodine status of that population. Spot urinary iodine concentrations have been found to be an adequate measurement for population iodine status (Soldin 2002).

A wide variability in plasma and UI concentrations of iodine has been reported during pregnancy (Liberman, *et al.* 1998). UI concentrations increase during pregnancy in iodine replete-areas (Silva and Silva 1981, Smyth, *et al.* 1997, Glinoer, De Nayer, *et al.* 1990, Glinoer, Delange and Laboureur, *et al.* 1992) and in marginally sufficient areas (Smyth, *et al.* 1997, A. W. Kung, T. T. Lao and M. T. Chau, *et al.* 2000). Higher UI concentrations (increased iodine loss) during pregnancy can be perceived as a "normal" level of iodine intake resulting in an under-estimation of the prevalence of IDD during pregnancy if the assessments are based on UI concentrations alone. UI concentrations are slightly decreased during pregnancy in moderately IDD areas (Pedersen, Borlum, *et al.* 1988, Glinoer, De Nayer, *et al.* 1990, Glinoer, Delange and Laboureur, *et al.* 1992, Burrow, Fisher and Larsen 1994, Caron, *et al.* 1997, Gartner, Manz and Grossklaus 2001, Thomson, *et al.* 2001) possibly reflecting marginally low iodine intake.

There are many techniques for urinary iodine measurement and the choice is dependent on the particular needs that are based on satisfactory removal of interfering substances, precision, speed, technical demands, complexity of instrumentations, safety and cost. Most methods are based on the Sandell-Kolthoff reaction (Sandell and Kolthoff 1937), in which iodide catalyses the reduction of ceric ammonium sulfate (yellow color) to the

cerous (colorless) in the presence of arsenious acid (Dunn 1993). One such method is named as "Method A" which Dunn created in 1993. The name of "Method A" is continuously used till now, even though it has been improved and developed when compared with the initial technique.

Recently, there is a new technique – rapid urinary iodide test kit (RUIT), which is validated by HPLC (Rendl, *et al.* 1998). This is a qualitative colorimetric method based on iodide catalyzed oxidation of 3, 3', 5, 5'- tetramethylbenzidine by per-acetic acid/H_2O_2. This method is straightforward and does not require any sophisticated apparatus or instruments.

Urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition when carried out with appropriate technology and sampling. This approach assesses iodine nutrition only at the time of measurement, whereas thyroid size reflects iodine nutrition over months or years. Therefore, even though populations may have attained iodine sufficiency on the basis of median urinary iodine concentration, goiter may persist, even in children.

In populations characterized by long-standing iodine deficiency and a rapid increase in iodine intake, median values for urinary iodine above 200 μg/L (and in pregnant women, above 250 μg/L) are not recommended because of the possible risk of iodine-induced hyperthyroidism. This adverse condition can occur during the 5 to 10 years following the introduction of iodized salt (Stanbury 1998). Beyond this period of time, median values up to 300 μg/L have not demonstrated side-effects, at least not in populations with adequately iodized salt.

2.5.2 Thyroid stimulating hormone (TSH)

Neonatal screening programs for hypothyroidism have been developed and have become popular worldwide since the 1970's. TSH had been recommend by WHO as the primary screening test for neonates because it detects not only permanent sporadic congenital hypothyroidism, but also compensated or transient primary hypothyroidism, whose incidence can be as high as 1 in 10 neonates and whose main cause is iodine deficiency (Vela, *et al.* 1999, LaFranchi 1999). However, data on possible influence on cord blood

TSH are scarce. Actually, there are a large number of women who enter into maternal wards of hospitals and who are usually discharged in about 24 hours. Thus, it is difficult to obtain whole blood samples after the age of 72 hours. In 1985, Franklin *et al* analyzed the effects of maternal diabetes mellitus, toxemia, fetal distress and other factors on TSH levels. They concluded that these factors did not affect cord serum TSH concentrations (Franklin, Carpenter and O'Grady 1985). Another early report from (Fuse, *et al.* 1991), also suggested that TSH values in cord blood could be less influenced by perinatal factors than T_4 values. Also, in 2000, Ward *et al.* 2000 confirmed that even severe maternal diseases do not affect a screening program using primary TSH from cord blood.

The WHO, UNICEF and ICCIDD have included neonatal TSH as one of the indicators for assessing iodine deficiency disorders and their control (Delange 1998). So far, WHO have not established the exact criterion for cord TSH. Most countries regard percentile value of cord blood TSH screening programme as the cut-off point when estimating iodine status in newborns (A. W. Kung, T. T. Lao and L. C. Low, *et al.* 1997).

Newborn TSH is an important measure because it reflects iodine status during a period when the developing brain is particularly sensitive to iodine deficiency. Compared with the adult, the newborn thyroid contains less iodine but has higher rates of iodine turnover. Particularly when iodine supply is low, maintaining high iodine turnover requires increased TSH stimulation. Serum TSH concentrations are therefore increased in iodine-deficient infants for the first few weeks of life, a condition termed transient newborn hyperthyrotropinemia.

The increase in the number of neonates with moderately elevated TSH concentrations (above 5 mIU/L whole blood) is proportional to the degree of iodine deficiency during pregnancy. It may be higher than 40% in severe endemic areas. When a sensitive TSH assay is used on samples collected three to four days after birth, a <3% frequency of TSH values >5 mIU/L indicates iodine sufficiency in a population (Zimmermann 2005).In areas of iodine deficiency, an increase in transient newborn hypothyroidism, indicated by more than 3% of newborn TSH values above the threshold of 5 mIU/L whole blood collected 3 to 4 days after birth, suggests iodine deficiency in the population (WHO/ UNICEF/ ICCIDD 2007).

In iodine-sufficient populations, about one in 4000 neonates has congenital hypothyroidism, usually because of thyroid dysplasia. Prompt correction with thyroid hormone is essential to avoid permanent mental retardation.

2.6 MANAGEMENT FOR THYROID DISORDERS

2.6.1 Trimester specific reference ranges for thyroid hormones

Pregnant women can have their thyroid hormones measured, with TSH and FT_4 typically used to define subclinical hypothyroidism (TSH > 97.5th percentile, FT_4 in normal reference range), overt hypothyroidism (TSH > 97.5th percentile and FT_4 < 2.5th percentile), and hypothyroxinemia (TSH in normal reference range, FT_4 < 2.5th percentile) (Costeira, *et al.* 2010). There is debate as to whether all pregnant women should be screened for abnormal thyroid hormone concentrations in the first trimester. Changes in thyroid hormones can be caused by a variety of factors such as thyroid disease, and is not specific to iodine deficiency. Indeed, WHO do not recommend the routine use of TSH, T_4 and T_3 for monitoring iodine status in the adult population as these tests are relatively insensitive in moderate to mild iodine deficiency; median UIC provides sufficient information to assess iodine status in these groups. However, because of the importance of maternal thyroid hormone concentrations for normal fetal brain development, the establishment of trimester specific international reference ranges for thyroid hormones in iodine sufficient and deficient pregnant women could be useful in identifying at-risk women (Skeaff 2011).

Physiological alterations in the homeostatic control of thyroid hormones cause changes in thyroid function tests in pregnant women. A lack of method, trimester and population-specific reference intervals for free triiodothyronine (FT_3), free thyroxine (FT_4) and thyrotropin (TSH) makes interpretation of FT_3, FT_4 and TSH levels in pregnancy difficult. Trends suggest that trimester specific measurements of FT_3, FT_4, TG and TSH are warranted (Soldin 2006).

During pregnancy the thyroid is hyper stimulated, resulting in changes in thyroid hormone concentrations. Accurate assessment of thyroid function during pregnancy is critical, for both the initiation of thyroid hormone therapy, and for the adjustment of thyroid hormone dose in those already receiving thyroid hormone. Trimester-specific

intervals are especially important during pregnancy when thyroid insufficiency may be associated with adverse obstetric outcome and fetal neurodevelopmental deficits. Gestational age-specific reference intervals are now available for thyroid function tests (Table 2.12). Knowing the expected normal changes in hormone concentrations throughout pregnancy allows individualized supplementation when necessary (Soldin 2006, Marwaha, *et al.* 2008).

Reference ranges provided by the manufacturers of most FT_4 measurement kits have been established using pools of nonpregnant normal sera. Such reference ranges are not valid during pregnancy because FT_4 assays are influenced by serum changes (mainly in TBG and serum albumin). So it has been proposed by various authors to adopt serum FT_4 reference ranges to "laboratory specific" or "trimester-specific" pregnancy ranges but, so far, no consensus has been reached (Soldin, Tractenberg, *et al.* 2004).

Serum TSH values are influenced by the thyrotropic activity of elevated circulating hCG concentrations, particularly (but not only) near the end of the first trimester. Thus, by using the classical reference range for serum TSH (0.4 mIU/L for the lower limit and 4.0 mIU/L for the upper limit), one might misdiagnose as "normal" women who already have a slight TSH elevation and, conversely, one might suspect hyperthyroidism in normal women who have a blunted serum TSH value (Panesar, Li and Rogers 2001). They proposed to use "trimester-specific" reference ranges for serum TSH during pregnancy.

Several studies have reported population based trimester specific thyroid hormone reference intervals. (Marwaha, *et al.* 2008) in a cross- sectional study established the reference ranges of FT_3, FT_4 and TSH for pregnant Indian women recruited in different trimesters of pregnancy using 5th and 95th percentile.

Dhatt, *et al.* 2006 established trimester specific reference intervals for TSH and FT_4 in a mixed ethnic population of pregnant women attending two antenatal clinics in the United Arab Emirates.

Gibert, *et al.* 2008 reported that the reference interval for TSH during the first trimester of pregnancy differs substantially from that for non-pregnant women, and applying the general laboratory reference range to pregnant women results in misclassification of thyroid status for 20.5% of women.

Table 2.12 Summary of studies concerning the reference intervals of TSH, FT_4 and FT_3 in pregnant women

First Author	Year	Type	Country	n	Method	TSH (mIU/ml)			Free T_4 (pmol/L)			Free T_3 (pmol/L)		
						1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim
Yan	2011	All trim Cross-sectional	China	505	IA, Advia Centaur	0.03-4.51	0.05-4.51	0.47-4.54	11.8-21	10.6-17.6	9.2-16.7	ND	ND	ND
Kuppens	2010	3rd trim Cross-sectional	Holland	1058	IA, Immulite	ND	ND	0.51-2.89	ND	ND	ND	ND	ND	ND
Fister	2010	3rd trim Cross-sectional	Silovenia	116	IA, Advia Centaur	ND	ND	0.61-3.96	ND	ND	7.91-14.15	ND	ND	3.30-4.53
Klajnbard	2010	2ns & 3rd trim Cross-sectional	Nordic countries	801	IA, Immulite	ND	0.3-4.1	0.4-3.7	ND	11.9-18.7	9.4-17.8	ND	ND	ND
Shan	2009	1st & 2nd trim Cross-sectional	China	4800	IA, Diagnostics products	0.09-2.96	0.35-3.88	ND	11.41-21.7	11.6-21.69	ND	ND	ND	ND
Silvio	2009	2nd trim Cross-sectional	U.S.	3102	IA, Elecsys E170	ND	0.18-4.07	ND	ND	9.5-15.8	ND	ND	ND	ND
Bocos-Terraz	2009	1st & 2nd trim Cross-sectional	Spain	1198	IA, Abbott Architect	0.10-2.65	0.12-2.64	ND	10.68-17.76	9.0-14.67	ND	ND	ND	ND

First Author	Year	Type	Country	n	Method	TSH (mIU/ml)			Free T_4 (pmol/L)			Free T_3 (pmol/L)		
						1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim
Marwaha	2008	All trim Cross-sectional	India	541	IA, Elecsys 1010	0.6-5.0	0.44-5.78	0.74-5.7	12.0-19.45	9.48-19.58	11.3-17.71	1.92-5.86	3.2-5.7	3.3-5.18
Larsson	2008	All trim Longitudinal	Sweden	52	IA, Abott Architect	0.09-3.39	0.37-3.4	0.40-3.88	ND	ND	ND	ND	ND	ND
Gilbert	2008	1st trim	Australia	2159	IA, Abott Architect	0.02-2.15	ND	ND	10.4-17.8	ND	ND	3.3-5.7	ND	ND
Yue	2008	1st & 2nd trim Cross-sectional	U.S.		Equilibrium dialysis-mass spectrometry	ND	ND	ND	13.9-23.42	11.07-19.69	ND	ND	ND	ND
Lambert-Messerlian*	2008	1st & 2nd trim Cross-sectional	U.S.	9562	IA, Immulite 2000	0.1-2.7	0.4-2.8	ND	0.9-1.4	0.8-1.3	ND	ND	ND	ND
Stricker	2007	All trim Cross-sectional	Switzer-land	2272	IA, Abott Architect	0.09-2.83	1.02-2.79	1.14-2.90	10.53-18.28	9.53-15.68	8.63-13.61	3.52-6.22	3.41-5.78	3.33-5.59
La'ulu	2007	2nd trim Cross-sectional	U.S.	3064	IA, Abott Architect	ND	0.15-3.11	ND	ND	9.3-15.2	ND	ND	ND	ND

First Author	Year	Type	Country	n	Method	TSH (mIU/ml)			Free T_4 (pmol/L)			Free T_3 (pmol/L)		
						1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim	1st trim	2nd trim	3rd trim
Dashe	2005	All trim Cross-sectional	U.S.	13599	IA, Siemens Immulite	0.01-5.09	0.02-4.09	0.20-6.40	ND	ND	ND	ND	ND	ND
Spencer	2005	1st trim Cross-sectional	U.S.		IA, Roche Elecsys & Tosoh	0.03-2.4	ND	ND	ND	ND	ND	ND	ND	ND
Haddow	2004	1st & 2nd trim Cross-sectional	U.S.	1126	IA, Siemens Immulite	0.08-3.61	0.39-3.71	ND	ND	ND	ND	ND	ND	ND
Price	2001	1st & 2nd trim Cross-sectional	Asians	-	IA, ACS 180	0.6-1.3	1.0-1.8	ND	11.8-13.4	10.9-12.1	ND	ND	ND	ND
Price	2001	1st & 2nd trim Cross-sectional	Caucasian	-	IA, ACS 180	0.7-1.1	1.2-1.5	ND	12.0-12.8	11.2-11.8	ND	ND	ND	ND
Panesar	2001	All trim Cross-sectional	China	343	IA, ACS 180	0.03-2.3	0.03-3.7	0.13-3.4	11.1-22.9	8.1-16.7	8.5-14.4	3.0-5.7	2.8-4.2	2.4-4.1

n= Number, IA= Immunoassay, ND= Not done, *the 5th and 95th percentile; Free T_4 in µg/L.

TPO-Ab status of pregnant women should be considered when constructing trimester-specific reference ranges because elevated serum TPO-Ab levels are associated with higher TSH and lower T_4 values (Pearce, *et al.* 2008).

2.6.2 Maternal hypothyroidism

The administration of levothyroxine is the treatment of choice for maternal hypothyroidism, if the iodine nutrition status is adequate. Hypothyroid pregnant women require larger thyroxine replacement doses than do nonpregnant patients, and women who already take thyroxine before pregnancy usually need to increase their daily dosage by, on average, 30-50% above preconception dosage (Mandel 2004). During pregnancy, because of the increased requirements, the full replacement thyroxine dose should be increased to 2.0-2.4 µg/kg bw.d.

In women who already receive thyroxine before conception, the need to adjust the preconception thyroxine daily dosage becomes manifest as early as by 4-6 wk gestation, hence justifying the adaptation of thyroxine replacement to ensure that maternal euthyroidism is maintained during early pregnancy. It is important to note that 25% of hypothyroid women who are able to maintain a normal serum TSH level in the first trimester, and 35% of those who maintain a normal serum TSH level until the second trimester without increasing their daily dosage, will still require an increment in thyroxine replacement during late gestation to maintain a euthyroid status (Mandel 2004, Alexander, *et al.* 2004, Rotondi, *et al.* 2004).

The etiology of hypothyroidism plays a pivotal role in determining the timing and magnitude of thyroid hormone adjustments during pregnancy. SCH patients needed a larger increase than OH and post-ablative hypothyroidism (PH) (Verga, *et al.* 2009, Loh JA, *et al.* 2009).

International task force created under the auspices of The Endocrine Society (Abalovich, Amino, *et al.* 2007) has recommended that:

i. If hypothyroidism has been diagnosed before pregnancy, adjustment of preconception thyroxine dose to reach before pregnancy a TSH level not higher than 2.5 mIU/L.

ii. The thyroxine dose often needs to be incremented by 4-6 weeks gestation and may require a 30-50% increment in dosage.

iii. If OH is diagnosed during pregnancy, thyroid function tests 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 mIU/L in the first trimester (or 3 mIU/L in second and third trimesters) or to trimester-specific normal TSH ranges.

iv. SCH has been shown to be associated with an adverse outcome for both the mother and offspring. Thyroxine treatment has been shown to improve the obstetrical outcome, but has not been proved to modify long-term neurological development in the offspring. However, panel recommended thyroxine replacement in women with TSH.

v. Women with thyroid autoimmunity (TAI) who are euthyroid in early stages of pregnancy are at risk of developing hypothyroidism and should be monitored for elevation of TSH above the normal range.

2.6.3 Maternal hyperthyroidism

Anti thyroid drugs (ATDs) are the main treatment for hyperthyroidism during pregnancy. Propylthiouracil (PTU), methimazole (MMI) and carbimazole have been used during gestation. They inhibit thyroid hormone synthesis via reduction in iodine organification and iodotyrosine coupling. A recommendation for the preferred use of PTU during pregnancy is based on report of reduced transplacental passage of PTU as compared with MMI. PTU is more extensively bound to albumin at physiological pH, whereas MMI is less bound, which might result in increased transplacental passage of MMI relative to PTU (Marchant, *et al.* 1977).

ATD treatment of pregnant women must be aimed to restore normal maternal thyroid function while ensuring that fetal thyroid function is minimally affected.

Recommendations of International task force (Abalovich, Amino, *et al.* 2007):

i. If a subnormal serum TSH concentration is detected during gestation, hyperthyroidism must be distinguished from both normal physiology of

pregnancy and hyperemesis gravidarum because of the adverse effects of overt hyperthyroidism on mother and the fetus.

ii. For overt hyperthyroidism due to Graves' disease, ATD therapy should be either initiated (for those with new diagnoses) or adjusted (for those with prior history) to maintain normal thyroid hormone levels for FT_4 in the upper nonpregnant reference range.

iii. PTU should be used as a first line drug, if available, especially during first-trimester organogenesis as MMI has been associated with congenital anomalies.

2.6.4 Gestational hyperemesis and hyperthyroidism

It is recommended (Abalovich, Amino, *et al.* 2007) that:

i. Thyroid function tests should be measured in all patients with hyperemesis gravidarum (5% weight loss, dehydration and ketonuria).

ii. Few women with hyperemesis gravidarum will require ATD treatment. Gestational hyperthyroidism with clearly elevated thyroid hormone levels (FT_4 above the reference range and TSH <0.1μIU/ml) and evidence of hyperthyroidism may require treatment as long as clinically necessary.

2.6.5 Autoimmune thyroid disease

Negro, *et al.* 2006 performed a prospective, randomized trial of 984 unselected women who were screened for TPO-Ab positivity and thyroid function tests at the first obstetrical visit. Women were divided into three groups; Group A: women who were TPO-Ab positive treated with levothyroxine (L-T4), Group B: women who were TPO-Ab positive and received no L-T4 treatment and Group C: TPO-Ab negative women and did not receive L-T4. The miscarriage rate was significantly higher in Group B (13.8%) than in Group A (3.5%) or C (2.4%) ($P<0.05$). Similarly, the preterm delivery rate was found to be higher in Group B (22.4%) than in Group A (7%) or C (8.2%) ($P<0.05$).

Although a positive association exists between the presence of thyroid antibodies and pregnancy loss, universal screening for thyroid antibodies, and possible treatment, has not been recommended till now.

2.6.6 Iodine nutrition during pregnancy

International Task force (Abalovich, Amino, *et al.* 2007) recommends that:

i. Women in the childbearing age should have an average iodine intake of 150µg/day. During pregnancy and breastfeeding, women should increase their daily iodine intake to 250µg on average.

ii. Iodine intake during pregnancy and breastfeeding should not exceed twice the daily recommended nutrient intake (RNI) for iodine, i.e. 500 µg iodine/day.

iii. To assess the adequacy of the iodine intake during pregnancy in a population, urinary iodine concentration (UIC) should be measured in a representative cohort of the population. UI should ideally range between 150 and 250 µg/L.

iv. To reach the daily RNI for iodine, multiple means must be considered, tailored to the iodine intake level in a given population. Different situations must therefore be distinguished (Andersson, *et al.* 2007):

    a. Countries with iodine sufficiency and/or without well-established universal salt iodization (USI) program.

    b. Countries without a USI program or with an established USI program where the coverage is known to be only partial.

    c. Remote areas with no accessible USI program and difficult socio-economic conditions.

2.6.7 Postpartum Thyroiditis

It is recommended (Abalovich, Amino, *et al.* 2007) that:

i. Women known to be TPO-Ab positive should have a TSH performed at 3 to 6 months postpartum.

ii. Symptomatic women and women with a TSH above normal and who are attempting pregnancy should be treated with L-T4. 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 remonitored in 4-8 wk.

2.7 SCREENING FOR THYROID DYSFUNCTION DURING PREGNANCY

International task force created under The Endocrine Society (Abalovich, Amino, *et al.* 2007) recommends case finding among the following groups of women at high risk for thyroid disease by measurement of TSH:

i. Women with a history of hyperthyroid or hypothyroid disease, PPT or thyroid lobectomy.
ii. Women with a family history of thyroid disease.
iii. Women with a goiter.
iv. Women with thyroid antibodies (when known).
v. Women with symptoms or clinical signs suggestive of thyroid underfunction or overfunction, including anemia, elevated cholesterol and hyponatremia.
vi. Women with Type I diabetes.
vii. Women with other autoimmune disorders.
viii. Women with infertility who should have screening with TSH as part of their infertility work-up.
ix. Women with previous therapeutic head or neck irradiation.
x. Women with a history of miscarriage or preterm delivery.

2.8 SEAWEED AND IODINE

Seaweeds are marine macro algae and primitive type of plants, growing abundantly in the shallow waters of sea, estuaries and backwaters. They flourish wherever rocky, coral or suitable substrata are available for their attachment. They belong to three groups namely green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) based on pigmentation, morphological and anatomical characteristics. There are about 900 species of green seaweed, 4000 red species and 1500 brown species found in nature. Some 221 species are utilized commercially. Of these, about 145 species are used for food and 110 species for phycocolloid production (eg. Agar). Seaweed has been a staple food in Japan and China for a very long time. The green seaweeds *Enteromorpha*, *Ulva*, *Caulerpa* and *Codium* are utilized exclusively as source of food. These are often eaten as fresh salads or cooked as vegetables (Khan and Satam 2003).

Many Indians may sneer at the thought of having algae as an alternate source of food, but algae are already extensively used as a food source in many countries. These algae are generally marine and are commonly termed as 'seaweeds'. The major users of seaweeds as food are the coastal Japanese, and about 25% of their daily diet consists of seaweeds. Seaweeds are used as food in many forms in several Asian countries, such as Myanmar, China, Thailand, Korea, Malaysia, Philippines and Indonesia, and are also considered a tasteful dish in England and Scotland. Seaweeds are also to be found in the diets of people in Australia, New Zealand, France, Chile, Hawaii, Brazil and several other Latin American countries. Species of the genera *Caulerpa, Durvillea, Laminaria, Monostroma, Nereocytstis, Oedogonium*, *Porphyra*, *Rhodymenia*, *Sargassum*, and *Spirogyra* are particularly commonly used as food in different parts of the world (Ghosh 2004).

The medicinal uses of seaweed are vast and range from topical burn therapy to goiter therapy to softening of tumors. In Japan, 21 species of seaweed are routinely included in the diet and in Korea more than 40 kinds of seaweed are commonly used as food. Elsewhere, in the Pacific basin, in Hawaii and other Polynesian islands, 29 kinds of seaweed have been reports as food, medicine, and as part of religious celebrations in precolonial times and seaweeds are still part of the diets of many indigenous people living in Asia, Polynesia and the Pacific Islands. Seaweeds are increasingly common foods and food supplements in the United States (Teas, *et al.* 2004).

Seaweeds are traditionally consumed in the orient as part of the daily diet. Currently, human consumption of green algae (5%), brown algae (66.5%) and red algae (33%) is high in Asia, mainly Japan, China and Korea. However demand for seaweed as food has now also been extended to North America, South America and Europe. The different species consumed present a great nutritional value as source of proteins, carbohydrates, minerals and vitamins (Karthiki Devi, *et al.* 2009).

Seaweeds have been used since ancient times as food, fodder, fertilizer and a source of medicine. They are the raw material for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. They are nutritionally valuable as fresh or dried vegetables or as ingredients in a wide variety of prepared foods. In particular, certain edible seaweeds contain significant quantities of

protein, lipids, minerals and vitamins, although nutrient contents vary with species, geographical location, season and temperature. The chemical composition of seaweeds varies with species, habitats, maturity and environmental conditions (Manivanna, *et al.* 2008).

Typically, algae consist of about 25-30% fats of its total dry weight; 10-20% proteins; 2-4% vitamins and 0.2-0.5% mineral salts. Algae are especially rich in vitamins A and E, and often are also rich in vitamin C and D. They are also rich in thiamine, niacin, riboflavin, choline, pantothenic acid, pyridoxine, biotin, etc. Mineral elements mainly include sodium, potassium, iodine, chlorine with a trace amount of copper, iron, manganese, zinc, etc. Thus, algae provide many elements essential for a well balanced diet (Ghosh 2004).

High carbohydrate (24-44%) and lipid (6-23%) contents were reported in seaweeds in Indian shores. The caloric value is more in seaweeds. Seaweeds like *Cladophora pinnulata*, *Levringia boergesenii*, *Sargassum wightii*, *Sarconema furcelatum* and *Asparagopsis taxiformis* are good sources of iodine (Manivanna, *et al.* 2008).

Seaweeds grow abundantly along the Tamil Nadu and Gujarat coasts and around Lakshadweep and Andaman and Nicobar islands. There are also rich seaweed beds around Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam and Pulicat in Tamil Nadu and Chilka in Orissa. Out of approximately 700 species of marine algae found in both inter-tidal and deep water regions of the Indian coast, nearly 60 species are commercially important. On the West coast, especially in the state of Gujarat, abundant seaweed resources are present on the intertidal and subtidal regions. These resources have great potential for the development of seaweed based industries in India (Khan and Satam 2003).

Iodine was first identified as an element based on the observations of Courtois in 1811 that sulphuric acid treated seaweed ash produced a purple vapor that condensed into purple crystals (Teas, *et al.* 2004).

Kesava Rao 1992 collected data on 176 algal species from Indian coast and found that iodine content ranged from 21 to 7, 400 mg /kg dry weight of the sample.

A study was conducted in the 18 species of algae collected from Cape Comorin during February 1993. *Sarconema filiforme* showed the highest iodine content (70.85 mg/100 g dry weight). Two species of *Caulerpa* (*C. racemosa* and *C. scalpelliformis*) varied widely in iodine content from 43.36 to 2.54 mg/ 100 g dry weight, respectively. No iodine was found in *Ulva fasciata* (Ganga Devi, Sobha and Nair 1996).

Five Indian seaweed species, viz. *Enteromorpha linza, E. prolifera, Ulva fasciata, Caulerpa taxifolia* and *Sargassum johnstonii*, from natural and cultivated sources were evaluated for safety and nutritional quality and feeding tests on rats did not produce any toxic effect on them (Naidu, *et al.* 1993).

Sobha, *et al.* 2008 prepared dishes from algae from the coastal waters of Kerala, particularly from Kovalam (Trivandrum) and Thankasseri (Kollam). Six species of seaweeds were selected because they were available in plenty throughout the year in the area. *Ulva reticulata, Ulva fasciata, Padina tretastromatica, Sargassum wightii, Gracilaria corticata* and *Caulerpa recemosa* are the algal species used for preparing dishes such as toffee, squash, pickle, cutlet, *biryani* and *thoran*.

There are few people suffering from iodine deficiency in Japan because people often consume seaweeds (or kelp) in addition to fish, chicken eggs, milk, and dairy products. Seaweeds are particularly rich in iodine, at 100-1,000 times the level in fish. For example, *kombu*, a typical and commonly consumed seaweed, contains more than 100,000μg of iodine/100g, while sardine and horse mackerel, as examples of fish with the highest iodine concentrations, feature only approximately 250μg of iodine/ 100g (Tokudome, *et al.* 2004).

Fortified salt and other foods clearly can prevent iodine deficiency. However, we need to limit salt consumption because it is related to hypertension. Intake of seaweeds is advised not only for prevention of lifestyle-related diseases, including cancer, cardiovascular and cerebrovascular disease but also of iodine deficiency. Furthermore, seaweed is a very palatable food because it contains glutamic acid providing a pleasant taste (Tokudome, *et al.* 2004).

Methods and Materials

3. METHODS AND MATERIALS

3.1 EXPERIMENTAL DESIGN

The study was divided into four phases:

Phase I	:	Enrollment of the pregnant subjects.
Phase II	:	Providing Nutrition Health Education (NHE)
Phase III	:	Follow up of the subjects during the three trimesters and also assessing neonatal status.
Phase IV	:	Identification and supplementation of iodine-rich seaweed.

In the present study 189 apparently normal, healthy pregnant women attending the antenatal clinics of various hospitals in Delhi: Gokalpuri Urban Health Centre (under Maulana Azad Medical College), St. Stephen's Hospital, Delhi and Endocrine clinic of Institute of Nuclear Medicine and Allied Sciences (INMAS) were included. The subjects were categorized as group I.

In Group II, 145 pregnant women who already had thyroid dysfunction either hypothyroidism or hyperthyroidism and were under treatment/ referrals were included.

All women in the age group of 18-45 years were enrolled in the first trimester (till 12-13 weeks) of pregnancy. The enrollment period was from June 2008 to November 2009 and subjects were enrolled randomly so as to exclude regional, nutritional, socioeconomic variation. The subjects were enrolled and advised to visit Division of Endocrinology and Thyroid Research, Institute of Nuclear Medicine and Allied Sciences, Delhi for clinical and biochemical evaluation including urine examination for iodine.

A written informed consent was taken from all the subjects (Annexure I). The study was approved by institutional ethical committee of Institute of Nuclear Medicine and Allied Sciences. All cases were clinically evaluated based on detailed history and physical examination with emphasis on following points.

Clinical history

- suggestive of hypo or hyperthyroidism before, during or after pregnancy.

- suggestive of visible enlargement of the gland
- suggestive of repeated abortions
- suggestive of any autoimmune disorder

General data

Included besides routine examination, a record of anthropometry and physiological parameters like

1. Height
2. Weight
3. Thyroid gland examination
4. Blood Pressure
5. Pulse

Serological and biochemical parameters

All the subjects were subjected to following investigations at the time of enrolment and during follow up till 6 months postpartum

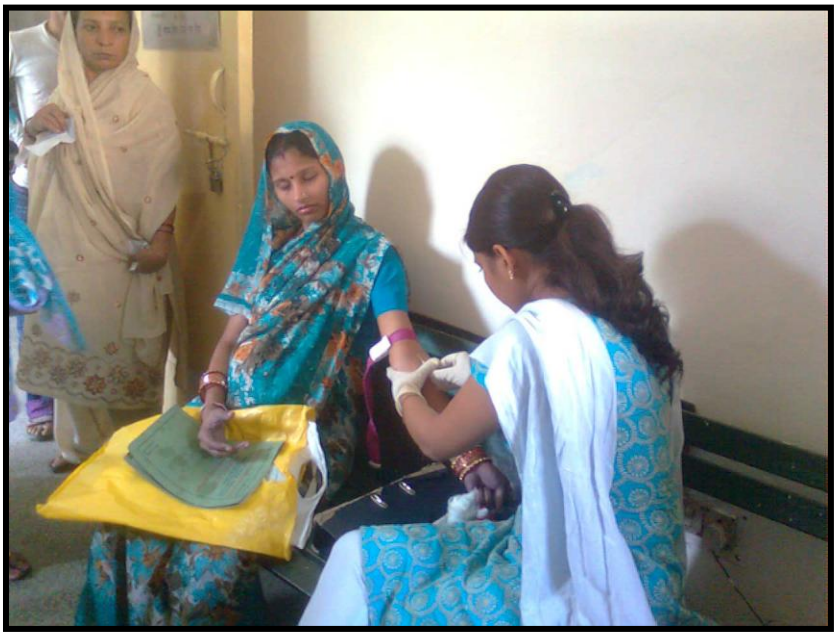
1. Free triiodothyronine (FT_3)
2. Free thyroxine (FT_4)
3. Thyroid stimulating hormone (TSH)
4. Thyroperoxidase antibodies (TPO-Ab)
5. Urinary iodine (UI)
6. Hemoglobin (Hb)

All the newborns were screened for transient and permanent thyroid dysfunction. Cord blood TSH and FT_4 was assessed in all the cases and newborns with TSH of >20 μIU/ml were recalled for repeat TSH and FT_4 to rule out congenital hypothyroidism.

The non-pregnant women in the same age group were also included. They formed the control group (Group IV). Their urine and serum samples were analyzed for FT_4, TSH, TPO-Ab, urinary iodine and hemoglobin.

(a)

(b)



(c)

Figure 3.1 (a, b, c) Subjects being enrolled for the study

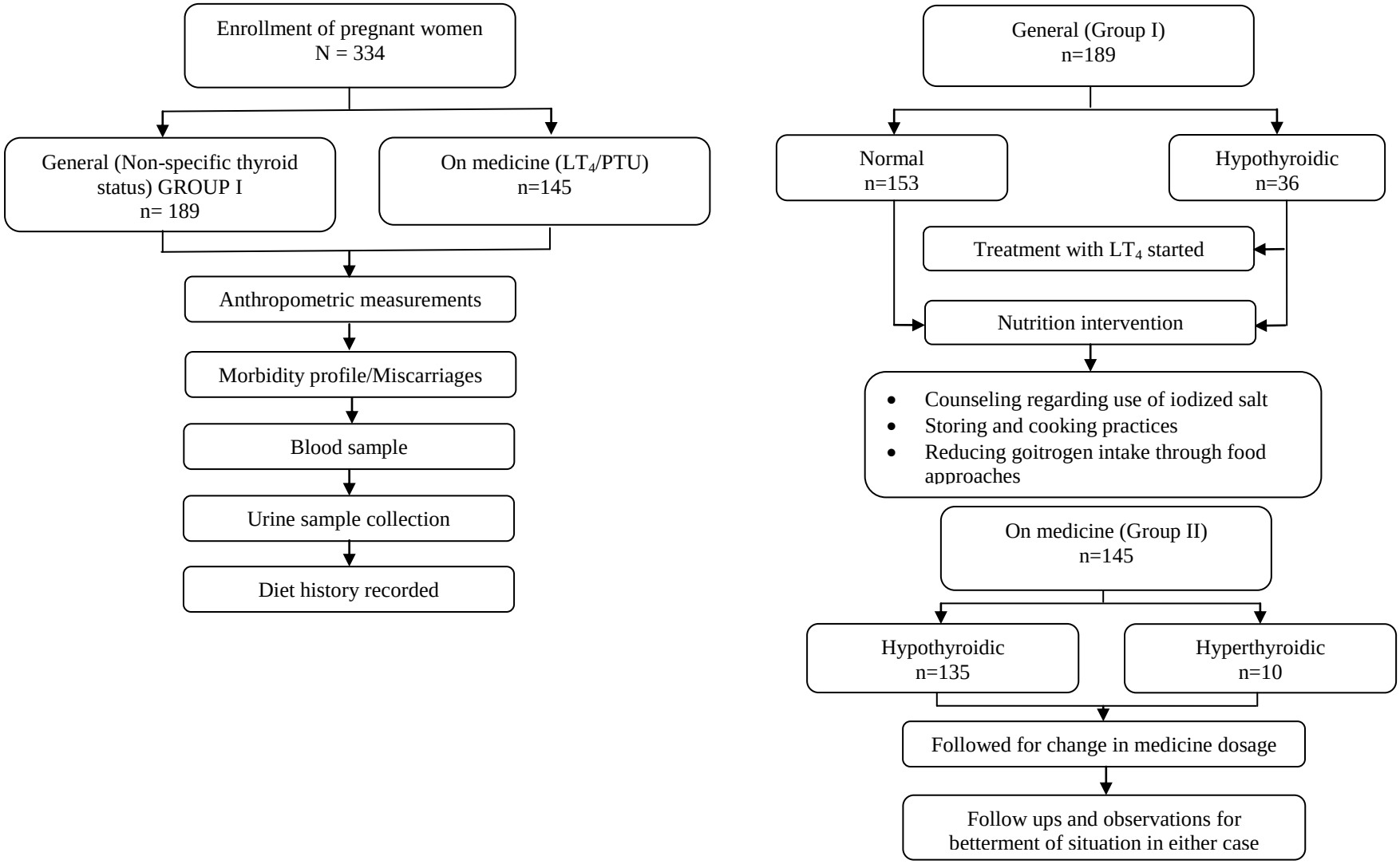


Figure 3.2 Experimental design: Phase I and II

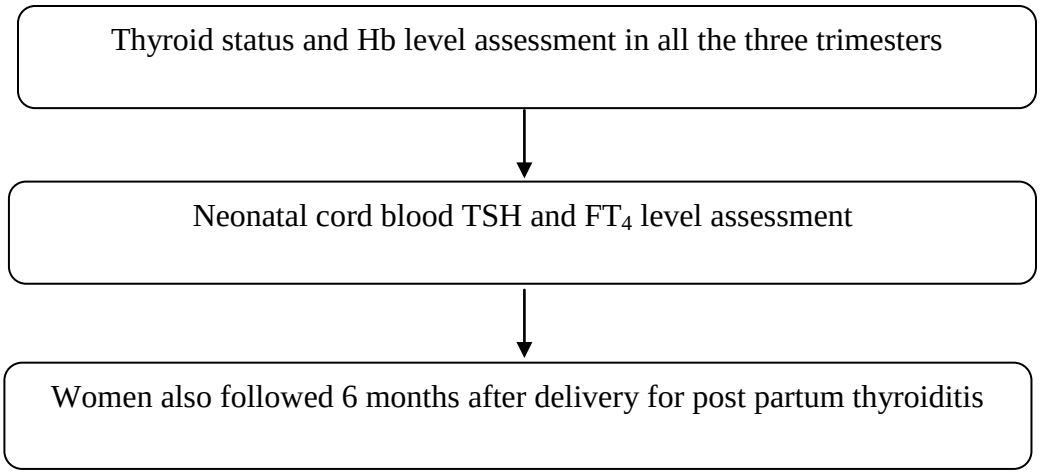


Figure 3.3 Experimental design: Phase III

Phase IV

Collection and Processing of seaweeds

The various group of marine macroalgae or seaweeds such as Chlorophyceae members (*Caulerpa racemosa, Caulerpa scalpelliformis, Caulerpa veravelense*) and Phaeophyceae member (*Padina tretastromatica*) were collected from Veraval, Adri and Porbandar Coast of Gujarat.

The seaweeds were handpicked and immediately cleaned with seawater to remove foreign particles, sand and epiphytes. Then the seaweed was kept in an ice box containing slush ice and immediately transported to the laboratory and cleaned thoroughly using tap water to remove the salt on the surface of the sample. It was spread on the blotting paper to remove excess amount of water. It was air dried and milled to a coarse powder and stored in refrigerator for chemical analysis and preparation of supplementation product (wheat flour *ladoos*).

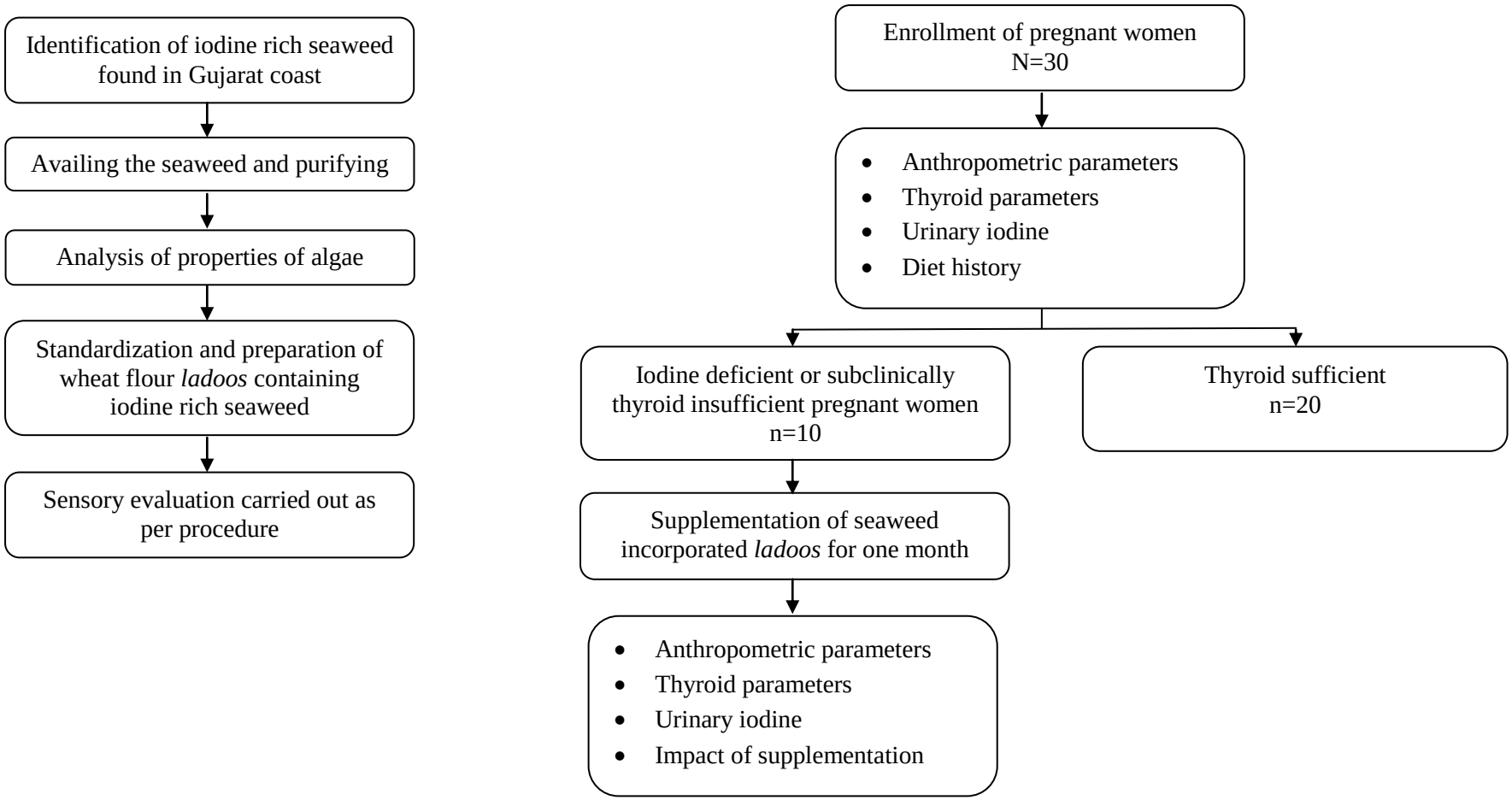


Figure 3.4 Experimental design: Phase IV

Recipe of wheat flour *ladoos*:

Ingredients

Wheat flour	100 g
Seaweed	1.02 g
Ghee	140 g
Powdered Sugar	69.5 g
Cardamom	½ teaspoon

Preparation

Step 1. Heat the ghee in *karahi* and add wheat flour, fry on a low flame stirring continuously till light brown.

Step 2. Now add sugar and stir fry for 5 minutes.

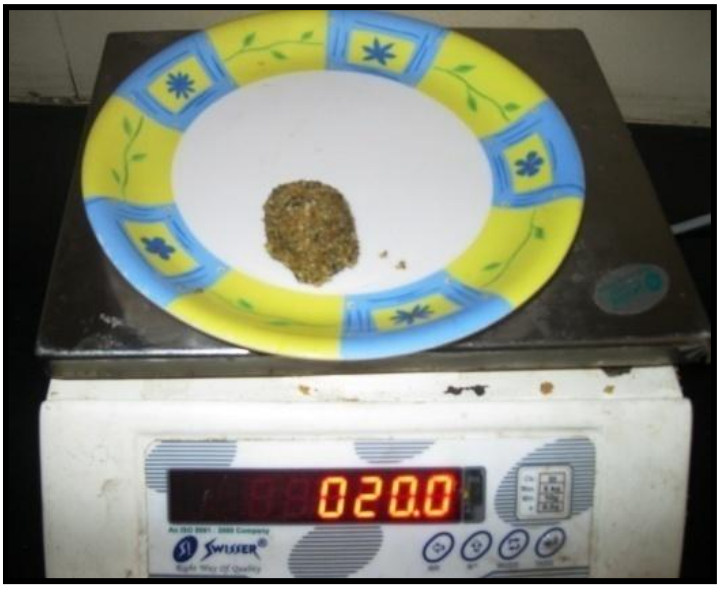
Step 3. Remove from the stove and cool for 10 minutes.

Step 4. Add seaweed powder, cardamom and mix well.

Step 5. While still hot, make round small balls out of the mixture.

Number of *ladoos* prepared : 6

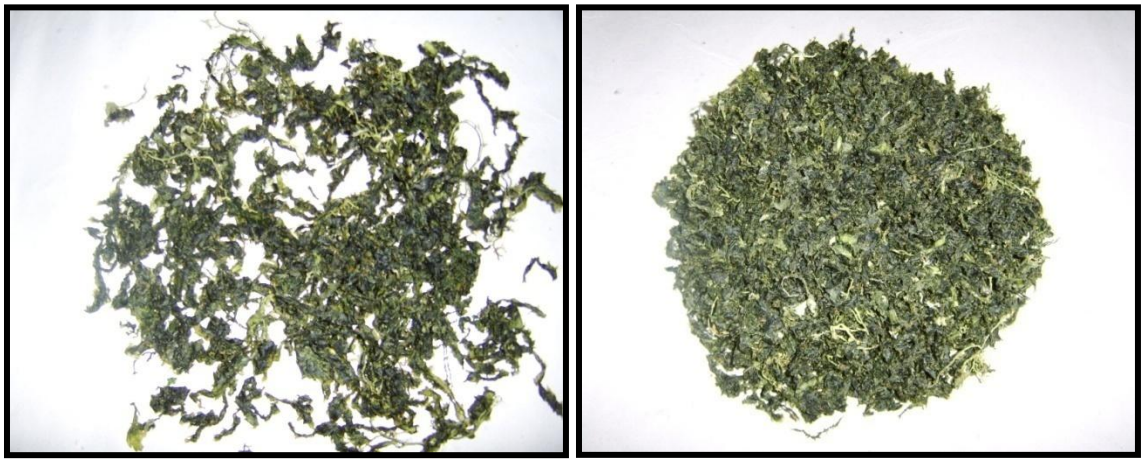
Weight of each *ladoo* : 20 g



(a) **(b)**

Figure 3.5(a, b) Collection of seaweeds

(a) Caulerpa racemosa

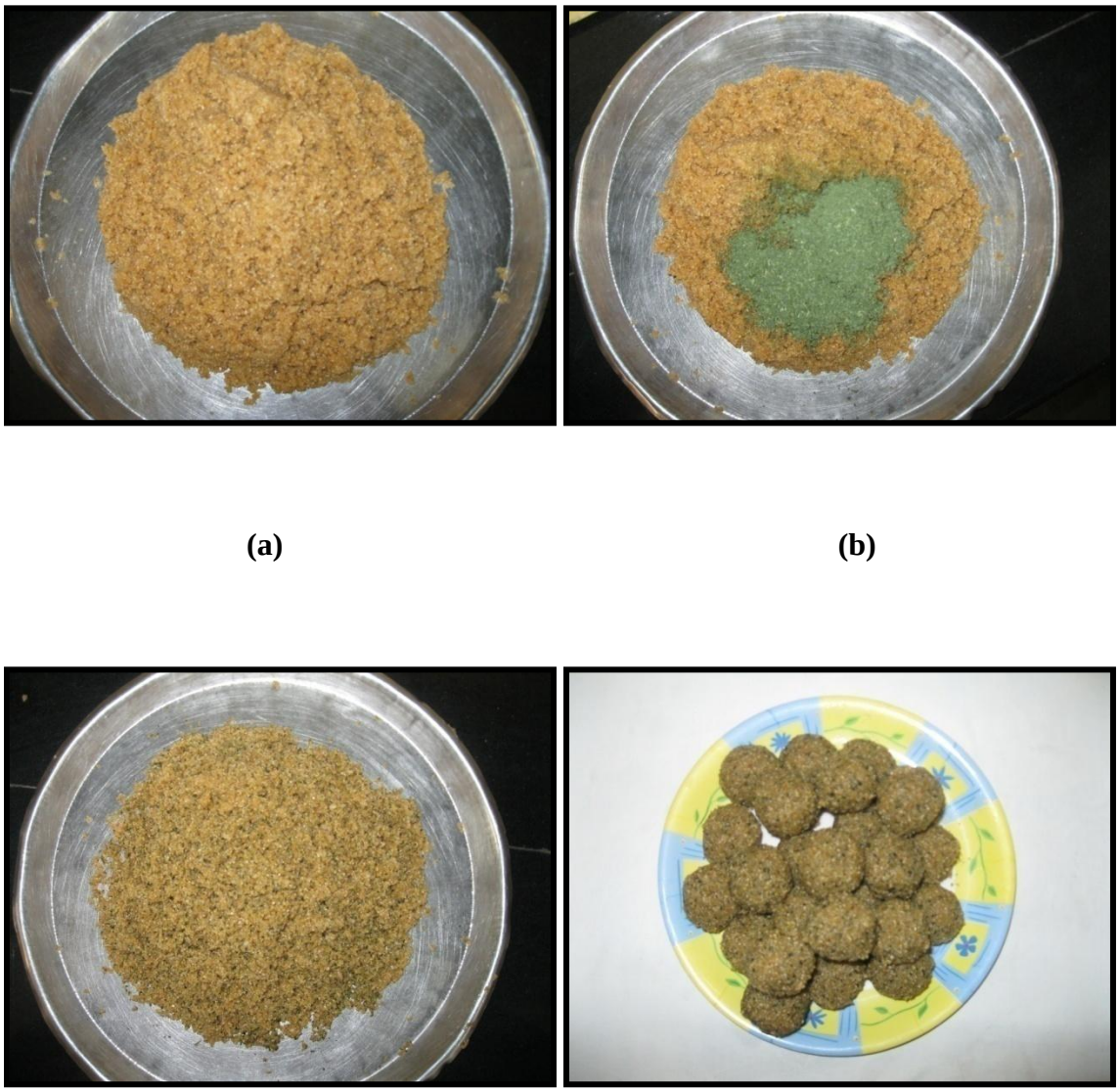


(b) Caulerpa scalpelliformis

(c) Caulerpa veravelense

(d) Padina teratostromatica

Figure 3.6(a, b, c, d) Drying of seaweeds



(a) **(b)**

(c) **(d)**

Figure 3.7(a) Wheat flour mix, (b) Addition of seaweed, (c) Wheat flour and seaweed mix, (d) Prepared wheat flour *ladoos* supplemented with seaweed.

3.2 DIET HISTORY AND KNOWLEDGE, ATTITUDE & PRACTICES (KAP)

The diet history of the subjects was recorded based on food frequency questionnaire and 24- hour dietary recall (Annexure II).

3.2.1 Twenty four (24) hour dietary recall

A single 24- hr recall gathered from a group of individuals can be used to characterize the 'usual' (foods that respondent consumes on a typical day) diet pattern of the population from which they are sampled, since intra- individual variation in diet is unimportant when examining group level dietary patterns. All the subjects were asked to recall all food items and beverages consumed on the previous day. It is used to estimate the food intake of an individual over the period of 24 hours, referring to previous day/ night. This questionnaire was interviewer administered, since it is seen that this method produce better results as the interviewer can probe for forgotten items or common diet patterns.

The 24- hr dietary recall was done for all the subjects to collect information on the intakes of calories, protein and fat. Subjects were asked to provide details of all the major meals consumed throughout the previous day, along with additional beverages, snacks, sweets, pickles, etc. along with added sugar and salt. The subject was probed to remember any forgotten item, and it was made sure that no expression of opinion, feelings or suggestions was made that could lead to respondent's answer. Subjects were shown the set of standardized set of utensils and encouraged to respond to the quantity, number and size of the food item consumed on the basis of the same. The portion size was estimated using volume measures, circular measures and numbers. A set of five vessels (ladle, *katori*, table spoon, tea spoon and glass) was used as an aid to estimate volumes. Items like *chapaties*, *puri* or *paranthas* were estimated using a set of four circular models- 8 cms, 12 cms, 16 cms and 18 cms.

3.2.2 Food Frequency Questionnaire (FFQ)

Details of the number of times the food items consumed on a daily, weekly, monthly or yearly basis was elicited using the FFQ. The FFQ emphasized on consumption of food items rich in iron, vitamin C and goitrogenic foods.

3.2.3 Knowledge, Attitude and Practice (KAP)

Questions regarding knowledge on iodized salt and its importance were asked from all the respondent both before and after providing NHE (Annexure II).

3.3 METHODS FOR ASSESSMENT

3.3.1 Anthropometric Measurements

Anthropometry is the measurement of body dimensions to characterize skeletal and tissue development, and effect relationship between nutrient level and well-being of the body is assessed.

a) ***Weight:*** It is the most widely used and simplest reproducible anthropometric measurement. It indicates the body mass and is a composite of all body constituents like water, minerals, fat, protein, bone etc. (Robinson, *et al.* 1986).

Technique- A platform weighing scale to the nearest 100 gm was used to measure weight. The subject was weighed in standard indoor clothing, bare feet and without leaning against or holding anything. Scale was 'zeroed' before taking any weight, and was calibrated using standard weights after every third subject.

b) ***Height****:* It is a linear measurement made up of the sum of four components i.e. legs, pelvis, spine and skull (Jelliffe 1966).

Technique- A spring- loaded non-stretchable tape was used to measure the height of the subjects. A convenient flat wall was identified at the clinic site for the measurement of height. The subject was made to stand barefoot with the arms hanging freely by the side. Heels of the feet were placed together with the medial (inner) border of the feet at an angle of 60 degrees. The scapula and the buttock were ensured to be in contact with the measuring wall. The head was held in the Frakfort plane (with the tragus of the ear and the lateral angle of the eye in a horizontal line). Height was recorded to the nearest 0.1 cm after the subject inhaled fully and maintained the erect position without altering the load on the heels. In this position, a mark was made on the wall and height was recorded with a measuring tape. Two consecutive reading were taken.

Computed Anthropometric Indices

c) ***Body Mass Index (BMI)*****:** The BMI is computed as follows:

$$BMI = \frac{Weight\ (kg)}{Height\ (m)^2}$$

3.3.2 Blood Pressure Measurement

The diastolic and systolic blood pressures were assessed. Blood pressure is the lateral pressure exerted by blood on vessel walls while flowing in it. Sitting blood pressure of subjects was measured using sphygmomanometer on the right arm.

Technique- Blood pressure measurements were taken after the subject was made to sit down quietly for at least 5 minutes. The bare arm of the subject was supported and positioned at heart level. A cuff of suitable size was evenly applied to the exposed upper arm, with the bladder of the cuff positioned over the brachial artery. The bladder length was at least 80% and width at least 40% of the circumference of the arm.

The cuff was snugly wrapped around the upper arm and inflated to 30 mmHg above the pressure at which the radial pressure disappears. The cuff was deflated at rate greater than 2 mmHg/ beat. If initial readings were high, several further readings were taken after 5 minute of rest. On each occasion two or more readings were averaged.

For diastolic reading, the disappearance of sound was used. Muffing of sound was used if sound continued towards zero (Adams, Burke and Beilin 2002).

3.3.3 Goiter Classification

A thyroid gland whose lateral lobes have a volume greater than the terminal phalanges of the thumbs of the person examined is considered goitrous.

The following stages classify goiter according to the size of the thyroid gland:

Stage 0	No goiter
Stage Ia	Goiter detectable only by palpation and not visible when the neck is fully extended.
Stage Ib	Goiter palpable and visible only when the neck is fully extended; this stage includes nodular glands, even if not goitrous.

Stage II	Goiter visible with the neck in normal position; palpation not needed for diagnosis.
Stage III	Very large goiter that can be recognized at a considerable distance.

The total goiter rate is the prevalence of stages I, II and III; the visible goiter rate is the prevalence of stages II and III.

This classification has been simplified as follows (WHO/ UNICEF/ ICCIDD 1994) and was used in the study.

Grade 0	No palpable or visible goiter
Grade 1	A mass in the neck that is consistent with an enlarged thyroid that is palpable but not visible when the neck is in the neutral position; it also moves upward in the neck as the subject swallows.
Grade 2	A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid when the neck is palpated.

3.3.4 Urinary Iodine Estimation

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

Principle

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

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

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

The ceric ion (Ce^{4+}) has a yellow color, while the cerous (Ce^{3+}) is colorless. The course of reaction can be followed by the disappearance of yellow color as the ceric ion is reduced and can be measured colorimetrically. With other reactants held stable, the speed

of this color disappearance is directly proportional to the amount of iodide catalyzing it. Because of its specificity and high sensitivity, this reaction has been the basis for almost all chemical methods for the detection of iodine in urine.

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

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

a. production of toxic wastes (>5ml/test) from arsenic trioxide in Sandell-Kolthoff reaction.
b. leakage of gas during sample digestion, requiring a special fumehood
c. difficulty in locating chloric acid from chemical vendors because of its instability.

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

Reagents

i. *Standards:* Measure 168.5 mg of KIO_3 and put in 100 ml volumetric flask. Make up the volume to 100 ml by deionized water. Mix it well. This is the stock solution 1.

Take out 100 µL of this stock solution 1 in 2nd 100 ml volumetric flask and make up the volume to 100 ml by deionized water. This is stock solution 2.

For preparation of working standards:

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

ii. *Ammonium persulphate solution:* Measure 5mg of ammonium persulphate and add in 15 ml of deionized water. Mix it properly. It should be prepared fresh.

iii. *Arsenous acid solution (500 ml):* Arsenic trioxide (5 g) was dissolved in 100 ml NaOH solution (3.5 w/v). Keep it in ice bath and add 16 ml of conc. H_2SO_4 slowly. After cooling add 12.5 g NaCl and dilute upto 500 ml with water. Filter and store in amber colored bottle.

iv. *Ceric ammonium sulphate solution (250 ml):* Weigh 3 g of ceric ammonium sulphate and add 250 ml of 3.5 N H_2SO_4. Store the solution in amber color bottle.

Procedure of simple microplate method for determination of urinary iodine

Fifty microliter (µL) each of calibrators (with known concentration of 0, 10, 20, 100, 150, 200, 300 and 400 µg/L) and urine samples are pipetted into the wells of a polypropylene (PP) plate, followed by the addition of 100 µL of 3% ammonium persulphate solution. The PP plate is set in a cassette. The cassette is tightly closed and kept for 60 min in an oven adjusted to 110ºC. After digestion, the bottom of the cassette is cooled to room temperature with tap water to avoid condensation of vapor on the top of wells and to stop the digestion. The cassette is opened and 50µL aliquots of the resulting digests are

transferred to the corresponding wells of a polystyrene 96 well microtiter plate. Arsenious acid solution (100 µL) is added to the wells and mixed; 50µL of ceric ammonium sulphate solution is then added quickly (within 1 min), using a microchannel pipette. The reaction mixture is allowed to sit for 30 min at 25ºC, and the absorbance is measured at 405nm with a ELISA reader.

Table 3.1 Epidemiological criteria for assessing iodine nutrition based on the median or range in urinary iodine concentrations of pregnant women [a] (WHO/ UNICEF/ ICCIDD 2007)

POPULATION GROUP	MEDIAN URINARY IODINE CONCENTRATION (µg/L)	IODINE INTAKE
Pregnant women	<150	Insufficient
	150-249	Adequate
	250-499	Above requirements
	≥500	Excessive [b]

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

[b] the term "excessive" means in excess of the amount required to prevent and control iodine deficiency.

3.3.5 Determination of Hemoglobin Concentration

Hemoglobin was assessed by Cyanmethemoglobin method (Cook 1985)**.**

Principle

When blood is mixed with Drabkin's reagent containing potassium cyanide and potassium ferricyanide, hemoglobin reacts with ferricyanide to form methemoglobin

which is converted to stable cyanmethemoglobin by the cyanide. The intensity of the color is proportional to hemoglobin concentration and it is compared with a known cyanmethemoglobin standard at 540 nm (green filter).

Requirement

1. Drabkin's reagent: It contains in 1000 ml of distilled water
    a. Potassium ferricyanide: 400 mg
    b. Potassium dihydrogen phosphate: 280 mg
    c. Potassium cyanide: 100 mg
    d. Ninidet: 1 ml
2. Cyanmethemoglobin standard: It is commercially available. This standard is directly pipetted in a cuvette and optical density is measured at 540 nm. The reading obtained, corresponds to 15 g/dl, hemoglobin.
3. Hb- pipette (20µl calibrated)
4. Test tubes (15 x 125 mm)
5. Photometer or spectrometer.

Procedure

1. Five ml of Drabkin's reagent was pipette in 2 tubes labeled as 'test' and 'blank'.
2. Blood sample (0.02 ml) was added in tube labeled 'test'. Content was thoroughly mixed and wait for 5 minutes.
3. Absorbance of test was read at 540 nm by setting blank to 100% T.
4. Absorbance of standard was read by pipetting directly in a cuvette.

Calculation

$$Haemoglobin\ (g/dl) = \frac{O.D.of\ test}{O.D.of\ standard} \times 15$$

Preparation of standard graphs

Pipette in the tubes, labeled as follows:

		Std. 5	Std. 10	Std. 15	Blank
1.	Drabkin's reagent	3.34	1.67	0.0	5.0
2.	Hb standard, ml	1.66	3.33	5.0	0.0

- Mix well and intensities of these standards is read by setting blank to 100 % T and 540 nm.
- Graph is plotted with O.D. reading on Y-axis and concentration of hemoglobin standards i.e. 5, 10and 15on X-axis.

Table 3.2 Hemoglobin cutoffs used to define anemia in people living at sea level

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

Source: WHO/ UNICEF/ UNU 2001

3.3.6 Determination of Thyroid Hormones

FT_3, FT_4, TSH and thyroperoxidase antibody (TPO-Ab/ anti-TPO) were estimated by the electrochemiluminescence (ECL) technique using commercially available kits from Roche Diagnostics (Mannheim, Germany) with Elecsys 1010 analyzer. The non-pregnant reference ranges for thyroid parameters is given in table 3.3.

Table 3.3 Laboratory specific normal ranges of thyroid parameters

Thyroid parameters	Reference ranges
FT_3 (pM/L)	2.8 – 7.1
FT_4 (pM/L)	12 – 22
TSH (µIU/ml)	0.27 – 4.2
TPO-Ab	>35 (positive)

ECL Assay Principles (Mathew BC 2005)

Electrochemiluminescence (ECL) processes are known to occur with numerous molecules including compounds of ruthenium, osmium, rhenium or other elements. ECL is a process in which highly reactive species are generated from stable precursors at the surface of an electrode. These highly reactive species react with one another producing light. The development of ECL immunoassays is based on the use of a ruthenium chelate as the complex for the development of light. The chemiluminescent reactions that lead to the emission of light from the ruthenium complex are initiated electrically rather than chemically. This is achieved by applying a voltage to the immunological complexes (including the ruthenium complex) that are attached to Streptavidin – coated micro particles. Streptavidin, isolated from Streptomyces avidinii is preferred to avidin in this biotin- mediated immunoassay since it has an affinity for biotin comparable to that of avidin, is less basic and had no carbohydrate residues, thus limiting non - specific reactions with acidic groups and lectins. The advantage of electrically initiating the chemiluminescent reaction is that the entire reaction can be precisely controlled.

The key elements and substances in the ECL process are:

- Measuring cell
- Voltage
- Platinum electrode
- Magnet
- Photomultiplier
- Antigen/ Antibody
- Biotin
- Paramagnetic microbeads coated with streptavidin
- ProCell solution (TPA-Tripropylamine with a phosphate buffer)
- CleanCell solution (KOH cleaning solution)

The Basic Principle

The core of the detection unit is the flow through ECL measuring cell.

Three processes steps are performed in the cell:

1. Bound/ free separation

- Streptavidin microbeads coated with antigen-antibody complex are aspirated.
- The magnet is activated.
- The antigen-antibody complex is captured by the magnet onto the working electrode.
- A TPA solution (ProCell) is aspirated to wash the microbeads on the working electrode.
- TPA (ProCell) is used to flush out the excess reagent and sample material.

2. ECL reaction
    - The ruthenium complex and TPA (ProCell) are involved in the reactions.
    - They remain stable as long as no voltage is applied.
    - Voltage is applied between the working and counting electrodes and an electrical field is created.
    - An ECL reaction of ruthenium-tris (bipyridyl)$^{2+}$ and TPA occurs on the surface of the electrode.
    - TPA is oxidized at the electrode.
    - This releases an electron and forms a TPA radical-cation.
    - This reacts by releasing a proton (H+) to form a TPA radical (TPA*).
    - The ruthenium complex also releases an electron.
    - The ruthenium complex then oxidizes to form the ruthenium cation Ru(bpy)33+ followed by the chemiluminescent reaction with the TPA radical.
    - The ECL reaction is initiated.
    - Peak light emission occurs for a short time interval (0.20-0.60 sec).
    - A photomultiplier detects and converts the ECL signal into an electric signal.
    - The corresponding signals are used for the calculation of results.
    - Each measuring cycle requires a total of 42 seconds for the following two stages:

      Preconditioning: 2 seconds (approximately)

      Bead capturing/ BF separation: 22 seconds (approximately)

The magnet is deactivated before the measurement is started, to avoid any interference.

Measuring: ~ 2 seconds

Cleaning: ~14 seconds

Re-conditioning: ~2 seconds

3. Release of microbeads and cell cleaning
    - Cleaning solution is aspirated to the measuring cell.
    - Microbeads are washed away from the electrode.
    - TPA (ProCell) is aspirated.
    - The surface of the measuring cell is regenerated by varying the electrode voltage.
    - The measuring cell is ready for another measurement.

Test Principles

Three test principles are used for the estimation of analytes and antibodies in the samples:

1. *Competitive principle*

    This principle is applied to analytes of low molecular weight such FT_3, FT_4, Cortisol, Testosterone and Estradiol. This type of assay is based on the competition between the analyte of interest and an enzyme- conjugated version of the same analyte for a limited number of specific antibody binding sites.

    The measurement is indirectly proportional to the sample concentration.

    High signal = low concentration

    Low signal = high concentration

2. *Sandwich principle*

    This priniciple is applied to high molecular weight antigens such as Thyroid stimulating hormone (TSH), Follicle stimulating hormone (FSH), Luteinizing hormone (LH). This assay involves two antibodies which “sandwich” the analyte between them.

The measurement is directly proportional to the sample concentration.

Low signal = low concentration

High signal = high concentration

3. *Bridging principle*

This principle is similar to the sandwich principle, except that the assay is designed to detect antibodies, not antigens in the sample (e.g. IgG, IgM and IgA). This is accomplished by including biotinylated and ruthenium- labeled antigens in the reagents for which the targeted antibody has the affinity.

The measurement is directly proportional to the sample concentration.

Low signal = low concentration

High signal = high concentration

3.3.6.1 Estimation of free triiodothyronine (FT_3)

Test Principle: Competition principle

Total duration of assay: 18 minutes

Procedure

- 1st incubation: 15 µL of sample and an anti-T_3-specific antibody labeled with a ruthenium complex are combined in an assay cup.
- 2^{nd} incubation: After addition of biotinylated T_3 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex is bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell.
- In the ECL reaction, the conjugate is a ruthenium- based derivative and the chemiluminescent reaction is electrically stimulated to produce light which is measured by a photomultiplier.

- The amount of light produced is indirectly proportional to the amount of antigen in the patient sample.
- The concentration of the antigen is evaluated and calculated by means of a calibration curve that was established using standards of known antigen concentration.

3.3.6.2 Estimation of free thyroxine (FT_4)

Test Principle: Competition principle

Total duration of assay: 18 minutes

Procedure

- 1st incubation: 15 µL of sample and a T_4-specific antibody labeled with a ruthenium complex.
- 2^{nd} incubation: After addition of biotinylated T_4 and streptavidin-coated microparticles, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex is bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration curve and a master curve provided via the reagent barcode.

3.3.6.3 Estimation of TSH

Principle: Sandwich principle

Total duration of assay: 18 minutes

Procedure

- 1st incubation (9 minutes): 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation (9 minutes): In this step, streptavidin- coated paramagnetic microbeads are added. During this nine minute incubation, the biotinylated antibody attaches to the streptavidin- coated surface of the microbeads via interaction of biotin and streptavidin.
- After the second incubation, the reaction mixture containing the immune complexes is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the working electrode. Unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is ruthenium- based derivative and the chemiluminescent reaction is electrically stimulated to produce light which is measured by a photomultiplier.
- The amount of light produced is directly proportional to the amount of TSH in the sample.
- Evaluation and calculation of concentration of the antigen or analyte are performed by means of a calibration curve that was established using standards of known antigen concentration.

3.3.6.4 Estimation of TPO-Ab

Test Principle: Competition principle

Total duration of assay: 18 minutes

Procedure

- 1st incubation: 20 µL of sample are incubated with anti-TPO-antibodies labeled with a ruthenium complex.

- 2nd incubation: After addition of biotinylated TPO and streptavidin-coated microparticles, the anti-TPO antibodies in the sample compete with the ruthenium-labeled anti-TPO antibodies for the biotinylated TPO antigen. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration curve and a master curve provided via the reagent barcode.

3.3.7 Chemical Analysis of Algae

Chemical analysis of the algae was carried out using standard methods Association of Official Analytical Chemists (AOAC) 2000. The estimation was carried out at National Institute of Nutrition (NIN), Hyderabad.

3.3.7.1 Estimation of Moisture (A.O.A.C. method 976.05)

- Moisture dishes were dried in an oven at 105°C for 30 minutes, allowed to cool in a dessicator and was weighed.
- Two gram of sample was placed in these dishes and kept in an oven at 130 °C for 2 hours until constant weight was obtained.
- It was allowed to cool in dessicator and was weighed.
- Loss in weight of the sample was taken as moisture and percentage of moisture was calculated.

$$Moisture\ \% = \frac{(W2 - W3)}{(W2 - W1)} \times 100$$

W1 = Weight of the empty aluminium dish

W2 = Weight of aluminium dish + Sample before drying

W3 = Weight of aluminium dish + Sample after drying

3.3.7.2 Ash Estimation (AOAC method 923.03)

- Set the temperature of muffle furnace at 600°C and place clean crucibles in the muffle furnace for one hour. Transfer them into a dessicator and cool them to a room temperature, then weigh (W_1) each crucible.
- Place about 2 gram of moisture free algae samples into a weighed crucible and weigh (W_2).
- Incinerate the sample at 600°C for about 2 hours.
- Transfer the crucibles into a dessicator and cool to room temperature and then weigh (W_3). The mass should be taken as quickly as possible to prevent moisture absorption.
- Repeat the incineration until a constant mass is obtained.

$$Ash\ \% = \frac{Mass\ of\ ash\ (W3 - W1)}{Mass\ of\ sample\ (W2 - W1)} \times 100$$

3.3.7.3 Protein Estimation (AOAC method 976.05)

Organic nitrogen was determined by Kjeldhal method and multiplied by the factor of 6.25 to estimate the protein content.

Principle: The estimation of nitrogen was done by Kjeldhal method, which is based on the principle that organic nitrogen when digested with sulphuric acid in the presence of a catalyst (selenium oxide, mercury or copper sulphate) is converted into ammonium sulphate. Ammonia liberated by making the solution alkaline is distilled into a known volume of a standard acid, which is then back- titrated. The protein is obtained by multiplying the nitrogen value with 6.25.

Reagents

a. Digestion moisture: 98 parts K_2SO_4 + 2 parts $CuSO_4$.
b. 40% NaOH

c. N/10 NaOH
d. N/10 H_2SO_4
e. Methyl red indicator: 0.1 g of the indicator dissolved in 60 ml of alcohol and water added to make to 100 ml.

Procedure

The sample (1 g) was weighed into dry Kjeldhal flask. About 5 g digestion mixture and 20 ml of pure conc. H_2SO_4 were added to the sample and the mixture was digested by heating for 4 to 5 hr. Glass beads were added to prevent bumping. After the content of the flask became clear, the digestion was continued for 1 hr. the contents of Kjeldhal flask were cooled, diluted with distilled water and the mixture made alkaline by adding excess of 40% NaOH (75 ml). A small quantity of pumice powder was added to prevent bumping during distillation. The ammonia liberated was distilled into a receiver containing 25 ml of N/10 H_2SO_4. The excess of acid in the receiver was back titrated against N/10 NaOH using 3 drops of methyl red indicator. A reagent blank was similarly digested and distilled. The titre value was subtracted from the value obtained for the sample to get the true titre value 'b'.

Calculation

If 'a' g of the sample is taken and if 'b' and 'c' ml of alkali of normality'd' were required for back titration and to neutralize 25 ml of N/10 H_2SO_4 respectively, then protein content per 100 g of sample is

$$Protein\ content = \frac{(c-b) \times 14\ d\ \times 6.25}{a\ \times 1000} \times 100$$

3.3.7.4 Total Lipids Estimation (AOAC method 923.07)

Fat was estimated as crude ether extract of the dry material. The dry sample (10 g) was weighed accurately into a thimble and plugged with cotton. The thimble is then placed in the Soxhlet apparatus and extracted with anhydrous ether for about 16 hr. The ether extract is filtered into a weighed conical flask. The flask containing the ether extract was washed 4 to 5 times with small quantities of ether and the washings were also transferred.

The ether was then removed by evaporation and the flask with the residue dried in an oven at 100°C, cooled in a dessicator and weighed.

3.3.7.5 Carbohydrate Estimation (by difference)

The carbohydrate was calculated from the equation:

% of protein + % of fat + % of carbohydrate = 100%

3.3.7.6 Iron (AOAC method 999.11)

Principle

Test portions are dried and then ashed at 450°C under a gradual increase (≤50°C/h) in temperature. 6M HCl (1+1) is added, and the solution is evaporated to dryness. The residue is dissolved in 0.1M HNO_3, and the analytes are determined by flame and graphite furnace procedures.

Apparatus

(a) Flame Atomic absorption spectrophotometer- With an air–acetylene burner or nitrous oxide–acetylene burner.

(b) Lamps- Hollow cathode or electrodeless discharge lamps for all elements determined.

(c) Furnace- Programmable, or muffle furnace with thermostat maintaining 450° ± 25°C. If muffle furnace is used, a separate pre-ashing device is required. See (d)–(h).

(d) Hot plate- With heating control, to heat up to about 300°C.

(e) Lamp- IR 250 W, fixed to a retort stand in a way that allows adjustment of the distance to the hot plate.

(f) Ceramic plate- e.g., Dessicator plate on a low stand, with a diameter that suits the hot plate.

(g) Glass cover- e.g., Crystallizing dish, 185 mm diameter, 100 mm height, to fit on (f) or equivalent.

(h) Wash-bottle- "Scrubber," containing H_2SO_4 for purification of air.

(i) Quartz or platinum crucibles- 50–75 ml.

(j) Polystyrene bottles- With leak-proof closures, 100 ml.

Carefully clean and rinse all glass ware and plasticware with HNO_3 or HCl to avoid metal contamination.

(k) Cleaning procedure for glass and plasticware-

Acid solution: 500 ml concentrated HNO_3 + 4500 ml deionized water.

Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with dilute acid. Finally rinse 4-5 times with deionized water.

Reagents

Reagents should be at least analytical reagent grade (p.a.), preferably ultrapure (suprapure), or equivalent.

(a) Water- Redistilled or deionized, resistivity $\geq$ 18 MΩcm.

(b) Hydrochloric acid- 6M. Dilute 500 ml HCl (37%, w/w) with water to 1 L.

(c) Nitric acid- 65% (w/w).

(d) Nitric acid- 0.1M. Dilute 7 ml HNO_3, (c), with water, (a), to 1 L.

(e) Iron standard solution- 1 mg/ml. Dissolve 1 g Fe in 14 ml water + 7 ml nitric acid in 1 L volumetric flask. Dilute to volume with water.

(j) Working standard solution- Dilute standard (e), with 0.1M HNO_3, (d), to a range of standards that covers the concentration of the element to be determined.

Procedure

(a) Pre treatment- Homogenize product if necessary, using noncontaminating equipment. Check for leaching metals if the apparatus consists of metal parts.

(b) Drying- In a crucible, weigh 10–20 g test portion to nearest 0.01 g. Dry in a drying oven, on a water-bath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace.

(c) Ashing- (i) Ashing in a programmable furnace: Place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of

50°C/h to 450°C. Let dish stand for at least 8 h or overnight. Continue according to (d).

(ii) Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described above in apparatus (d)–(h).

Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp down at the cover. Pre-ash product by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200°–250°C and slowly raise temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool.

(d) Solution- Wet ash with 1–3 ml water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50°–100°C/h) to 450°C. Proceed with ashing at 450°C for 1–2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product. Add 5 ml 6M HCl, to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0–30.0 ml, to the nearest 0.1 ml, of 0.1M HNO_3. Swirl crucible with care so that all ash comes into contact with acid. Cover with watch glass and let stand for 1–2 h. Then stir solution in crucible thoroughly with stirring rod and transfer contents to plastic bottle. Treat blanks in the same way as products. Include 2 blanks with each analytical batch.

(e) Atomic absorption spectrophotometry- Iron determined by flame AAS.

Flame technique- Prepare calibration curves from a minimum of 3 standards.

Calculations and Evaluation of Results

Detection limit- Calculate the detection limit, DL as

DL = 3 * standard deviation of the mean of the blank determinations ($n \geq 20$)

Calculate the concentration, c, of metal in the test sample according to the formula:

$$c = \frac{[(a - b) \times V]}{m}$$

where, c = concentration in the test sample (mg/kg);

a = concentration in the test solutions (mg/L);

b = mean concentration in the blank solutions (mg/L);

V = volume of the test solution (ml);

m = weight of the test portion (g).

If (a – b) is lower than the DL, then (a – b) is substituted with DL for calculation of the limit of detection in the test portion.

If test solution has been diluted, dilution factor has to be taken into account. When running replicates, the average of the results should be given with 2 significant figures.

3.3.7.7 Iodine

- The iodine content was estimated by the alcoholic potash method (MB 1958).The algal powder (5 to 10 g) was refluxed for 24 hours with 30 ml absolute alcohol and 10 g potassium hydroxide.
- The dried mass was ashed at 450-500°C for about 4 hours.
- The hot water extract of the ash was made up to 100 ml.
- About 25 ml of the solution obtained was acidified to the acid point of methylred, oxidized with bromine water and the excess bromine was boiled off.
- Potassium iodide (1 g) was then added and the liberated iodine was titrated with 0.001 N sodium thiosulphate using starch as indicator.

3.4 LEVOTHYROXINE (L-T4) DOSE ADJUSTMENT

Dose adjustment of the subjects on levothyroxine (L-T4) was done and delta value (Δ%) is calculated using following formula.

$$\Delta\% = \left(\frac{L - T4\ final\ dose}{L - T4\ dose\ before\ pregnancy} \times 100\right) - 100$$

3.5 NUTRITIONAL STATUS OF NEONATES

Three standard indices of physical growth that describe the nutritional status of children were used.

1. Height-for-age (stunting)
2. Weight-for-height (wasting)
3. Weight-for-age (underweight)

Each of the three nutritional status indicators was expressed in standard deviation units (Z-scores) from the median of the reference population. Each index provides different information about growth and body composition, which is used to assess nutritional status. It was calculated using WHO Anthro for personal computers, version 3.1 2010.

The height-for-age index is an indicator of linear growth retardation and cumulative growth deficits. Children whose height-for-age Z-score is below minus two standard deviations (-2 SD) from the median of the reference population are considered short for their age (stunted) and are chronically malnourished. Children below minus three standard deviations (-3 SD) from the median of the reference population are considered to be severely stunted. Stunting reflects failure to receive adequate nutrition over a long period of time and is also affected by recurrent and chronic illness. Height-for-age, therefore, represents the long-term effects of malnutrition in a population and does not vary according to recent dietary intake.

The weight-for-height index measures body mass in relation to body length and describes current nutritional status. Children whose Z-score is below minus two standard deviations (-2 SD) from the median of the reference population are considered thin (wasted) for their height and are acutely malnourished. Wasting represents the failure to receive adequate nutrition in the period immediately preceding the survey and may be the result of inadequate food intake or a recent episode of illness causing loss of weight and the onset of malnutrition. Children whose weight-for-height is below minus three standard deviations (-3 SD) from the median of the reference population are considered to be severely wasted.

Weight-for-age is a composite index of height-for-age and weight-for-height. It takes into account both acute and chronic malnutrition. Children whose weight-for-age is below minus two standard deviations from the median of the reference population are classified as underweight. Children whose weight-for-age is below minus three standard deviations (-3 SD) from the median of the reference population are considered to be severely underweight.

Table 3.4 Cut-off points used in classifying nutritional status of children based on WHO reference standards for growth (World Health Organization Multicenter Growth Reference Study Group 2006)

Classification	Cut-off points
Weight-for-age	
Underweight	<-2SD
Normal	-2SD to +2SD
Overweight	>+2SD
Height-for-age	
Underheight or short	<-2SD
Normal	-2SD to +2SD
Above average/ Tall	>+2SD
Weight-for-height	
Thin	<-2SD
Normal	-2SD to +2SD
Overweight	>+2SD

3.6 STATISTICAL ANALYSIS

The Statistics Package for Social Sciences for Windows version 14.0 (SPSS Inc., IL, USA) was used for data processing and analysis. Simple descriptive analysis of the data was carried out. Statistical analysis was performed using Chi-square (χ^2) when appropriate for categorical data.

To check the normality of data, the Kolmogorov-Smirnov test was used. Where indicated, the data was normalized using log transformation to facilitate the use of normal-theory analytic methods and summarized as geometric mean.

Nonparametric (Mann-Whitney U test and Kruskal-Wallis test) or parametric (student's t-test and ANOVA) statistical tests, depending on the normality of the data, were used to detect within- group and between- group differences. Further post-hoc Scheffe analysis was done. To determine associations between analytes Pearson's correlation or Spearman's rank correlation were calculated.

A two-tailed p value <0.05 was considered statistically significant.

Results and Discussion

4. RESULTS AND DISCUSSION

In this chapter the results are presented and discussed under the following sub headings:

4.1 Classification of the study subjects into various groups

4.1.1 General profile of the study groups

4.1.2 Medical study of the study groups

4.2 General with non-specific thyroid status group (Group I)

4.2.1 Normal subjects (Group I A) with complete three trimesters data

4.2.1.1 Baseline characteristics

4.2.1.2 Hemoglobin profile

4.2.1.3 Urinary iodine status

4.2.1.4 Thyroid function parameters

4.2.1.5 Pregnancy outcome of Group I A subjects

4.2.2 Medicine started (Group I B) with complete three trimesters data

4.2.2.1 Baseline characteristics

4.2.2.2 Hemoglobin profile

4.2.2.3 Urinary iodine status

4.2.2.4 Thyroid function parameters

4.2.2.5 Levothyroxine treatment

4.2.2.6 Pregnancy outcome of Group I B subjects

4.3 Subjects already on hormone replacement therapy (Group II)

4.3.1 Hypothyroidic subjects (Group II A)

4.3.1.1 Baseline characteristics

4.3.1.2 Groups formed according to dose

4.3.1.3 Adjustment of levothyroxine dose

4.3.1.4 Urinary iodine status

4.3.1.5 Pregnancy outcome of Group II A subjects

4.3.2 Hyperthyroidic subjects (Group II B)

4.3.2.1 Disease history

4.3.2.2 Urinary iodine status

4.3.2.3 Pregnancy outcome of Group II B subjects

4.4 Seaweed group (Group III)

4.4.1 Baseline characteristics

4.4.2 Proximate composition and mineral content of seaweed

4.4.3 Urinary iodine status

4.4.4 Thyroid function parameters of seaweed supplemented *vs.* non-supplemented subgroup

4.4.5 Hemoglobin profile of supplemented *vs.* non-supplemented subgroup

4.5 Non-pregnant control group (Group IV)

4.5.1 Baseline characteristic

4.5.2 Hemoglobin profile

4.5.3 Urinary iodine status

4.5.4 Thyroid function parameters

4.6 Establishment of thyroid hormones trimester- specific reference intervals

4.7 Prevalence of thyroid dysfunction in Group I subjects based on different methods

4.8 Nutritional status of the study subjects

4.8.1 Nutrient intake of the respondents

4.8.2 Food frequency

4.9 Knowledge, attitude and practices regarding iodized salt

4.10 General discussion

4.1 CLASSIFICATION OF THE STUDY SUBJECTS INTO VARIOUS GROUPS

Total of three hundred and thirty four pregnant women in the first trimester of pregnancy between 18- 45 year of age with and without thyroid dysfunction were enrolled for the study from various hospitals of Delhi.

Subjects in the study groups were again divided into subgroups according to the thyroid status (Table 4.1). The subjects with non-specific thyroid status (Group I) were subdivided into normal (Group I A) and medicine started during the course of pregnancy (Group I B).

Subjects who were already on hormone replacement therapy (Group II) were categorized into hypothyroidic (Group II A) and hyperthyroidic (Group II B).

The subjects in both the groups were serially followed throughout pregnancy and up to six months postpartum. However, there were drop outs in the study due to abortion, miscarriage or unwillingness to participate in the study. Only 92 women in Group I and one hundred and two women in Group II regularly visited in all the three trimesters of the pregnancy (Fig. 4.1).

Thirty pregnant women in any trimester were enrolled from five *anganwadis* of Vadodara (Group III). These women were followed for a period of one month.

One hundred and fifty non-pregnant women (Group IV) in the same age group were also recruited for the study to compare the difference in thyroid function between pregnant and non-pregnant women.

Table 4.1 Various groups formed during study

GROUPS	NUMBER (N)	COMPLETE THREE TRIMESTERS DATA
I General with non-specific thyroid status	189	92
I A: Normal	153	68
I B: Medicine started during pregnancy	36	24
II Already on Hormone replacement therapy	145	102
II A: Hypothyroidic	135	92
II B: Hyperthyroidic	10	10
III Seaweed Study	30	-
III A: Normal	20	-
III B:Seaweed supplemented	10	-
IV Non-pregnant control	150	-

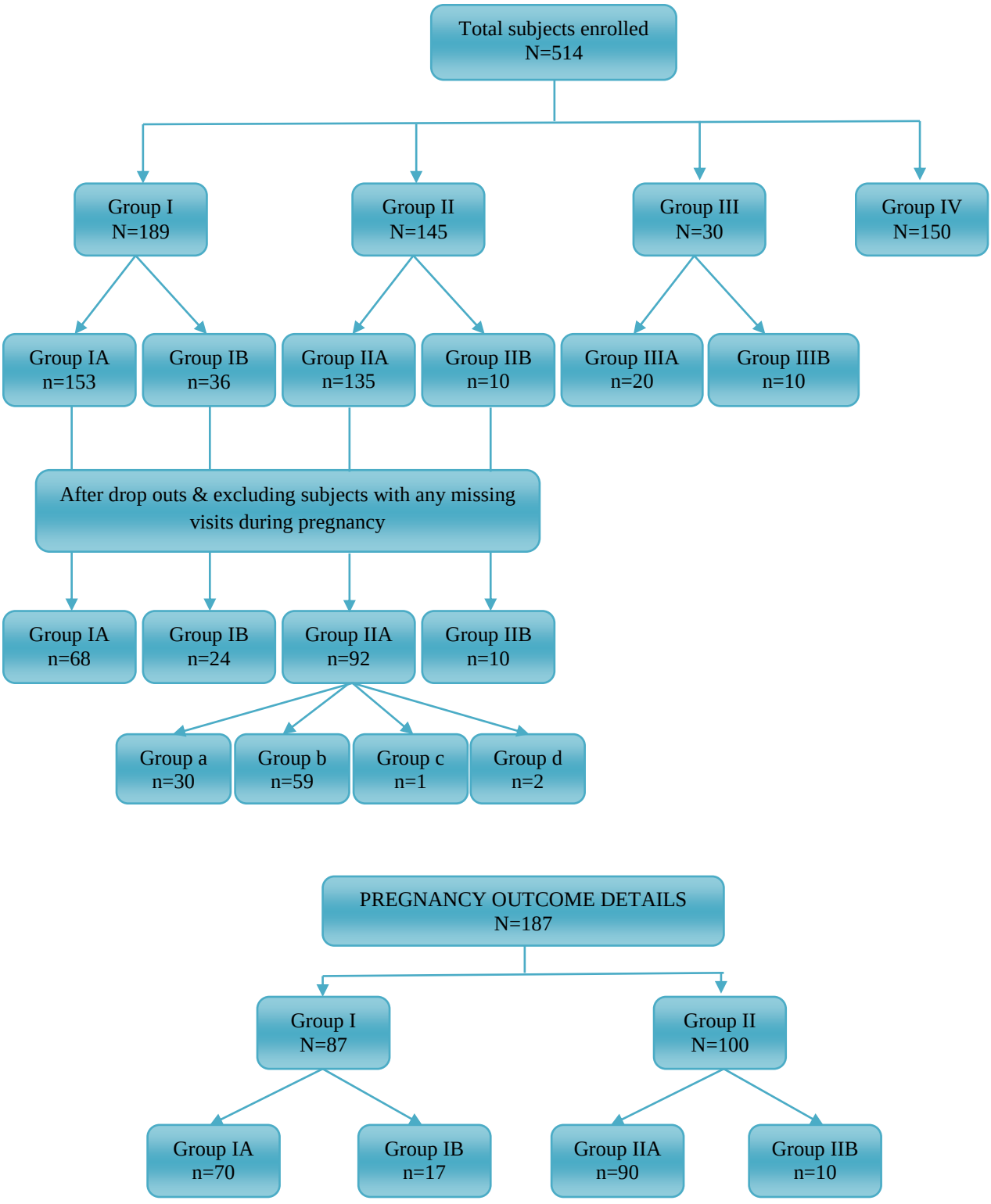


Figure 4.1 Flowchart depicting the groups and subgroups and pregnancy outcome details of the study subjects

4.1.1 General profile of the study groups

Women in almost all the groups belonged to urban area and majority of them were Hindus. Most of the subjects had attained primary education. Approximately 40 percent of the subjects had joint family. The monthly family income of majority of subjects in all the groups ranged between <5000 to 10000 (Table 4.2).

Table 4.2 General profile of the study subjects in various groups

	Group I		Group II		Group III	Group IV
	I A	I B	II A	II B		
Area of residence						
Urban	153 (100)	36 (100)	132 (98.5)	10 (100)	30 (100)	150 (100)
Rural	-	-	2 (1.5)	-	-	-
Religion						
Hindu	136 (88.9)	32 (88.9)	116 (86.6)	10 (100)	22 (73.3)	141 (94)
Muslim	12 (7.8)	4 (11.1)	12 (9)	-	8 (26.7)	6 (4)
Sikh	5 (3.3)	-	5 (3.7)	-	-	3 (2)
Christian	-	-	1 (0.7)	-	-	-
Education						
Illiterate	28 (18.3)	1 (2.8)	1 (0.7)	-	11 (36.7)	13 (8.7)
Primary education	63 (41.2)	12 (33.3)	27 (20.1)	4 (40)	16 (53.3)	32 (21.3)
High school	30 (19.6)	9 (25)	23 (17.2)	3 (30)	3 (10)	57 (38)
Intermediate	19 (12.4)	4 (11.1)	26 (19.4)	1 (10)	-	37 (24.7)
Graduate	10 (6.5)	5 (13.9)	46 (34.3)	1 (10)	-	11 (7.3)
Postgraduate	3 (2)	5 (13.9)	11 (8.2)	1 (10)	-	-
Type of family						
Nuclear	100 (65.4)	23 (63.9)	59 (44)	7 (70)	16 (53.3)	97 (64.7)
Joint	53 (34.6)	13 (36.1)	75 (56)	3 (30)	14 (46.7)	53 (35.3)
Monthly family income (Rs.)						
<5000	82 (53.6)	14 (38.9)	17 (12.7)	-	17 (56.7)	48 (32)
5000-10000	65 (42.5)	12 (33.3)	45 (33.6)	7 (70)	13 (43.3)	85 (56.7)
10000-15000	3 (2)	4 (11.1)	31 (23.1)	1 (10)	-	17 (11.3)
15000-20000	1 (0.7)	3 (8.3)	15 (11.2)	1 (10)	-	-
>20000	2 (1.3)	3 (8.3)	26 (19.4)	1 (10)	-	-

Value in parenthesis indicate percentage

4.1.2 Medical history of the study subjects in various groups

Around 60 percent of the subjects in Group I was primiparous whereas 70 percent of the subjects in group II and III were multiparous (Fig. 4.2). History of repeated abortions, known complications like diabetes and hypertension, autoimmune disorder and family history of thyroid disorder was present more in group II subjects (Table 4.3).

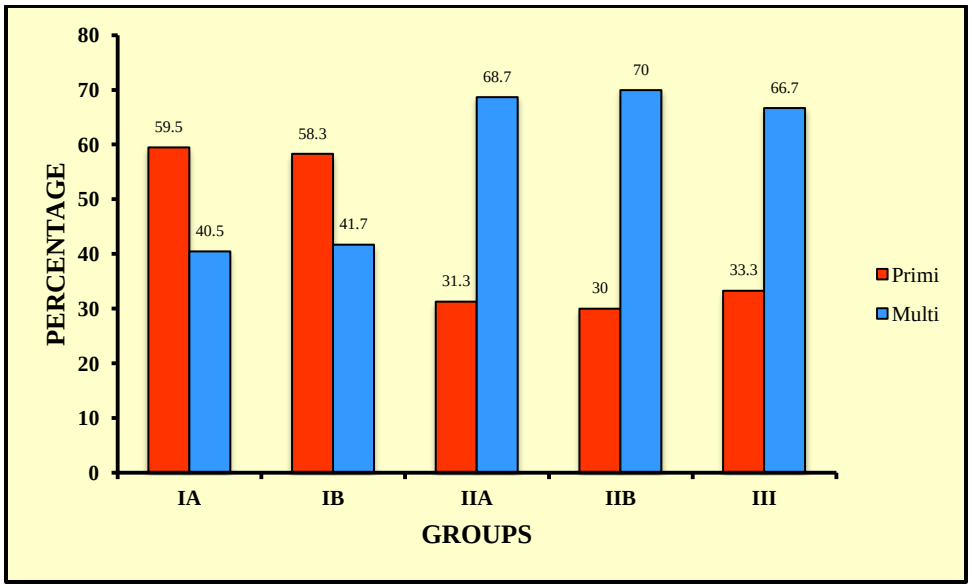


Figure 4.2 Group-wise distribution of study subjects according to parity

Table 4.3 Past histories of the study subjects

Past Histories	Group I		Group II		Group III
	I A	I B	II A	II B	
Repeated abortions	35 (22.9)	14 (38.9)	62 (45.9)	2 (20)	4 (13.3)
Any known complication	8 (5.2)	2 (5.6)	10 (7.4)	2 (20)	-
Autoimmune disorder	-	-	85 (63)	6 (60)	-
Thyroid disease family history	9 (5.9)	2 (5.6)	49 (36.3)	-	-

Value in parenthesis indicate percentage

4.2 GROUP I: GENERAL WITH NON- SPECIFIC THYROID STATUS GROUP

The subjects in this group were categorized into two subgroups. The baseline characteristics of both the groups was almost similar but the mean abortion rate was little higher in the group whose medicine had to be started (Group I B) during pregnancy (Table 4.4).

Table 4.4 Baseline characteristics of the group I

Characteristic	Group I A (n=153)	Group I B (n=36)
Age (yrs)	24.53 ± 3.54	25.81 ± 4.36
Height (cms)	152.91 ± 5.65	154.43 ± 5.9
Parity	0.55 ± 0.79	0.64 ± 0.96
Abortion	0.27 ± 0.59	0.44 ± 0.69

Overall 24.18 percent and 30.56 percent women in Group IA and Group IB, respectively, were having body mass index (BMI) <18.5 kg/m^2. Majority of women around 60 percent in both the groups were having BMI in the range of 18.5 – 25.0 kg/m^2 (Table 4.5).

Table 4.5 Distribution of Group I A and Group I B pregnant women in relation to BMI at the time of registration

Gestational age	GROUP I A Level of BMI (kg/m^2)				GROUP I B Level of BMI (kg/m^2)			
	< 18.5	18.5-25	>25	Mean	< 18.5	18.5-25	>25	Mean
< 1 month	1 (50)	1 (50)	-	20.41 ± 3.5	-	-	-	-
1-2 month	3 (10)	18 (60)	9 (30)	23.66 ± 5.1	2 (11.8)	10 (58.8)	5 (29.4)	22.87 ± 4.6
2-3 month	31 (25.6)	70 (57.9)	20 (16.5)	21.44 ± 4.4	9 (47.4)	9 (47.4)	1 (5.2)	21.2 ± 4.7

Value in parenthesis indicate percentage

The average weight gain of pregnant women throughout pregnancy was below the recommended weight gain (Institute of Medicine 2009) for subjects who had low and normal BMI at the time of registration (Table 4.6).

Table 4.6 Average weight gain of pregnant women in group I A & I B throughout pregnancy

BMI at the time of registration	Group I A	Group I B	Recommended weight gain
Low (< 18.5)	8.73 ± 1.58	7.64 ± 2.58	12.5 - 18 kg
Normal (18.5-25)	8.04 ± 2.29	7.13 ± 4.02	11.5 - 16 kg
High (>25)	8.29 ± 1.68	8.04 ± 2.92	7 - 11.5 kg

Sixty three percent of the subjects in group I A and 48 percent in group I B had normal hemoglobin level (>11g/dl) (Table 4.7). None of the subjects had severe anemia.

Table 4.7 Distribution of pregnant women in group I A and I B according to hemoglobin level at the time of registration

Severity of anemia	Hemoglobin level (g/dl)	Group I A	Group I B
Severe	<7	-	-
Moderate	7.0 - 9.9	30 (19.6)	7 (19.4)
Mild	10 - 10.9	27 (17.7)	12 (33.3)
Normal	>11	96 (62.7)	17 (47.3)

Value in parenthesis indicate percentage

The goiter grade of the subjects was assessed and it was found that majority of the subjects in group I A did not have goiter while, 50 percent subjects in group I B had goiter (Fig. 4.3).

The thyroid peroxidase antibody (TPO-Ab) status of the subjects revealed that 12 out of 36 subjects in Group IB had TPO-Ab >35 IU/ml (Fig. 4.4).

The urinary iodine concentration (UIC) of the groups showed iodine sufficiency with median being 159.9 µg/Land 174.5 µg/L, respectively, in Group I A and Group I B. The data also revealed that almost half of the subjects in Group I A had UIC less than 150 µg/L whereas 60 percent of the subjects in Group I B had UIC between 150- 249 µg/L (Table 4.8).

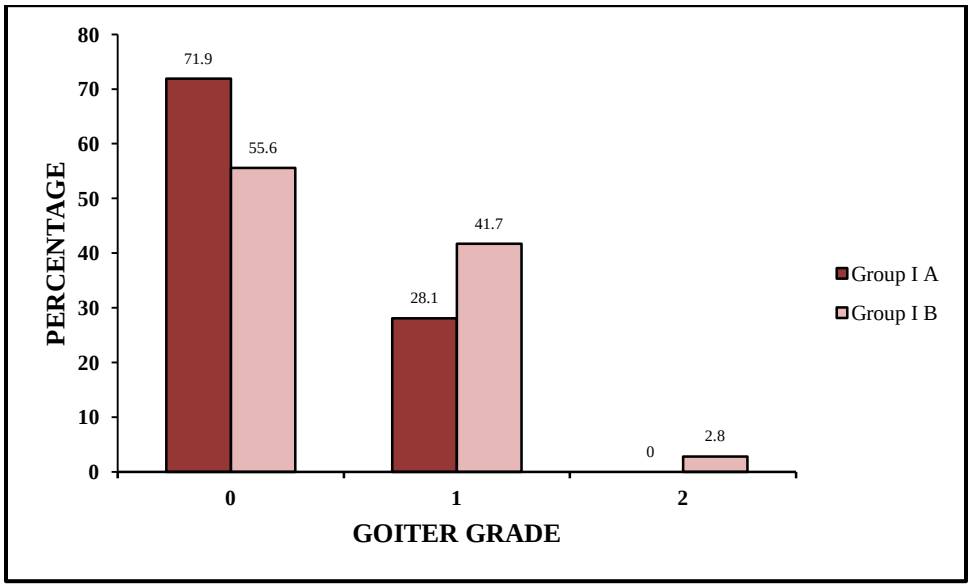


Figure 4.3 Distribution of group I A and I B subjects according to goiter grade

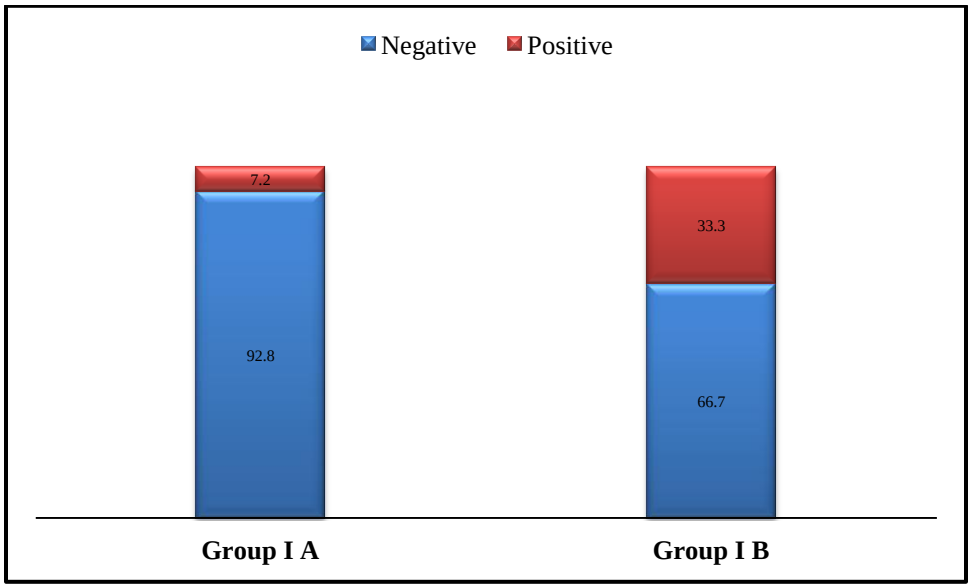


Figure 4.4 Distribution of group I A and I B subjects according to TPO-Ab

Table 4.8 Distribution of pregnant women in group I A & I B according to urinary iodine concentration

UIC (µg/L)	Group I A	Group I B
<150	70 (45.8)	8 (22.2)
150-249	55 (35.9)	22 (61.1)
250-499	28 (18.3)	6 (16.7)

Value in parenthesis indicate percentage

Out of the total 189 subjects enrolled who were followed throughout pregnancy, there was a drop-out of about 45 percent subjects due to various reasons like abortion, miscarriage, missing the follow-up visits. Total three trimester data of sixty eight subjects in group I A was available; hence, further analysis of these subjects were carried out.

4.2.1 GROUP I A: NORMAL SUBJECTS WITH COMPLETE THREE TRIMESTERS DATA (n= 68)

4.2.1.1 Baseline characteristics

The mean age and height of the subjects were 24.34 ± 2.95 years and 153.48 ± 5.4 cm. 27.9 percent of the subjects were underweight at the time of registration (Table 4.9).

Table 4.9 Distribution of Group I A pregnant women with complete three trimesters data in relation to BMI at the time of registration

Gestational age	GROUP I A (n=68) Level of BMI (kg/m^2)			
	< 18.5	**18.5-25**	**>25**	**Mean ± SD (Range)**
< 1 month	-	-	-	-
1-2 month	3 (37.5%)	2 (25%)	3 (37.5%)	24.66 ± 7.27 (17.07-36.1)
2-3 month	16 (26.7%)	34 (56.7%)	10 (16.7%)	21.5 ± 4.59 (15.36-38.31)

The subjects who had above normal BMI at the time of registration showed weight gain throughout pregnancy within the recommended levels (Table 4.10).

Table 4.10 Average weight gain of Group I A pregnant women with complete three trimesters data throughout pregnancy

BMI at the time of registration	Weight gain (kg)	Recommended weight gain (kg)
Low (< 18.5)	8.82 ± 1.58	12.5-18
Normal (18.5-25)	8.44 ± 1.94	11.5-16
High (>25)	8.43 ± 1.66	7-11.5

4.2.1.2 Hemoglobin profile

Majority (60 percent) of the subjects had normal hemoglobin concentration throughout pregnancy and postpartum. None of them had moderate or severe anemia (Fig. 4.5).

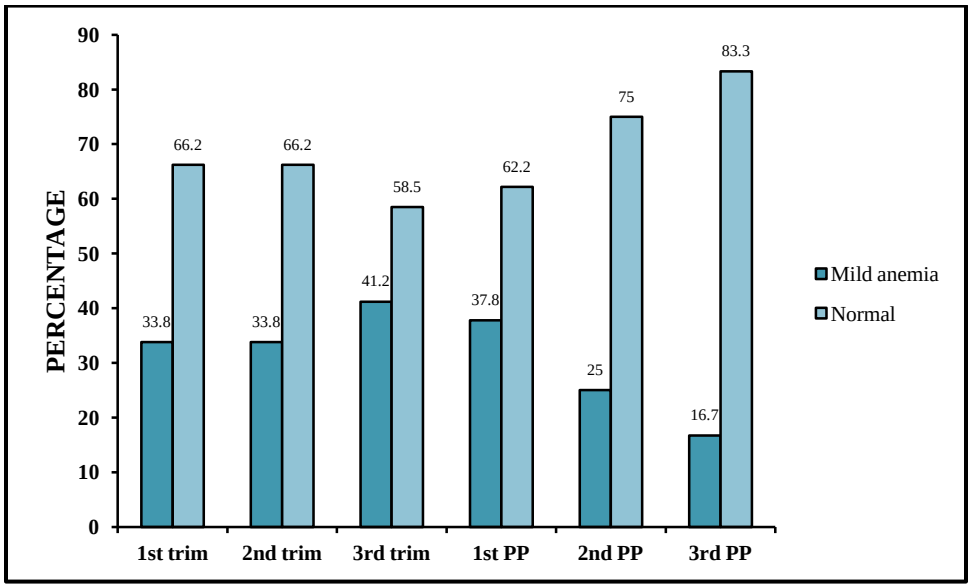


Figure 4.5 Distribution of Group I A pregnant women with complete three trimesters data according to hemoglobin level

The mean and median hemoglobin of the subjects was above normal hemoglobin level cut-off (>11g/dl for pregnant women) both during pregnancy and postpartum as shown below.

Hb (g/dl)	1st trim	2nd trim	3rd trim	1st PP	2nd PP	3rd PP
Mean (±SD)	11.39±1.3	11.45±1.1	11.47±1.1	11.59±0.8	11.51±0.7	11.74±0.6
Median	11.55	11.6	11.45	11.8	11.7	12

4.2.1.3 Urinary iodine status

Urinary iodine excretion during the course of pregnancy and postpartum are presented in figure 4.6.The median urinary iodine showed that the population under study is iodine sufficient in all the trimesters (UIC being 189.2, 200 and 196.9 µg/L in first, second and third trimester, respectively) of the pregnancy. During pregnancy the urinary iodine concentration increased as compared to postpartum (UIC was 139.1, 142 and 141 µg/L after 6 weeks, 12 weeks and 24 weeks postpartum, respectively). In pregnant women,

UIC was significantly ($p<0.001$) elevated, compared with postpartum. During the first postpartum months the decrease in maternal iodine levels is due to iodine elimination through breast milk, which contributes to maintaining homeostasis in the newborn.

Fister, *et al.* 2011 showed that UIC was significantly higher during pregnancy than after delivery ($p=0.044$). Ardawi, Nasrat and Mustafa 2002 reported that UIC was significantly elevated in pregnant women but decreased during the second and third trimesters as compared to the first trimester values. Azizi, *et al.* 2003 reported similar results as our study that there was no significant difference in urinary iodine excretion between the three trimesters of pregnancy in the pregnant women residing in iodine sufficient region.

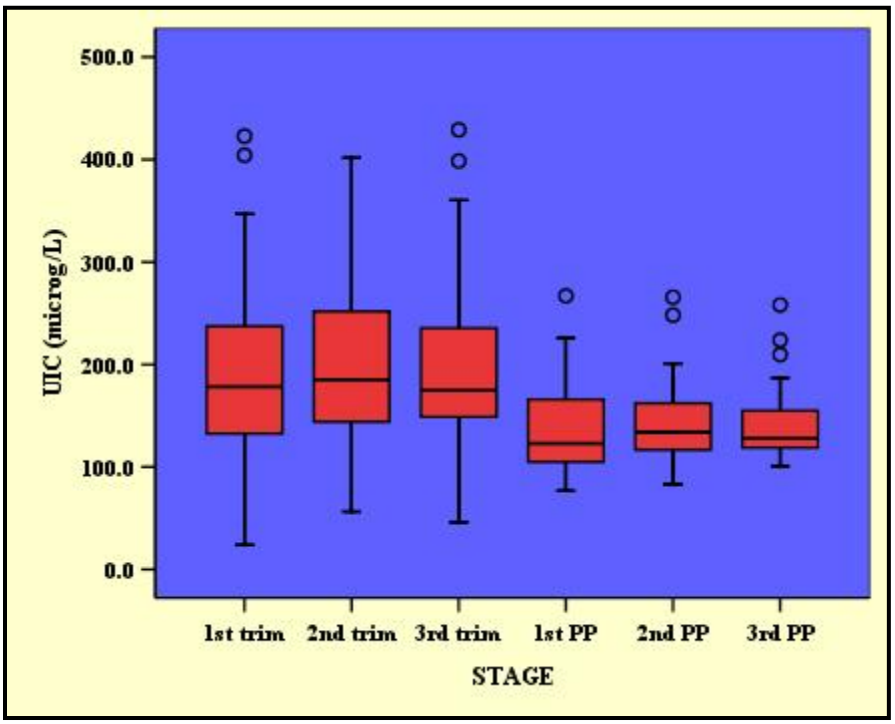


Figure 4.6 Changes in median urinary iodine concentration in group I A pregnant women (1st, 2nd and 3rd trimester) and lactating women (1st PP=6 weeks postpartum, 2nd PP=12 weeks postpartum, 3rd PP=24 weeks postpartum)

It was observed that during pregnancy 30-40 percent of the subjects were iodine deficient whereas postpartum the number decreased to 6 percent (Table 4.11 a & b). A statistically significant association was observed between UIC in the first trimester and second trimester (Spearman correlation $\rho=0.854$, $p<0.001$), first trimester and third trimester (Spearman correlation $\rho=0.794$, $p<0.001$) and second and third trimester (Spearman

correlation ρ=0.849, p<0.001). Ten percent of the women in first trimester who were iodine deficient shifted to iodine sufficient category in second and third trimester. Alvarez-Pedrerol, *et al.* 2009 also observed a statistically significant association between UIC in the first and third trimesters (r=0.24, p<0.001), although only 25 percent of the women with UIC at the first and third trimesters had the same category of UIC in both trimesters.

Table 4.11(a) Trimester-wise distribution of group I A pregnant women according to urinary iodine

UIC (µg/L)	1st trimester	2nd trimester	3rd trimester
<150	27 (39.7)	20 (29.4)	19 (27.9)
150-249	26 (38.3)	30 (44.1)	38 (55.9)
250-499	15 (22.1)	18 (26.5)	11 (16.2)

Table 4.11(b) Distribution of group I A lactating women according to urinary iodine

UIC (µg/L)	1st postpartum	2nd postpartum	3rd postpartum
50-99	4 (5.8)	4 (5.8)	-
100-199	59 (86.8)	59 (86.8)	63 (92.6)
200-299	5 (7.4)	5 (7.4)	5 (7.4)

Value in parenthesis indicate percentage

4.2.1.4 Thyroid function parameters

Five subjects (7.4 percent) were TPO-Ab positive as shown below. Majority of the subjects (76.5 percent) did not have goiter. Rest of them had grade 1 goiter. Chi-sq test between goiter and TPO-Ab was non-significant (χ^2=0.045, df=1, p=0.832) which shows that there is no interrelationship between goiter and TPO-Ab status.

Goiter	TPO-Ab	
	Negative	Positive
0	48 (70.5%)	4 (5.9%)
1	15 (22.1%)	1 (1.5%)

A study by Das, *et al.* 2011 reported that thyroid autoimmunity did not contribute to high prevalence of goiter. On multiple regression analysis, considering goiter as a dependent variable and gender, urinary iodine, hemoglobin, serum ferritin, anti-TPO antibody positivity, serum selenium and smoking as independent variables, low serum ferritin had a significant odds ratio (OR) of 2.8 whereas results of other variables did not achieve statistical significance

***Serum FT_3*:** The mean FT_3 decreased significantly ($p<0.05$) in second trimester by 9.5 percent and further decreased non-significantly in third trimester (Table 4.12). Postpartum the concentration increased significantly ($p<0.05$) when compared to second and third trimester and almost reached the first trimester concentration (Fig. 4.7). Ardawi, Nasrat and Mustafa 2002 showed that serum FT_3 levels showed a continuous decrease throughout gestation. Fister, *et al.* 2011 reported that FT_3 level was significantly lower ($p<0.001$) in third trimester than after delivery.

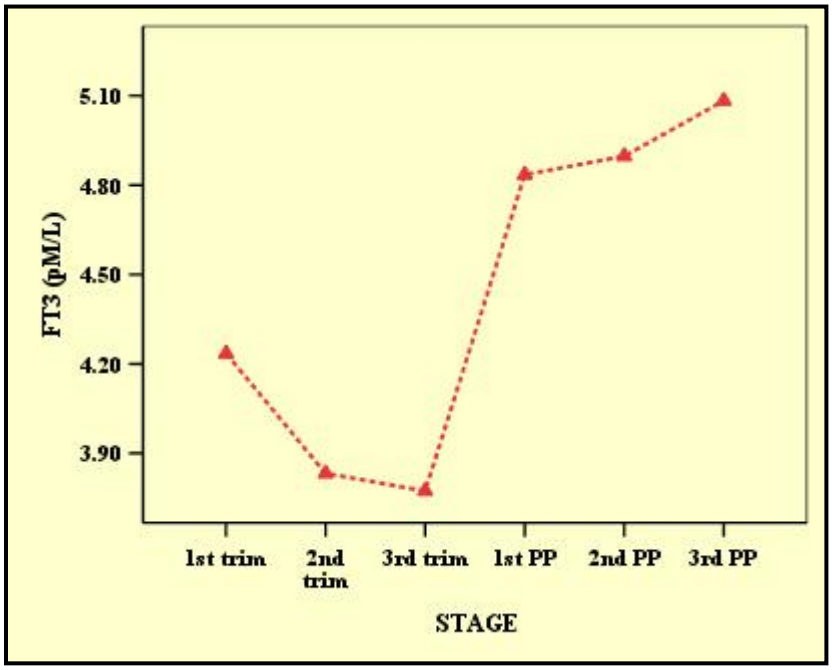


Figure 4.7 Changes in serum free triiodothyronine levels in Group I A pregnant women and lactating women (1st PP=6 weeks postpartum, 2nd PP=12 weeks postpartum, 3rd PP=24 weeks postpartum)

Table 4.12 Mean thyroid function tests of group I A subjects (n=68) during gestation and postpartum

Parameters	Stage	Mean ±SD	Median
FT_3 (pM/L)	1st trimester	4.23 ± 0.7	4.23
	2nd trimester	3.83 ± 0.82*	3.91
	3rd trimester	3.77 ± 1.12*	3.65
	1st postpartum	4.83 ± 1.29†‡	4.67
	2nd postpartum	4.89 ± 0.8*†‡	4.93
	3rd postpartum	5.08 ± 1.01*†‡	5.04
FT_4 (pM/L)	1st trimester	14.19 ± 3.65	13.4
	2nd trimester	12.83 ± 2.8*	12.64
	3rd trimester	12.69 ± 2.35*	12.48
	1st postpartum	15.36 ± 4.26†‡	14.43
	2nd postpartum	14.85 ± 1.71	14.66
	3rd postpartum	16.14 ± 5.00†‡	14.77
TSH (µIU/ml)	1st trimester	1.99 ± 1.23	2
	2nd trimester	2.49 ± 1.16*	2.49
	3rd trimester	2.46 ± 1.00*	2.32
	1st postpartum	2.35 ± 1.18	2.1
	2nd postpartum	2.45 ± 0.96*	2.27
	3rd postpartum	2.34 ± 1.44	2.09

* $p<0.05$ *vs.* 1st trimester; † $p<0.05$ *vs.* 2nd trimester; ‡ $p<0.05$ *vs.* 3rd trimester

***Serum FT_4*:** The FT_4 concentrations were lower during pregnancy than in the postpartum period (Fig. 4.8). The mean FT_4 decreased significantly ($p<0.05$) by about 9.5 percent from first to second trimester and by 10.6 percent from first to third trimester. Postpartum at 6 weeks the concentration significantly increased by 21 percent as compared to third trimester (Table 4.12). Fister, *et al.* 2011 reported that FT_4 level was significantly lower ($p<0.001$) in third trimester than after delivery. Similarly Soldin, Tractenberg, *et al.* 2004

observed that mean FT_4 decreased by about 15 percent from the first trimester to the second and then remained stable during the remainder of pregnancy. Ardawi, Nasrat and Mustafa 2002 also showed that serum FT_4 levels significantly decreased in the second and third trimesters as compared to first trimester. Eltom, *et al.* 2000 reported that median FT_4 concentration increased from 9.7 pM/L three months after delivery to 10.4 pM/L nine months after delivery. Both these levels were significantly higher than the median value in the third trimester of pregnancy ($p<0.001$).

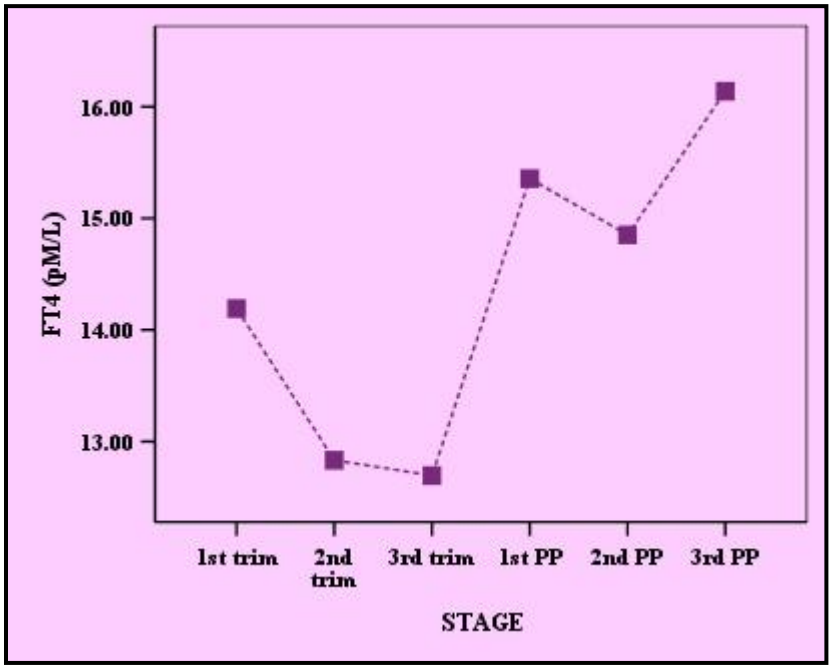


Figure 4.8 Changes in serum free thyroxine levels in group I A pregnant women and lactating women (1st PP=6 weeks postpartum, 2nd PP=12 weeks postpartum, 3rd PP=24 weeks postpartum)

Berghout and Wiersinga 1998 revealed that FT_4 significantly decreased by about 30 percent to low normal values in the second and third trimester of pregnancy in both iodine- depleted and iodine- replete areas. Similar trend was observed in FT_3 concentration during pregnancy. The decrease in serum FT_4 in pregnancy cannot be explained by changes in plasma volume or concentrations of albumin, TBG and free fatty acids in the serum. Although renal iodine clearance is increased during pregnancy, probably by increased glomerular filtration rate, absolute thyroidal iodine intake remains

unchanged. Moreover, relative iodine deficiency cannot fully explain the decrease in FT_4, because it is observed in both iodine-deficient and iodine- replete areas. The decrease in FT_4 is associated with a similar decrease in FT_3, which also argues against an iodine-related phenomenon, since, in iodine deficiency, T_3 values are normal even increased. Lastly, net T_4 turnover and presumably also thyroid hormone requirements are unaltered in human pregnancy i.e. 90 µg/day in non-pregnant women *vs.* 97 µg/day in pregnant women.

The changes in thyroid hormones can be related to energy balance during pregnancy. In a study on energy requirements of pregnancy in The Netherlands (Raaij, *et al.* 1987), it was found that the energy intake during the second and third trimesters of pregnancy is lower than the calculated need for energy. It was calculated that the extra energy needs of pregnancy can be 1020 kJ/day, which comprises the energy required for the synthesis of new tissues together with related increments in basal metabolism. However, in pregnancy, the increase in energy intake was found to be very small, approximating 80 kJ/day, giving rise to an estimated energy gap of 940 kJ/day. This is only partially compensated for by a decrease in physical activity, thus, saving 355 kJ/day. A shortfall of 585 kJ/day has still to be met, and it is in this respect that down-regulation of thyroid hormone action as indicated by the decrease in the levels of FT_4 and FT_3 may contribute to the saving of energy (Ardawi, Nasrat and Mustafa 2002).

***Serum TSH*:** The mean TSH increased significantly by 25.1 percent in second trimester when compared to first trimester (Fig. 4.9) and then decreased non-significantly in third trimester. Postpartum the TSH concentration showed non-significant decline but the mean remained higher than the first trimester value (Table 4.12). Our results are similar to those reported by Soldin, Tractenberg, *et al.* 2004 that TSH concentrations in first trimester were slightly lower (about 16 percent) than the postpartum values, but remained above the postpartum baseline during the second and third trimester. TSH increased between the first and third trimester ($p<0.05$), but not between the second and third trimester ($p>0.90$), implying that the increase took place between the first and second trimesters. Fister, *et al.* 2011 found that in the third trimester TSH was significantly

higher (p=0.003) than after delivery. These changes reflect thyroidal stimulation by hCG which peaks in the first trimester and decreases during the second and third trimesters. Normal TSH values were progressively restored during the second trimester and it was both the amplitude and duration of peak hCG values that geared the changes in thyroid function.

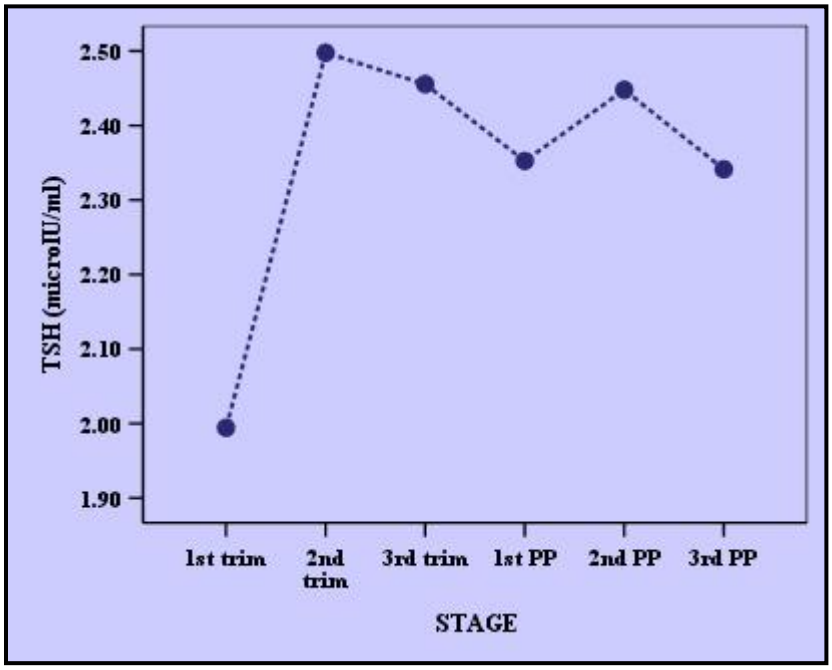


Figure 4.9 Changes in serum thyrotropin levels in group I A pregnant women and lactating women (1st PP=6 weeks postpartum, 2nd PP=12 weeks postpartum, 3rd PP=24 weeks postpartum)

A significant positive correlation was found between FT_3 and FT_4 and a significant negative correlation was found between FT_4 and TSH in all the trimesters and postpartum (Table 4.13). In the first trimester, a significant negative correlation ($r=-0.251$, $p<0.05$) was found between urinary iodine and TSH ($p<0.05$) (Fig. 4.10) whereas in all the other trimesters the correlation was non-significant. A non-significant correlation was observed between TPO-Ab and all other thyroid parameters.

Fister, *et al.* 2011 found no significant correlations between UIC and TSH or between UIC and both free thyroid hormones during pregnancy or after delivery.

Wang, *et al.* 2009 reported that the percentage of TSH outside the normal range in women with lower urinary iodine was higher than in women with higher urinary iodine, which indicated that women with lower urinary iodine concentrations may have a higher risk of developing thyroid function disorders than those with higher urinary iodine concentrations. Ardawi, Nasrat and Mustafa 2002 found a significant negative correlations between urinary iodine and maternal TSH ($r=0.228$, $p<0.05$).

According to Soldin, Tractenberg, *et al.* 2004 the concentrations of analytes (T_3, T_4, FT_4, TG and TSH) at each trimester and at 1-year postpartum tended not to correlate with each other, with two exceptions. One exception was that T_3 and T_4 tended to be associated ($p<0.05$) at all time points except the second trimester; the second exception was that levels of T_4 and FT_4 tended to correlate positively during pregnancy ($p<0.05$), but not postpartum ($p>0.05$).

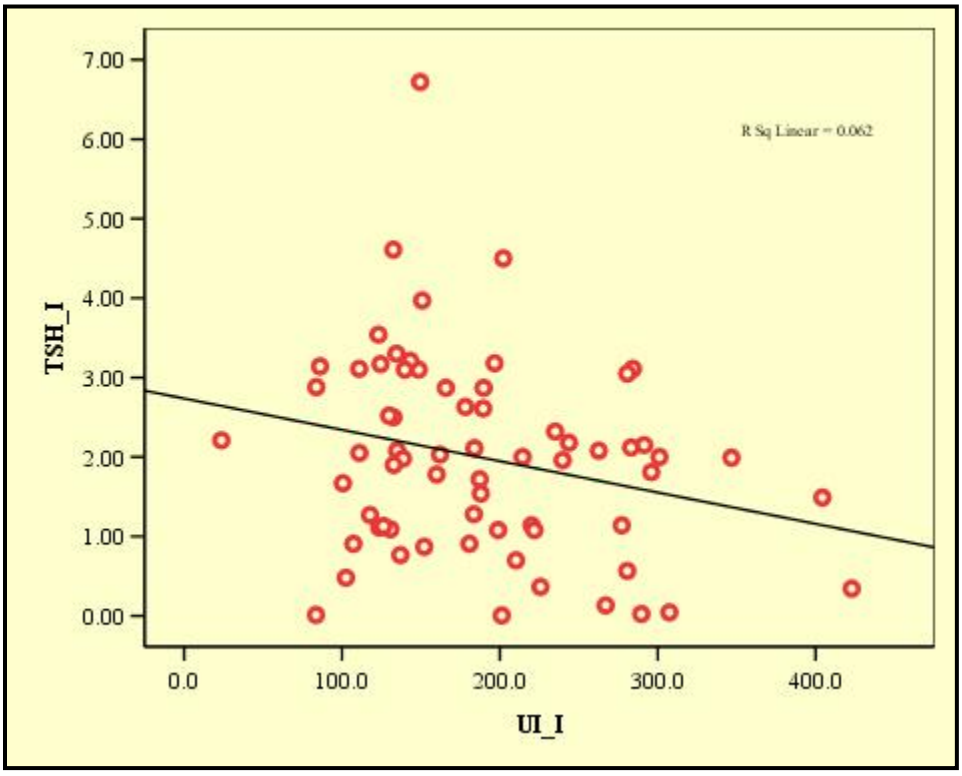


Figure 4.10 Relationship between concentrations of first trimester urinary iodine concentration (µg/L) and serum concentrations of TSH (pM/L) in group I A

Table 4.13 Correlation coefficients for thyroid analytes and urinary iodine by trimester and postpartum in group I A subjects

	FT_3	FT_4	TSH	TPO-Ab	UIC
1st trimester					
FT_3	1				
FT_4	0.342**	1			
TSH	-0.198	-0.284*	1		
TPO-Ab	0.003	-0.035	0.036	1	
UIC	-0.022	-0.032	-0.251*	-0.172	1
2nd trimester					
FT_3	1				
FT_4	0.416**	1			
TSH	-0.268**	-0.315**	1		
TPO-Ab	-0.143	-0.120	0.189	1	
UIC	-0.117	0.124	-0.044	-0.179	1
3rd trimester					
FT_3	1				
FT_4	0.231	1			
TSH	0.024	0.042	1		
TPO-Ab	-0.085	-0.034	0.114	1	
UIC	-0.098	-0.052	-0.089	-0.209	1
1st postpartum					
FT_3	1				
FT_4	0.805**	1			
TSH	-0.307	-0.377**	1		
TPO-Ab	0.383**	0.409*	-0.387*	1	
UIC	0.010	0.167	0.066	0.332*	1
2nd postpartum					
FT_3	1				
FT_4	-0.058	1			
TSH	-0.146	-0.368*	1		
TPO-Ab	-0.360*	0.049	0.016	1	
UIC	-0.449**	0.176	0.378*	0.221	1
3rd postpartum					
FT_3	1				
FT_4	0.111	1			
TSH	-0.251	-0.45**	1		
TPO-Ab	-0.235	0.471**	-0.148	1	
UIC	-0.231	0.092	0.241	-0.080	1

** $p<0.01$; * $p<0.05$

Comparison of iodine deficient vs. iodine sufficient subjects

The subjects in group I A were subdivided based on their iodine status in first trimester. The median UIC of the iodine deficient and iodine sufficient group in first trimester of pregnancy was 126.3 and 220.95 µg/L, respectively (Fig. 4.11). There was no relationship found between goiter grade and urinary iodine status (χ^2=0.068, df=1, p=0.794). There might have been individual differences, but mean differences did not show significance.

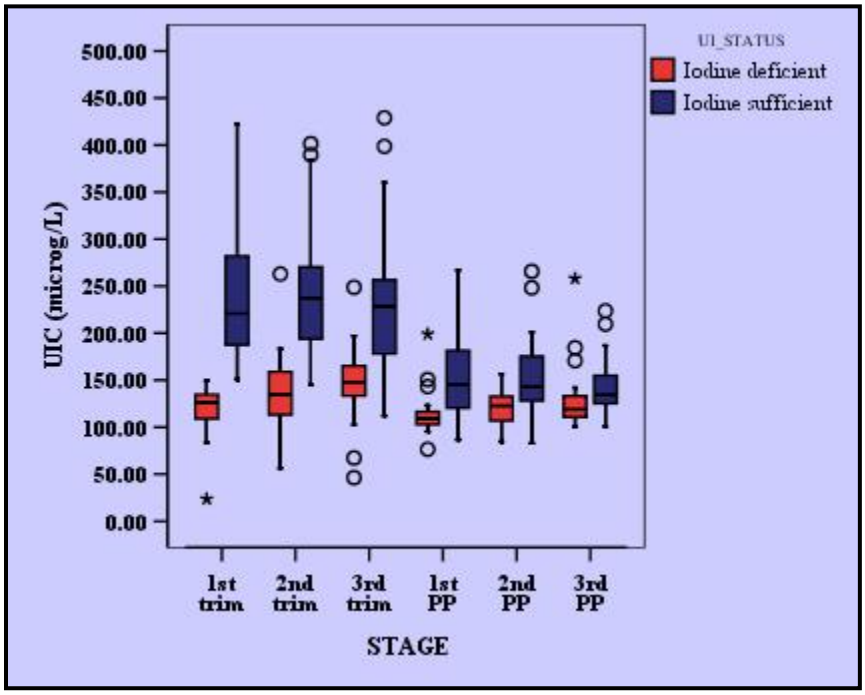


Figure 4.11 Trimester-wise and postpartum median urinary iodine concentration of group I A iodine deficient and iodine sufficient subjects

The thyroid function parameters of iodine sufficient (n=41) and iodine deficient (n=27) were compared in all the three trimesters and postpartum. All the parameters were within the normal ranges in both the groups. There was no significant difference in mean FT_3 and FT_4 during trimesters and postpartum between the two groups (Table 4.14). Serum TSH was significantly ($p<0.05$) lower in iodine sufficient group in first trimester whereas non-significantly lower in all the other trimesters and postpartum. This could be due to thyroidal stimulation by low levels of iodine in iodine-deficient women which is reflected by slightly increased TSH though within the reference range as it is more sensitive to

iodine status. There was no change observed in FT_4 values. This could be suggestive that women with low iodine levels may be more prone to subclinical hypothyroidism as compared to iodine- sufficient women.

Table 4.14 Mean thyroid function test of group I A iodine deficient *vs.* iodine sufficient subjects

Parameters	Stage	Iodine deficient (n=27)		Iodine sufficient (n=41)	
		Mean ± SD	Median	Mean ± SD	Median
FT_3 (pM/L)	1st trimester	4.39 ± 0.57	4.5	4.14 ± 0.77	4.11
	2nd trimester	3.79 ± 0.7	3.78	3.82 ± 0.89	3.95
	3rd trimester	3.85 ± 1.6	3.7	3.76 ± 0.63	3.63
	1st postpartum	4.85 ± 0.76	4.78	4.89 ± 1.66	4.7
	2nd postpartum	5.22 ± 0.51	5.12	4.66 ± 0.93	4.63
	3rd postpartum	5.09 ± 1.15	5.11	5.09 ± 0.96	5.05
FT_4 (pM/L)	1st trimester	14.14 ± 4.28	13.2	14.07 ± 3.28	13.52
	2nd trimester	12.27 ± 2.59	12.56	13.15 ± 2.93	12.67
	3rd trimester	12.49 ± 2.39	12.09	12.69 ± 2.31	12.48
	1st postpartum	14.95 ± 1.83	14.97	15.49 ± 1.76	14.01
	2nd postpartum	14.53 ± 1.61	14.25	14.93 ± 1.65	14.66
	3rd postpartum	15.27 ± 1.25	14.79	15.99 ± 1.24	14.17
TSH (µIU/ml)	1st trimester	2.35 ± 1.4	2.21	1.74 ± 1.07*	1.88
	2nd trimester	2.54 ± 1.15	2.55	2.41 ± 1.14	2.48
	3rd trimester	2.69 ± 1.00	2.56	2.31 ± 0.9	2.21
	1st postpartum	2.08 ± 0.81	1.99	2.38 ± 1.01	2.43
	2nd postpartum	2.44 ± 1.03	2.22	2.35 ± 0.77	2.29
	3rd postpartum	2.6 ± 1.19	2.2	1.95 ± 0.95	1.98

* $p<0.05$ iodine sufficient *vs.* iodine deficient

Wang, *et al.* 2009 carried out a study on pregnant women, lactating women and infants in the regions where iodized salt coverage rate was more than 90% since 2000. They found that 15.4 percent women's TSH were abnormal, among them 11 percent was above the high value of reference range. Most of these women had UIC lower than 150 µg/L. Free T_4 in all the pregnant women were within the reference range. This indicated that TSH is

more sensitive to low iodine status than FT_4 and a low iodine status increases the risk of thyroid function disorders.

A study by Teng, *et al.* 2008 found that among the subjects with mildly deficient iodine intake, those with adequate intake, and those with more than adequate intake, the prevalence of clinical and subclinical hypothyroidism was 0, 1.13 and 2.84 percent, respectively ($p=0.014$); that of thyroid goiter was 24.88, 5.65 and 11.37 percent, respectively ($p<0.01$); and that of serum thyrotropin values was 1.01, 1.25 and 1.39 mIU/L, respectively. However, authors observed an iodine related increase in serum TSH values ($p<0.001$) and this iodine-induced high serum TSH level may be a result from the feedback of lower triiodothyronine contents in pitutary due to decreased 5' deiodinase activity in thyroid and pitutary glands.. The authors concluded that median urinary iodine 100-200 µg/L may reflect the safe range of iodine intake levels.

4.2.1.5 Pregnancy outcome of Group I A subjects

The pregnancy outcome of subjects under study (seventy subjects of Group I A) are presented in table 4.15. All details of only these neonates are available as some subjects had miscarriage or dropped out from the study or had home delivery. Out of 98 subjects, who reported of giving birth, 30.6 percent had home delivery. One subject had preterm delivery.

Table 4.15 Characteristics of neonates of group I A (n=69)

	Frequency (Percentage)
Sex of the baby	
Male	30 (43.5)
Female	39 (56.5)
Type of delivery	
Normal	44 (63.8)
Cesarean	19 (27.5)
Other	6 (8.7)

Table 4.16 shows the mean parameters of group I A neonates. The mean birth weight of the neonates was above normal and the birth weights ranged between 2.7 kg to 4.24 kg. The mean APGAR score of the neonates at 1 min, 5 min and 10 min was 7.48±1.32, 8.11±1.05 and 8.57±1.04, respectively.

Table 4.16 Mean parameters of group I A neonates (n=69)

Parameters	Mean ± SD
Gestational age at delivery (weeks)	39.11 ± 1.44
Birth weight (kg)	2.83 ± 0.46
Birth length (cms)	47.73 ± 2.98
Head circumference (cms)	33.11 ± 2.00

Nutritional status of neonates

Percentage of neonates classified as undernourished according to three anthropometric indices of nutritional status: height-for-age (stunting), weight-for-length (wasting) and weight-for-age (underweight), by sex, when compared with WHO child growth standards using (WHO Anthro for personal computers, version 3.1 2010).

The data when classified according to Z-scores revealed that 16.7 percent and 23 percent of males and females, respectively, were stunted. Eighty five percent of both males and females had normal weight for height. Six subjects in both the sexes were underweight (Table 4.17).

Thyroid function tests of neonates

Cord blood FT_4 (CFT_4) and cord blood TSH (CTSH) levels showed a wide scatter or variability. Important characteristics of both FT_4 and TSH levels are shown in table 4.18. Cord TSH levels were asymmetrical and skewed towards right side (positively skewed) which means their trends shifted towards higher values. A study by Abbas, *et al.* 2003 also showed cord blood TSH values trend towards higher values.

Elevated serum TSH in the neonates indicates insufficient supply of thyroid hormones to the developing brain. WHO/UNICEF/ICCIDD has included neonatal TSH as one of the indicators for assessing IDD and their control in a population (Delange 1998). In the absence of iodine deficiency, the frequency of neonatal serum TSH above 10 µIU/ml is less than 3%. A frequency of 3%-19.9% indicates mild IDD. Frequencies of 20%-39.9% and above 40% indicate moderate and severe IDD, respectively.

According to our data 23 (32.3 percent) neonates had TSH level above 10 µIU/ml which indicates moderate iodine deficiency (Fig. 4.12). It means substantial number of fetuses

Table 4.17 Nutritional status of group I A neonates based on Z-scores

Sex	n	Height-for-age				Weight-for-height				Weight-for-age			
		<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD
M	30	2 (6.7)	3 (10)	24 (80)	1 (3.3)	2 (6.7)	2 (6.7)	24 (80)	2 (6.7)	1 (3.3)	5 (16.7)	24 (80)	-
F	39	4 (10.3)	5 (12.8)	30 (76.9)	-	4 (10.3)	2 (5.1)	32 (82)	1 (2.6)	1 (2.6)	5 (12.8)	33 (84.6)	-
T	69	6 (8.7)	8 (11.6)	54 (78.3)	1 (1.4)	6 (8.7)	4 (5.8)	56 (81.2)	3 (4.3)	2 (2.9)	10 (14.5)	57 (82.6)	-

Value in parenthesis indicate percentage

Table 4.18 Serum FT_4 and TSH levels in cord blood of group I A neonates

Parameters	Mean ± SD	Median	Range
Cord blood FT_4 (pM/L)	15.43 ± 5.47	15.15	8.2-51.8
Cord blood TSH (µIU/ml)	8.52 ± 5.05	5.05	0.48-20.40

were iodine deficient. A study by Chakraborty, *et al.* 2006 carried out in a rural hospital of West Bengal found that only 2.9 percent of the neonates had cord blood TSH > 5 µIU/ml. According to this the population fell just below the recommended criteria for mild endemicity of IDD.

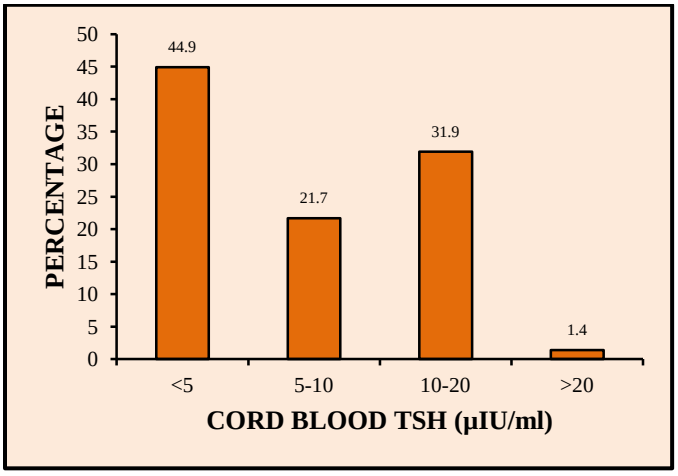


Figure 4.12 Distribution of group I A neonates according to cord blood TSH level

Relationship between neonatal parameters and neonatal thyroid function tests

The correlation between neonatal parameters and thyroid function tests revealed that cord blood FT_4 is positively correlated with birth weight (r=0.408; p<0.001) (Fig. 4.13) while cord blood TSH is negatively correlated with head circumference (Fig. 4.14) (Table 4.19). Head circumference was found to be highly correlated with birth length (r=0.517, p<0.01) (Fig. 4.15). This indicates that thyroid hormones are essential for normal growth and bone development.

Table 4.19 Correlation between neonatal parameters and thyroid function tests in group I A neonates

Neonatal parameters	Cord blood FT_4	Cord blood TSH
Head Circumference	.099	-0.321*
Length	.034	-0.135
Weight	.408**	-0.189

* p<0.05; ** p<0.01

Thyroid hormones play a key role in growth and bone development either indirectly by increasing the secretion of growth hormone and insulin like growth factor 1, or directly by influencing target genes via specific nuclear receptors. The actions of growth hormone fully manifest only when enough thyroid hormone is present. As a result, growth is severely stunted in hypothyroid children. It is known that the disruption of hypothalamic-pitutary-thyroid axis during growth profoundly influences skeletal development. The expression of TH receptors in bone cells as the responsiveness to TH in cell culturing systems is the evidence of effect of TH on bone tissue (Abu, *et al.* 1997, Bassett and Williams 2008).

Shields, *et al.* 2011 studied normal, healthy pregnancies to assess whether fetal thyroid hormone at birth as measured in cord blood is associated with fetal growth. They found that cord FT_4 was associated with birth weight ($r=0.25$; $p<0.001$), length ($r=0.17$; $p<0.001$), head circumference ($r=0.11$; $p<0.009$). There was no association between cord TSH and birth measurements. Similar result were reported by Jaruratanasirikul, *et al.* 2009 that there is no correlation between neonatal TSH concentration and neonatal outcome, i.e. gestational age, birth weight, birth length or head circumference.

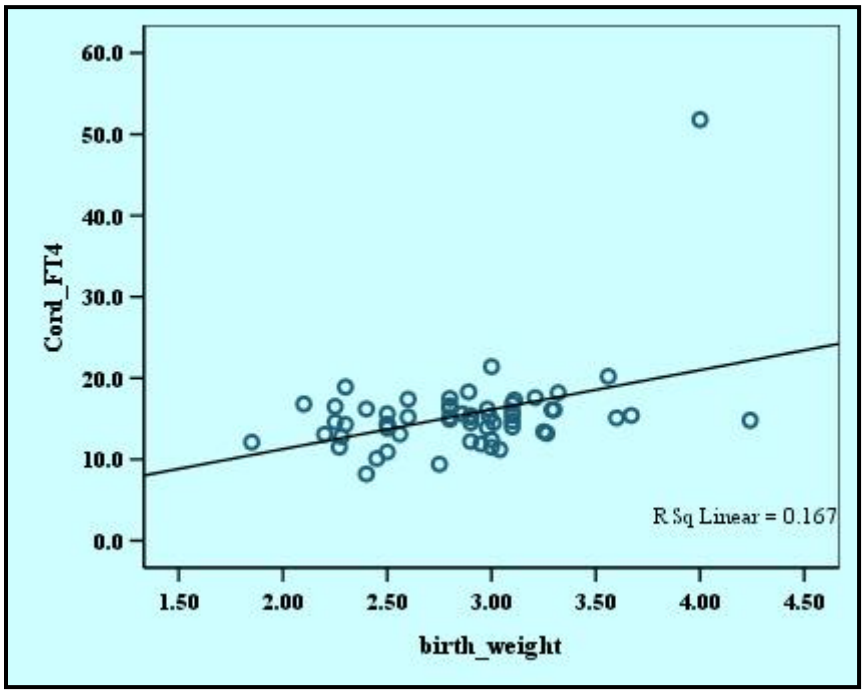


Figure 4.13 Relationship between cord blood thyroxine and birth weight of group I A neonates

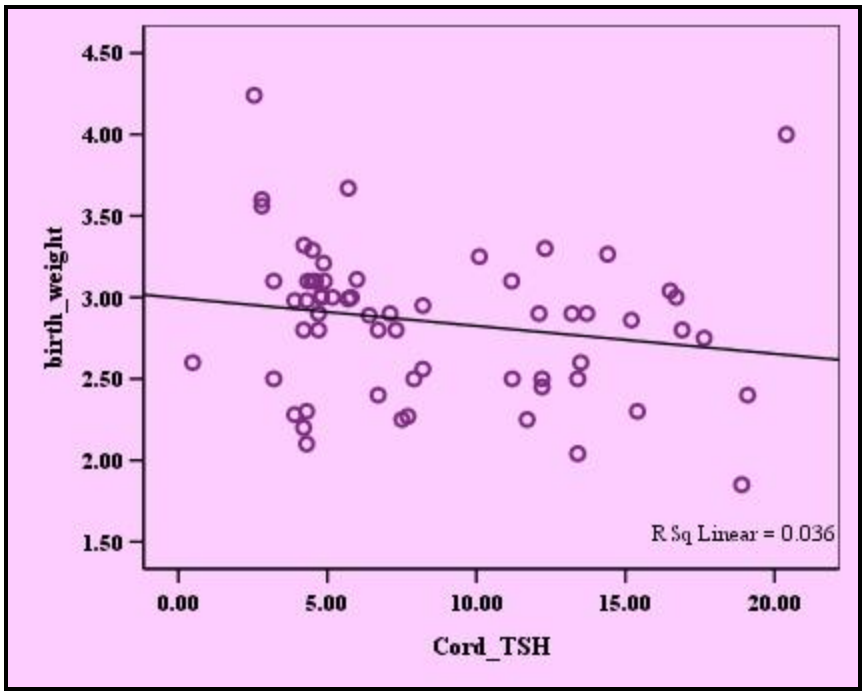


Figure 4.14 Relationship between cord blood thyroid stimulating hormone and birth weight of group I A neonates

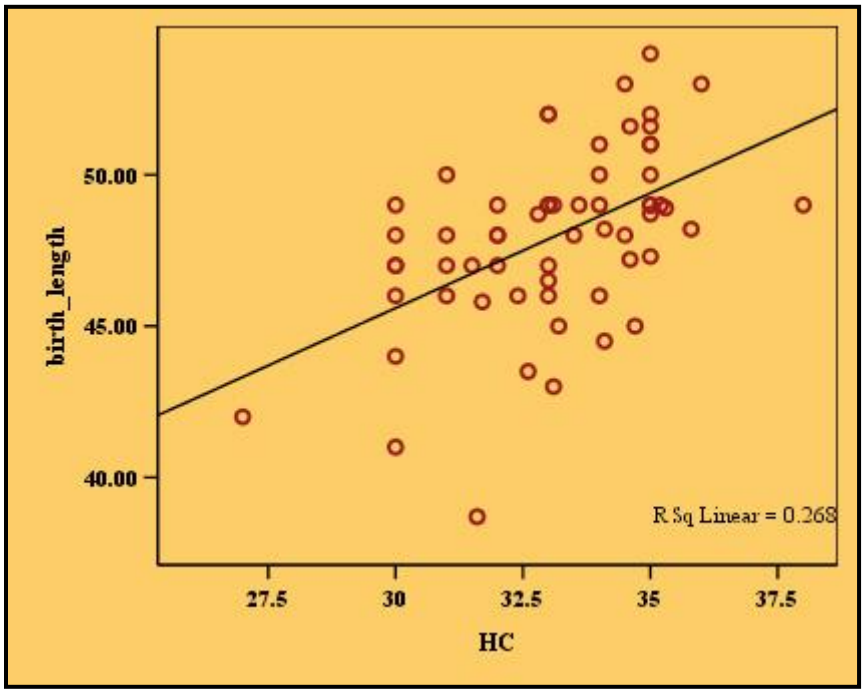


Figure 4.15 Relationship between birth length and head circumference of group I A neonates

Maternal thyroid function parameters and neonate thyroid function

Cord blood FT_4 was positively correlated with maternal first trimester FT_4 ($r=0.645$, $p<0.01$) (Fig. 4.16 a) and third trimester FT_4 ($r=0.345$, $p<0.05$) (Fig. 4.16 b) whereas significant negative correlation was found between first trimester maternal TSH and cord blood TSH (Table 4.20 & 4.21). A non-significant negative correlation was found between maternal TSH and cord FT_4. No correlation was found between maternal UIC and cord blood parameters.

Shields, *et al.* 2011 reported weaker negative associations between maternal TSH and cord FT_4 ($r=-0.10$; $p=0.02$) and FT_3 ($r=-0.10$; $p=0.02$). A highly significant correlation ($r=0.14$; $p=0.001$) was found between maternal FT_4 and cord blood FT_4.

Ardawi, Nasrat and Mustafa 2002 found negative correlation between maternal FT_4 and the neonatal TSH ($r=-0.70$; $p<0.001$). However, Orito, *et al.* 2009 concluded that serum concentrations of TSH during early pregnancy in normal women had no relevance to parameters in neonates. Chan, *et al.* 2003, Jaruratanasirikul, *et al.* 2009 showed that there was no significant correlation between neonatal TSH and maternal urine iodine content. Alvarez-Pedrerol, *et al.* 2009 found that the UIC levels during the third trimester of pregnancy were related to birth weight of the neonates.

Table 4.20 Correlation between maternal thyroid function tests (first trimester) and fetal thyroid function tests of group I A subjects

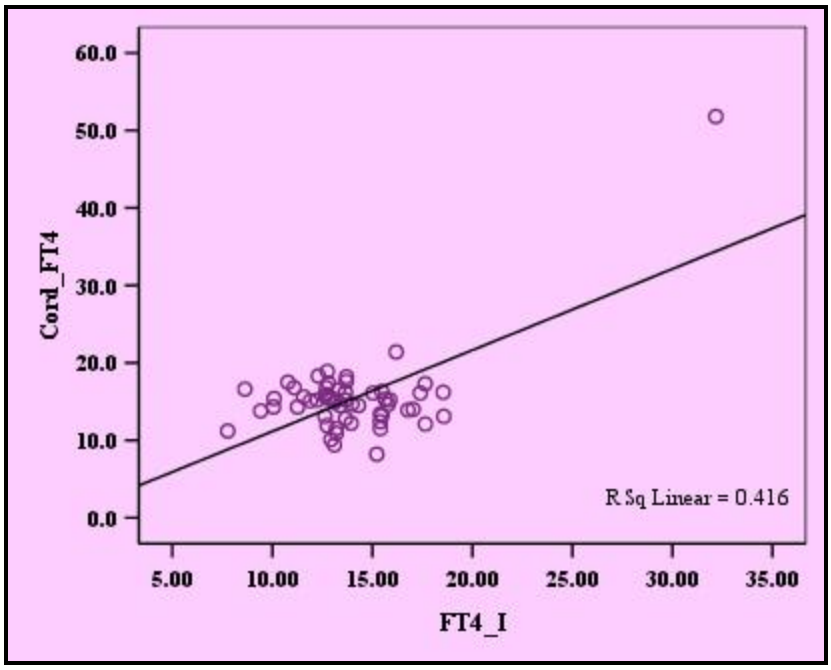
	FT_3	**FT_4**	**TSH**	**UIC**	**CFT_4**	**CTSH**
FT_3	1					
FT_4	0.326**	1				
TSH	-0.197*	-0.279**	1			
UIC	0.119	0.049	-0.131	1		
CFT_4	0.094	0.645**	-0.210	-0.088	1	
CTSH	0.001	0.182	-0.254*	0.130	0.069	1

** $p<0.01$; * $p<0.05$

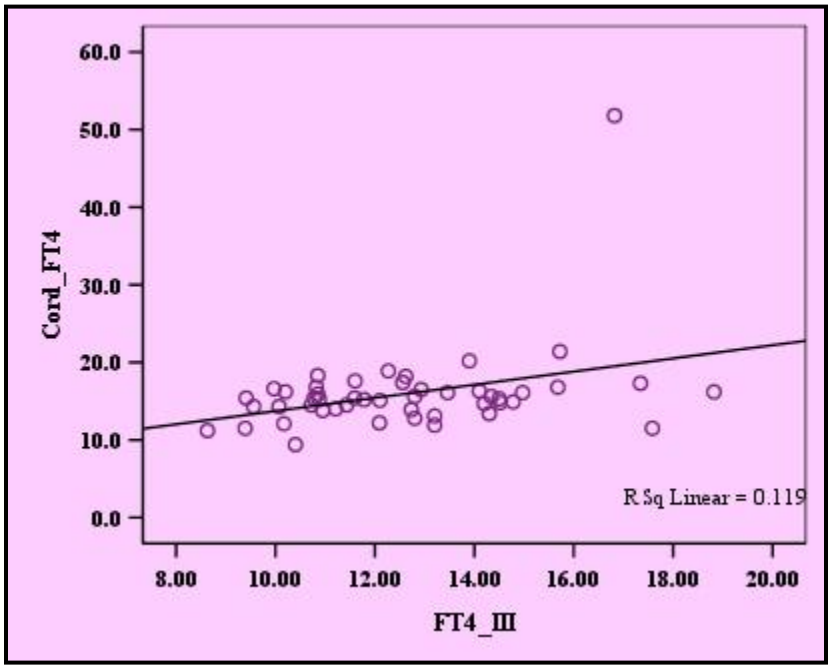
Table 4.21 Correlation between maternal thyroid function tests (third trimester) and fetal thyroid function tests of group I A subjects

	FT_3	**FT_4**	**TSH**	**UIC**	**CFT_4**	**CTSH**
FT_3	1					
FT_4	0.272*	1				
TSH	-0.003	-0.012	1			
UIC	-0.112	-0.038	-0.155	1		
CFT_4	0.048	0.345*	-0.183	-0.259	1	
CTSH	-0.254	-0.147	-0.204	0.132	0.069	1

* $p<0.05$



(a)



(b)

Figure 4.16 Relationship between cord blood free thyroxine and (a) first trimester maternal free thyroxine (b) third trimester maternal free thyroxine of group I A subjects

4.2.2 GROUP I B: MEDICINE STARTED DURING PREGNANCY WITH COMPLETE THREE TRIMESTERS DATA (n=24)

4.2.2.1 Baseline characteristics

The mean age and height of the subjects were 25.21±3.2 years and 154.18±5.88 cm, respectively. At the time of registration 20.8 percent of the subjects were underweight and 60 percent were normal (Table 4.22).

Table 4.22 Distribution of group I B pregnant women with complete three trimesters data in relation to BMI at the time of registration

Gestational age	GROUP I B Level of BMI (kg/m^2)			
	< 18.5	**18.5-25**	**>25**	**Mean**
< 1 month	-	-	-	-
1-2 month	1 (10)	5 (50)	4 (40)	23.59 ± 5.34
2-3 month	4 (28.6)	9 (64.3)	1 (7.1)	21.30 ± 4.13

Value in parenthesis indicate percentage

The average weight gain of all the women was between 7 to 8 kg irrespective of their BMI status (Table 4.23). This shows that women who were underweight remained underweight throughout pregnancy.

Table 4.23 Average weight gain of group I B pregnant women with complete three trimesters data throughout pregnancy

BMI at the time of registration	GROUP I B	Recommended weight gain
Low (< 18.5)	7.64 ± 2.58	12.5-18 kg
Normal (18.5-25)	8.02 ± 3.23	11.5-16 kg
High (>25)	8.04 ± 2.92	7-11.5 kg

4.2.2.2 Hemoglobin profile

One-third of the subjects in this group were anemic but none of them had severe anemia (Fig. 4.17). The mean hemoglobin of the subjects throughout pregnancy and postpartum was approximately 11 g/dl.

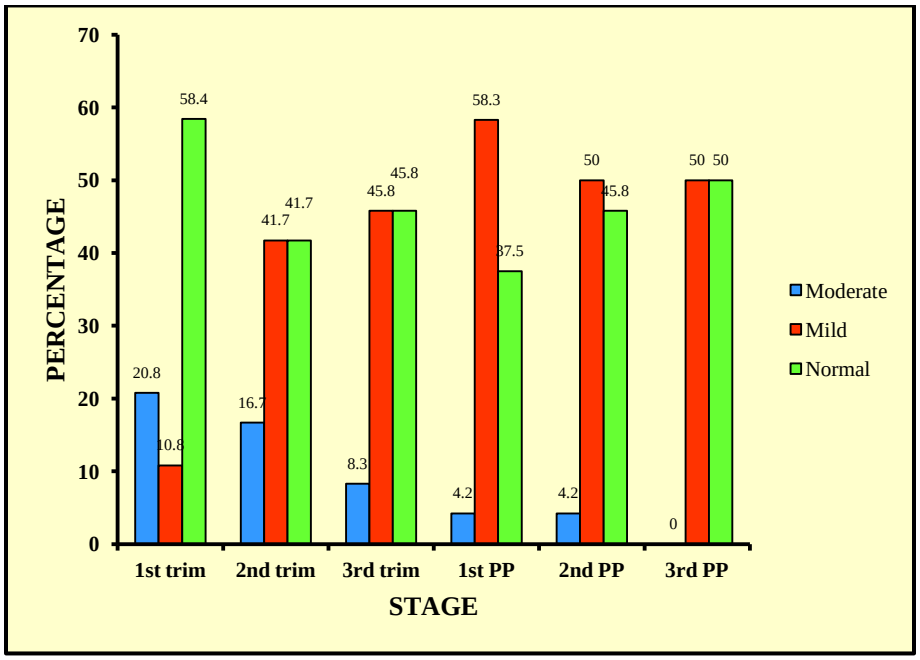


Figure 4.17 Distribution of Group I B pregnant women with complete three trimesters data according to hemoglobin level

4.2.2.3 Urinary iodine status

The median urinary iodine concentration of the group showed iodine sufficiency throughout pregnancy and postpartum (Fig. 4.18). The median urinary iodine concentration remained almost constant throughout pregnancy being 180.2, 179.9 and 199.05 µg/L in first, second and third trimester, respectively. It decreased after pregnancy in 6 weeks, 12 weeks and 24 weeks postpartum to 134.41, 123.8 and 140.75 µg/L, respectively.

When the subjects were divided according to the WHO/UNICEF/ICCIDD classification for UIC, it was found that during pregnancy 21 percent of the subjects had UIC below normal; whereas postpartum data of these subjects revealed that 8 percent had below normal urinary iodine concentration (Table 4.24 a & b).

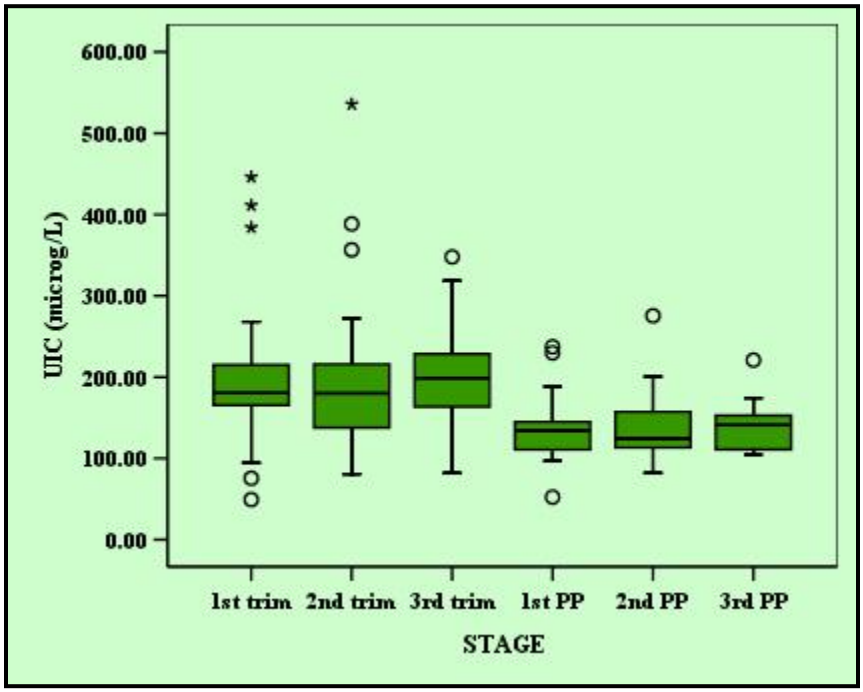


Figure 4.18 Changes in median urinary iodine concentration in group I B pregnant women (1st, 2nd and 3rd trimester) and lactating women (1st PP=6 weeks postpartum, 2nd PP=12 weeks postpartum, 3rd PP=24 weeks postpartum)

Table 4.24a Trimester-wise distribution of group I B pregnant women according to urinary iodine concentration

UIC (µg/L)	1st trimester	2nd trimester	3rd trimester
<150	5 (20.8)	7 (29.2)	5 (20.8)
150-249	15 (62.5)	13 (54.2)	15 (62.5)
250-499	4 (16.7)	3 (12.5)	4 (16.7)
>500	-	1 (4.2)	-

Table 4.24b Distribution of group I B lactating women according to urinary iodine concentration

UIC (µg/L)	1st postpartum	2nd postpartum	3rd postpartum
50-99.9	2 (8.3)	1 (4.2)	-
100-199.9	20 (83.4)	21 (87.5)	23 (95.8)
200-299.9	2 (8.3)	2 (8.3)	1 (4.2)

Value in parenthesis indicate percentage

4.2.2.4 Thyroid function parameters

Majority of the subjects (66.7 percent) were TPO-Ab negative while the rest of them showed positivity. At the initial stages of pregnancy 37 percent of the subjects had goiter while in the third trimester 46 percent of them had goiter. Postpartum 67 percent of the subjects were normal (Fig. 4.19). No significant interrelationship was found between TPO-Ab positivity and goiter.

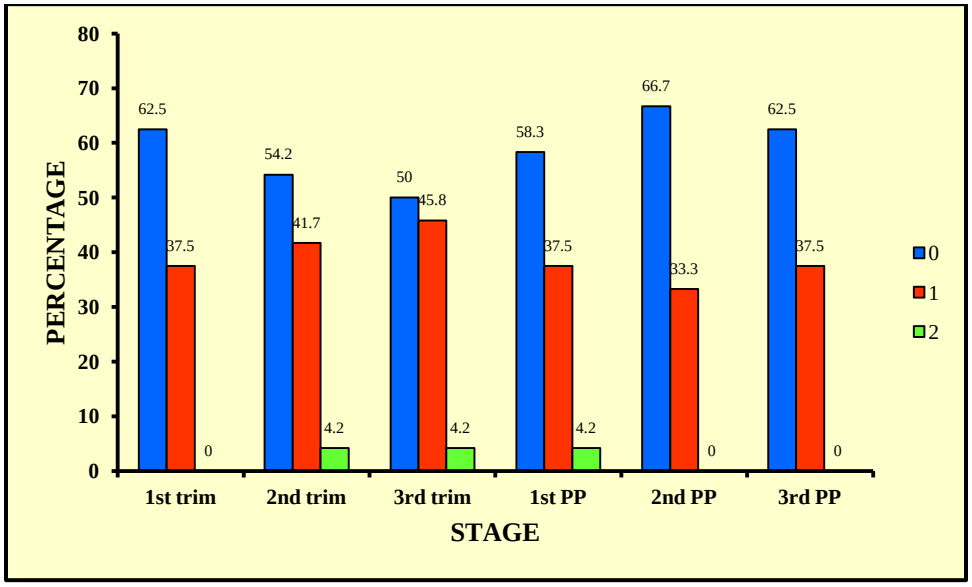


Figure 4.19 Trimester-wise and postpartum distribution of group I B subjects according to goiter grade

***Serum FT$_3$*:** The mean FT_3 decreased throughout pregnancy and significantly by 16.2 percent in third trimester when compared to first trimester. The median FT_3 increased significantly ($p<0.05$) postpartum as compared to pregnancy.

***Serum FT$_4$*:** The mean serum FT_4 increased by 27 percent after delivery when compared to third trimester. A significant increase ($p<0.05$) in median was observed postpartum as compared to pregnancy levels.

***Serum TSH*:** The subjects had abnormal TSH in the first trimester ranging from 0.03 to 100 µIU/ml and it decreased significantly ($p<0.05$) in second and third trimester when compared to first (Table 4.25). The mean TSH in six months postpartum was slightly higher than the normal since some subjects discontinued with the medicine. The median

TSH decreased significantly (p=0.026) by 29 percent from first to third trimester. A further significant (p<0.05) decrease in median TSH was observed postpartum.

Table 4.25 Mean thyroid function tests of group I B subjects (n=24) during gestation and postpartum

Parameters	Trimester	Mean ±SD	Median
FT_3 (pM/L)	1st trimester	4.21 ± 1.02	4.36
	2nd trimester	3.84 ± 0.67	3.89
	3rd trimester	3.53 ± 0.6	3.58*
	1st postpartum	5.33 ± 1.79	5.56*†‡
	2nd postpartum	5.41 ± 1.06	4.3†‡
	3rd postpartum	3.77 ± 1.1	3.66
FT_4 (pM/L)	1st trimester	12.07 ± 5.06	12.8
	2nd trimester	13.59 ± 2.46	14.4
	3rd trimester	13.26 ± 2.5	12.73
	1st postpartum	17.07 ± 3.65	17.18*†‡
	2nd postpartum	15.59 ± 2.36	15.5*‡
	3rd postpartum	15.51 ± 2.4	16.09*‡
TSH (µIU/ml)	1st trimester	13.54 ± 26.9	5.34
	2nd trimester	3.96 ± 2.09	4
	3rd trimester	3.86 ± 2.05	3.78*
	1st postpartum	1.22 ± 1.99	0.7*†‡
	2nd postpartum	1.97 ± 1.91	1.54*†‡
	3rd postpartum	5.32 ± 7.88	2.84*

* p<0.05 *vs.* 1st trimester; † p<0.05 *vs.* 2nd trimester; ‡ p<0.05 *vs.* 3rd trimester

The Pearson's correlation coefficient showed a significant positive correlation (r=0.452, p<0.05) between first trimester TSH and TPO-Ab and significant negative correlation between 12 weeks postpartum TPO-Ab and FT_3 (r=-0.688, p<0.01), FT_4 (r=-0.857, p<0.01), respectively. A significant negative correlation (p<0.01) was observed between urinary iodine and FT_4 in first and second trimester (Table 4.26).

Table 4.26 Correlation coefficients for thyroid analytes and urinary iodine by trimester and postpartum in group I B subjects

	FT_3	FT_4	TSH	TPO-Ab	UIC
1st trimester					
FT_3	1				
FT_4	0.562**	1			
TSH	-0.368	-0.546**	1		
TPO-Ab	-0.422	-0.407	0.452*	1	
UIC	-0.141	-0.633**	0.375	-0.105	1
2nd trimester					
FT_3	1				
FT_4	0.280	1			
TSH	0.078	-0.492*	1		
TPO-Ab	0.229	0.288	-0.204	1	
UIC	-0.084	-0.509*	0.061	-0.107	1
3rd trimester					
FT_3	1				
FT_4	0.096	1			
TSH	-0.333	-0.464*	1		
TPO-Ab	-0.261	-0.055	0.191	1	
UIC	0.149	-0.273	0.172	-0.014	1
1st postpartum					
FT_3	1				
FT_4	0.605*	1			
TSH	-0.429	-0.551*	1		
TPO-Ab	0.434	0.395	-0.232	1	
UIC	0.09	0.275	-0.182	0.074	1
2nd postpartum					
FT_3	1				
FT_4	0.934*	1			
TSH	-0.402	-0.378	1		
TPO-Ab	-0.688**	-0.857**	0.122	1	
UIC	0.122	0.148	-0.359	0.041	1
3rd postpartum					
FT_3	1				
FT_4	0.423	1			
TSH	-0.337	-0.699**	1		
TPO-Ab	-0.465	-0.169	-0.146	1	
UIC	-0.010	-0.007	-0.198	0.169	1

** $p<0.01$; * $p<0.05$

Comparison between TPO-Ab positive vs. TPO-Ab negative subjects

The comparison between TPO-Ab positive and TPO-Ab negative subjects using Mann-Whitney nonparametric test showed that median FT_3 (Fig. 4.20) and median FT_4 (Fig. 4.21) of the two groups in first trimester was significantly different (Table 4.27). The median FT_3 and FT_4 of TPO-Ab positive subjects was significantly ($p<0.05$) lower when compared to TPO-Ab negative subjects. The median TSH (Fig. 4.22) of TPO-Ab positive subjects was non-significantly higher than the other group.

Pearce, *et al.* 2008 reported that first trimester serum TSH levels were higher ($p<0.001$) and serum T_4 levels and free T_4 index values were marginally lower in TPO-Ab positive women ($p=0.03$ and $p=0.06$, respectively) compared with TPO-Ab negative women.

Table 4.27 Median thyroid function tests in group I B TPO-Ab positive *vs.* TPO-Ab negative subjects during gestation

Analytes	Trimester	TPO-Ab negative (n=16)	TPO-Ab positive (n=8)
FT_3 (pM/L)	1st	4.44	3.48*
	2nd	3.95	3.64
	3rd	3.69	3.46
FT_4 (pM/L)	1st	14.65	10.74*
	2nd	14.41	15.03
	3rd	13.41	12.43
TSH (µIU/ml)	1st	4.53	5.51
	2nd	3.86	3.97
	3rd	3.52	3.31
TPO-Ab	1st	12.86	222.65**
	2nd	12.16	152.04**
	3rd	13.54	136.68**

* $p<0.05$; ** $p<0.001$

A study by Negro, *et al.* 2006 showed that antibody positive subjects had a higher TSH at baseline (though within the euthyroid range) as compared with antibody negative group. The results of this study suggest that subjects with the autoimmune thyroid disease may have a subtle deficiency of thyroid hormones due to impaired adaptability, and also implies that the judicious use of levothyroxine, at least in subjects with a high-normal TSH, could improve pregnancy outcomes. They suggested that the pregnant women with high anti-TPO titers should probably be treated even if the TSH is mildly raised or even in the high normal range.

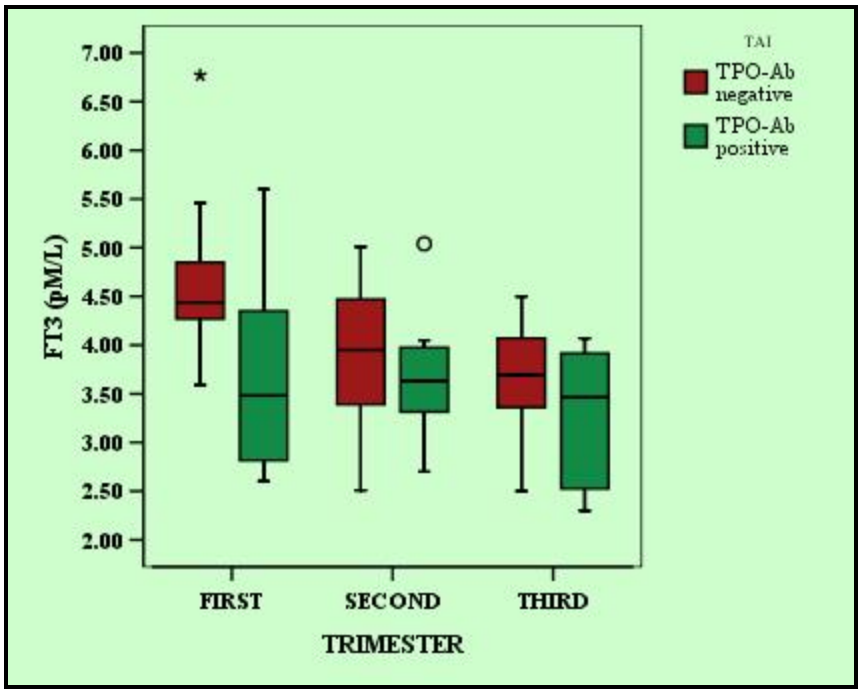


Figure 4.20 Median free triiodothyronine levels of group I B TPO-Ab negative *vs.* TPO-Ab positive subjects

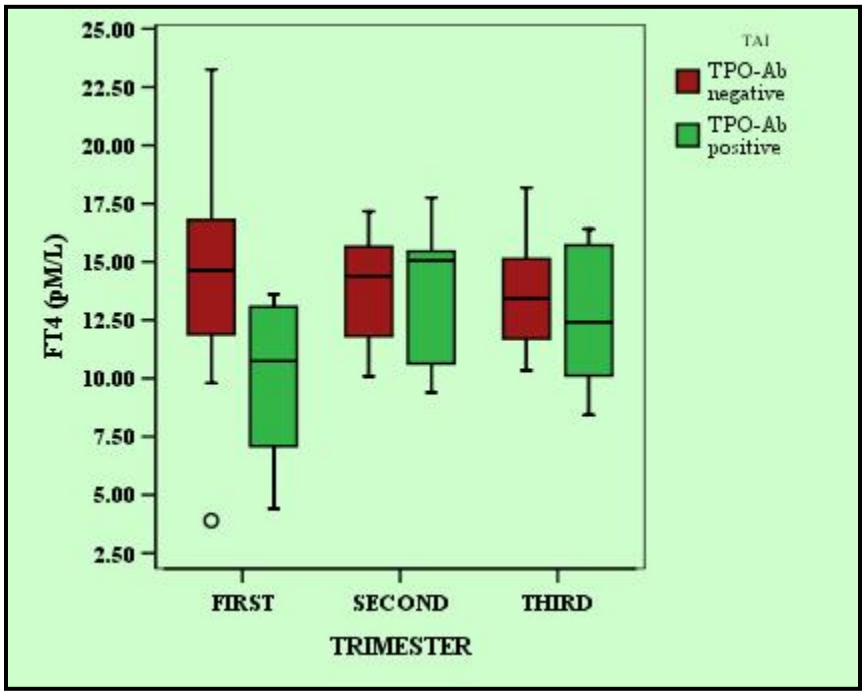


Figure 4.21 Median free thyroxine levels of group I B TPO-Ab negative *vs.* TPO-Ab positive subjects

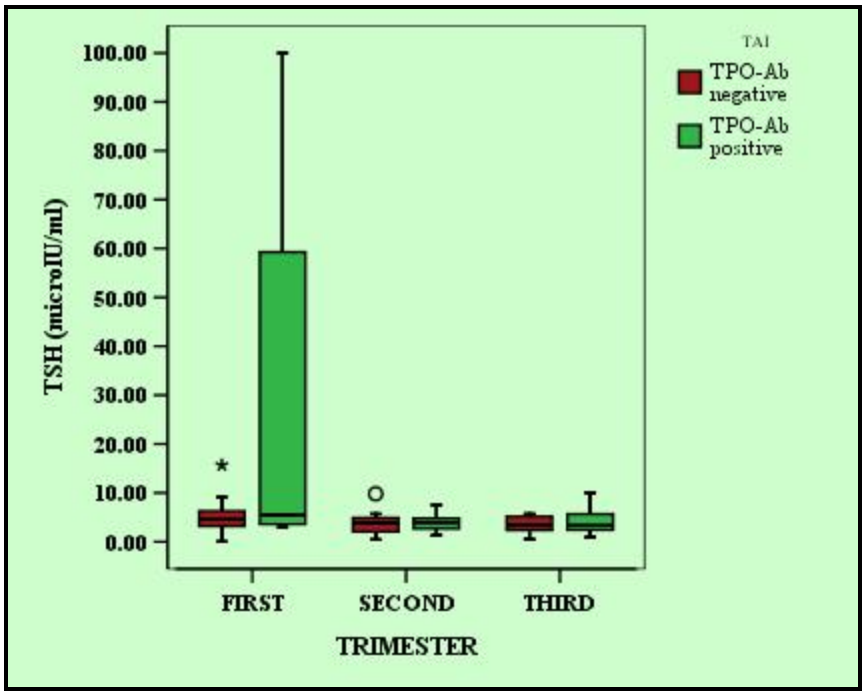


Figure 4.22 Median thyrotropin levels of group I B TPO-Ab negative *vs.* TPO-Ab positive subjects

4.2.2.5 Levothyroxine treatment

The levothyroxine dose was started in first trimester in majority (80%) of the subjects while only one subject received treatment in third trimester.

The L-T4 minimum dosage in first trimester was 0 µg/day whereas at the end of pregnancy it was 25 µg/day. The absolute percentage increase in dose during pregnancy was 45.58 µg/day and 25.09 µg/kg day (Table 4.28). L-T4 dose was increased in 16 out of 24 (66.7 percent) subjects and unchanged in rest of the subjects. Among the 16 patients who needed further increases of L-T4, seven (43.7 percent) reached the definitive therapeutic dosage within the second trimester and nine (56.3 percent) within the third. Final L-T4 doses in patients who reached a definitive therapeutic dosage within the second and third trimester of gestation were not statistically different (93.11±34.9 *vs.* 81.94±26.6 µg/day; p=ns).

However, Verga, *et al.* 2009 showed that the final L-T4 doses in patients who reached a definitive therapeutic dosage within the second and third trimester of gestation were statistically different ($p<0.05$).

Table 4.28 Initial and final L-T4 doses, TSH levels and FT_4 levels in patients (group I B) starting substitutive therapy during gestation

	Mean ± SD	Range
L-T4 starting doses (µg/day)	46.88 ± 32.39	0-100
L-T4 doses at the end of pregnancy(µg/day)	75.6 ± 32.2	25-150
Δ of L-T4 absolute doses (µg/day) (%) [#]	45.58 ± 49.4	0-150
L-T4 starting doses (µg/kg day)	0.94 ± 0.7	0-2.85
L-T4 doses at the end of pregnancy(µg/kg day)	1.3 ± 0.63	0.28-3.06
Δ of L-T4 absolute doses (µg/kg day) (%)	25.09 ± 43.01	18.55 to 121.84
TSH levels at the first evaluation(µIU/ml)	13.54 ± 26.9	0.03-100
TSH levels at the end of pregnancy (µIU/ml)	3.86 ± 2.05	0.51- 10.02
FT_4 levels at the first evaluation (pM/L)	12.07 ± 5.06	9.9-23.26
FT_4 levels at the end of pregnancy (pM/L)	13.25 ± 2.5	8.42-18.19

[#] $\Delta\% = [(L-T4\ final\ dose/L-T4\ starting\ dose\ \times 100) - 100]$

Ref: (Verga, et al. 2009)

4.2.2.6 Pregnancy Outcome of Group I B subjects

Details of 17 subjects were available out of which one had preterm delivery. The characteristics of these subjects are presented in table 4.29. All of them delivered in hospital.

Table 4.29 Characteristics of the group I B neonates (n=16)

	Frequency (Percentage)
Sex of the baby	
Male	9 (58.8)
Female	7 (41.2)
Type of delivery	
Normal	10 (58.8)
Cesarean	5 (29.4)
Other	1 (11.8)

Value in parenthesis indicate percentage

The mean APGAR score of the neonates was 7.71, 8.5 and 8.93 at 1, 5 and 10 minute, respectively. The mean birth weight and length of the neonates was above normal (Table 4.30).

Table 4.30 Mean parameters of group I B neonates (n=16)

Parameters	Mean ± SD
Gestational age at delivery (weeks)	39.38 ± 1.02
Birth weight (kg)	3.07 ± 0.42
Birth length (cms)	48.88 ± 1.89
Head circumference (cms)	33.02 ± 1.82

The data when classified according to Z-scores showed that all the neonates had normal height-for-age and weight-for-age whereas one male and one female neonate was respectively, severely and moderately stunted (Table 4.31).

Thyroid function tests of neonates

There was a wide variability in cord blood FT_4 and TSH. Table 4.32 shows the important characteristics of both FT_4 and TSH.

When the neonates were categorized according to WHO criteria to define severity of IDD and their control in a population, 37.5 percent of the subjects had >10 µIU/ml cord blood TSH (Fig. 4.23). This indicates moderate iodine deficiency.

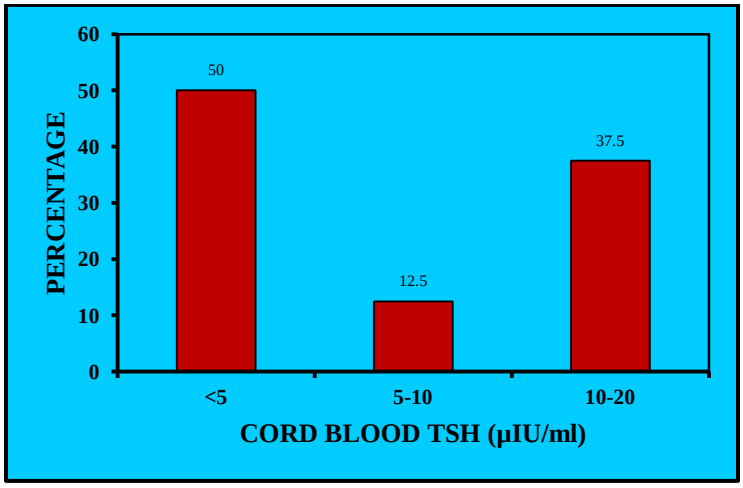


Figure 4.23 Distribution of group I B neonates according to cord blood TSH level

Table 4.31 Nutritional status of group I B neonates based on Z-scores

Sex	n	Height-for-age				Weight-for-height				Weight-for-age			
		<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD
M	9	-	-	9 (100)	-	1 (11.1)	-	8 (88.9)	-	-	-	9 (100)	-
F	7	-	-	7 (100)	-	-	1 (14.3)	6 (85.7)	-	-	-	7 (100)	-
T	16	-	-	16 (100)	-	1 (6.3)	1 (6.3)	14 (87.4)	-	-	-	16 (100)	-

Value in parenthesis indicate percentage

Table 4.32 Serum FT_4 and TSH levels in cord blood of group I B neonates

Parameters	Mean ± SD	Median	Range
Cord blood FT_4 (pM/L)	14.88 ± 2.73	15.2	9.87-19.5
Cord blood TSH (µIU/ml)	9.52 ± 5.36	9.95	1.4-19.2

Relationship between neonatal parameters and neonatal thyroid function tests

There was no significant correlation between neonatal parameters such as head circumference, birth length, birth weight and thyroid function tests i.e. cord blood FT_4 and TSH (Table 4.33). A significant positive correlation was found between length and weight ($r=0.766$, $p<0.01$) (Fig. 4.24), length and head circumference ($r=0.806$, $p<0.01$) (Fig. 4.25) and birth weight and HC ($r=0.590$, $p<0.05$) (Fig. 4.26).

However, Shields, *et al.* 2011 reported that cord FT_4 is significantly associated with birth weight, length and head circumference whereas there was no association between birth measurements and cord TSH.

Table 4.33 Correlation between neonatal parameters and thyroid function tests of group I B subjects

Parameters	Cord blood FT_4	Cord blood TSH
Head Circumference	0.217	-0.121
Length	0.042	-0.050
Weight	0.1	0.009

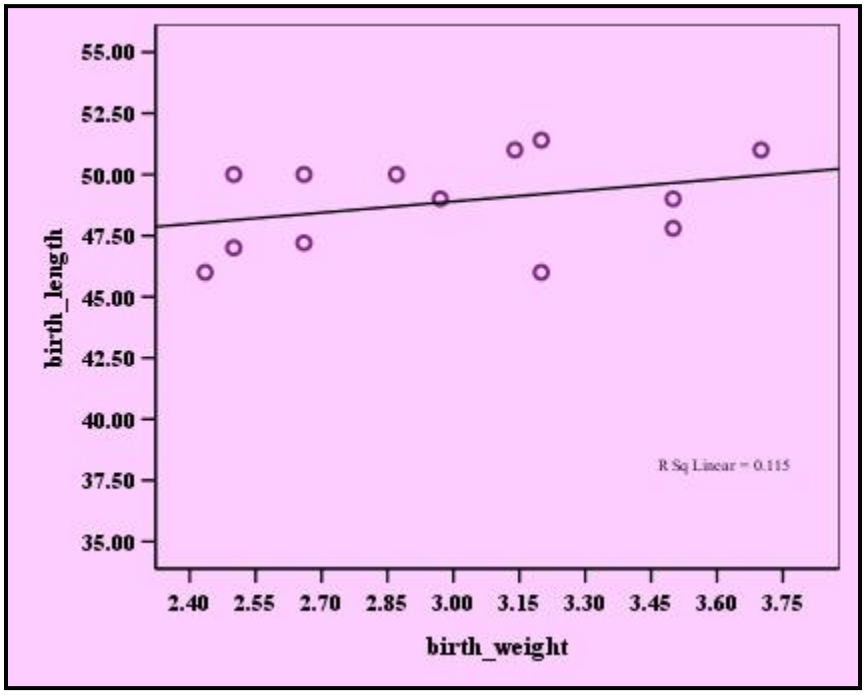


Figure 4.24 Relationship between birth length and weight of group I B neonates

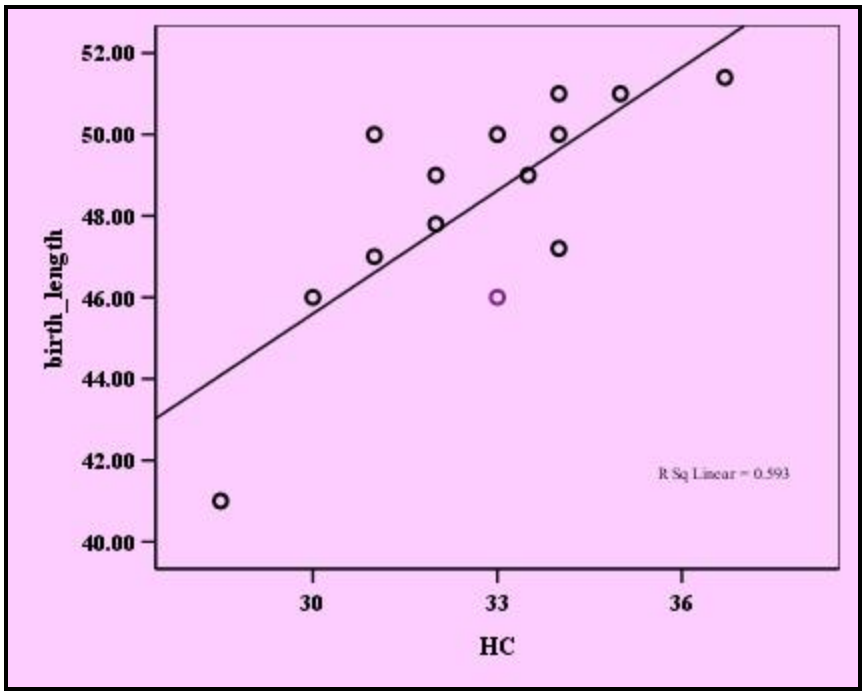


Figure 4.25 Relationship between birth length and head circumference of group I B neonates

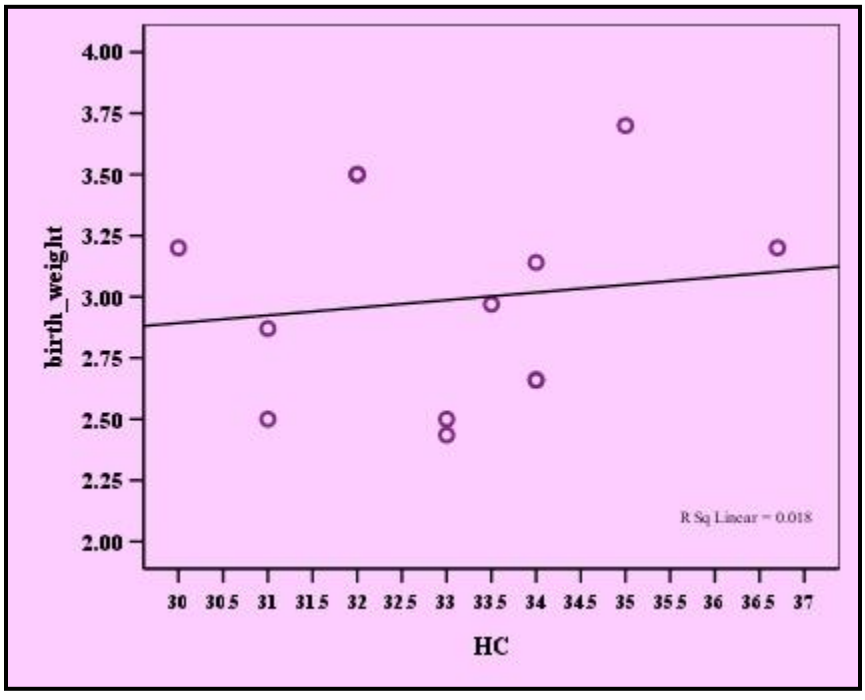


Figure 4.26 Relationship between birth weight and head circumference of group I B neonates

Maternal thyroid function parameters and neonate thyroid function

A significant negative correlation was observed between cord blood FT_4 and cord blood TSH while there was no significant correlation between maternal thyroid parameters and cord blood thyroid function tests (Table 4.34 & 4.35). Neonatal thyroid status was non-significantly correlated with urinary iodine concentration of mother.

Shields, *et al.* 2011 reported weaker negative associations between maternal TSH and cord FT_4 and FT_3 whereas a highly significant correlation was found between maternal FT_4 and cord blood FT_4.

Alvarez-Pedrerol, *et al.* 2009 found that the UIC levels during the third trimester of pregnancy were related to birth weight of the neonates. In the multivariate model, women with UICs between 100 and 149 µg/L in the third trimester had the lowest risk of having a small for gestational age (SGA) infant, and their babies had higher mean birth weights than those with UICs below 50 µg/L. Birth weights among women with UICs between 50 and 99 µg/L in the third trimester were also significantly higher than those among women with UICs below 50 µg/L. However, for women with UICs in categories above 150 µg/L, the increase in adjusted mean birth weight and the reduced probability of having an SGA infant did not reach significance. UICs at the first trimester showed similar findings than UICs at the third trimester, particularly for birth weight, although differences were not statistically significant. Higher TSH levels were also associated with a higher risk of having an SGA baby or having a newborn with a lower birth weight; nonetheless, this association was observed only when women with all the measurements were analyzed. The iodine requirements during pregnancy increases since there is an increment in TH synthesis to provide for the needs of the fetus, and there is an increased loss of iodine in the urine resulting from an increased renal clearance during pregnancy. Thyroid hormones play a key role in growth and bone development either indirectly or directly and the disruption of the hypothalamic- pitutary- thyroid axis during growth profoundly influences skeletal development. The expression of TH receptors in bone cells as the responsiveness of TH in cell culturing systems is the evidence of an effect of TH on bone tissue.

Orito, *et al.* 2009 concluded that serum concentrations of TSH during early pregnancy in normal women had no relevance to parameters in neonates. Chan, *et al.* 2003, Jaruratanasirikul, *et al.* 2009 showed that there was no significant correlation between neonatal TSH and maternal urine iodine content. However, Ardawi, Nasrat and Mustafa 2002 found negative correlation between maternal FT_4 and the neonatal TSH.

Table 4.34 Correlation between maternal thyroid function tests (first trimester) and fetal thyroid function tests of group I B subjects

	FT_3	**FT_4**	**TSH**	**UIC**	**Cord blood FT_4**	**Cord blood TSH**
FT_3	1					
FT_4	0.628**	1				
TSH	-0.523**	-0.638**	1			
UIC	-0.102	-0.536**	0.248	1		
Cord blood FT_4	-0.247	-0.239	0.073	0.486	1	
Cord blood TSH	0.120	0.131	0.024	-0.202	-0.634**	1

** $p<0.01$; * $p<0.05$

Table 4.35 Correlation between maternal thyroid function tests (third trimester) and fetal thyroid function tests of group I B subjects

	FT_3	**FT_4**	**TSH**	**UIC**	**Cord blood FT_4**	**Cord blood TSH**
FT_3	1					
FT_4	0.277	1				
TSH	-0.266	-0.385*	1			
UIC	0.123	-0.280	0.209	1		
Cord blood FT_4	0.100	0.024	-0.106	-0.335	1	
Cord blood TSH	-0.163	0.069	-0.050	0.564	-0.634*	1

* $p<0.05$

4.3 GROUP II: SUBJECTS ALREADY ON HORMONE REPLACEMENT THERAPY

The subjects in this group were subdivided into two groups based on the type of thyroid dysfunction. Majority of the patients were hypothyroidic (Group II A) and were taking levothyroxine. The baseline characteristics of both the groups were almost similar (Table 4.36).

Table 4.36 Baseline characteristics of the group II subjects (Mean ± SD)

Characteristic	Hypothyroidic patients Group II A (n=135)	Hyperthyroidic patients Group II B (n=10)
Age (yrs)	27.93 ± 4.1	25.6 ± 2.7
Height (cms)	154.7 ± 5.9	151.6 ± 4.5
Parity	0.89 ± 0.78	0.7 ± 0.48
Abortion	0.75 ± 1.01	0.3 ± 0.67
Baseline mean FT_4 (pM/L)	16.95 ± 2.3	17.32 ± 4.7
Baseline mean TSH (μIU/ml)	2.27 ± 1	1.62 ± 1.2

The body mass index of the subjects at the time of registration revealed that 34 percent in Group II A and 60 percent in Group II B were normal (Table 4.37).

Table 4.37 Distribution of group II subjects in relation to body mass index at the time of registration

Gestational age	GROUP II A Level of BMI (kg/m^2)				GROUP II B Level of BMI (kg/m^2)			
	< 18.5	18.5-25	>25	Mean	< 18.5	18.5-25	>25	Mean
< 1 month	-	3 (42.9)	4 (57.1)	26.48 ± 4.1	1 (50)	1 (50)	-	18.46 ± 0.24
1-2 month	20 (25)	23 (28.7)	37 (46.3)	26.36 ± 4.9	-	4 (66.7)	2 (33.3)	23.52 ± 2.3
2-3 month	4 (8.5)	19 (40.4)	24 (51.1)	24.5 ± 8.04	-	1 (50)	1 (50)	22.65 ± 4.9

Value in parenthesis indicate percentage

The average weight gain of pregnant women throughout pregnancy was below the recommended weight gain (Institute of Medicine 2009) for both the groups (Table 4.38). Symptoms of hypothyroidism, such as tiredness and weight gain are also quite common in pregnant women so these should not be overlooked. In our study the thyroid hormone levels of the subjects was constantly monitored and kept within the reference limits so we can say that the weight gain during pregnancy was due to increased energy intake and not due to thyroid hormone deficiency. If there are insufficient amount of thyroid hormones, the metabolism decreases and the person gains weight.

Table 4.38 Average weight gain of group II pregnant women throughout pregnancy

BMI at the time of registration	GROUP II A	GROUP II B	Recommended weight gain (kg)
Low (< 18.5)	7.67 ± 3.1	5	12.5 - 18
Normal (18.5-25)	7.80 ± 2.7	5.32 ± 2.3	11.5 - 16
High (>25)	6.91 ± 3.1	8.70 ± 0.4	7 - 11.5

Half of the subjects in both the groups at the time of registration were anemic but only 2 subjects in group II A had severe anemia (Fig. 4.27).

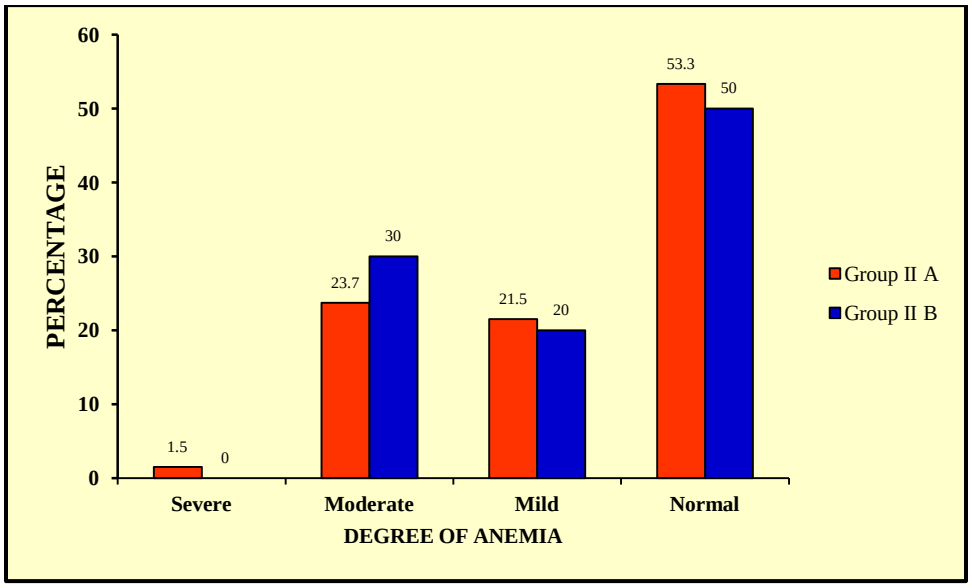


Figure 4.27 Distribution of group II pregnant women according to hemoglobin level at the time of registration

4.3.1 GROUP II A: HYPOTHYROIDIC SUBJECTS (n=135)

The median duration of hypothyroidism before conception was four years (<1 year to 25 year). The disease history of the following subjects revealed that 62 subjects had history of repeated abortions and 36 percent had family history of thyroid disease (Table 4.39). Abalovich, Gutierrez, *et al.* 2002 have reported that miscarriage rate was as high as 60-70 percent in hypothyroidic subjects whereas Mannisto, Varrasmaki, *et al.* 2009 showed that 16 percent of subclinical hypothyroidic and 22.2 percent of overt hypothyroidic subjects had miscarriage.

Table 4.39 Prior disease history of the Group II A subjects

Prior disease history	Frequency	Percentage (%)
Any known Complication	10	7.4
History of repeated abortions	62	45.9
Any known autoimmune disorder	85	63
Known thyroid disease	-	100
Family history of thyroid disorder	50	36.3

Out of all the subjects, 99 (73.9 percent) had overt hypothyroidism while 26.1 percent had subclinical hypothyroidism (SCH) according to the first time they were diagnosed and prior to starting of medicine. The baseline (before conception) levothyroxine (L-T4) dose was 87.94 ± 37.6 (range 25 to 200 µg/day).

The median urinary iodine (169.9 µg/L) of the population at the time of registration showed that the population was iodine sufficient but 35.1% of the subjects had urinary iodine concentration below normal levels (<150 µg/L).

Out of 134 subjects, 18 had abortions in third or fourth month and some subjects dropped out of study after one or two visits. Therefore, complete data of 92 subjects was available which was analyzed further.

GROUP II A WITH COMPLETE THREE TRIMESTERS DATA (n=92)

4.3.1.1 Baseline characteristics

The mean age and height of these subjects was 27.8 ± 4.1and 155.1±6.1 cm, respectively. The baseline (pre pregnancy) mean TSH was within the reference range (Table 4.40). The number of thyroid function tests performed in these women ranged from 3 to 8 (median 5).

Table 4.40 Baseline characteristics of group II A hypothyroidic patients with complete three trimesters data (n=92)

Characteristic	Mean ± SD (Range)
Age (yrs)	27.8 ± 4.1 (19-39)
Height (cms)	155.1 ± 6.1 (136.5-167.5)
Parity	0.89 ± 0.75 (0-4)
Abortion	0.72 ± 0.95 (0-5)
Baseline mean TSH (µIU/ml)	2.3 ± 0.99 (0.6-4.2)
Baseline mean FT_4 (pM/L)	16.88 ± 2.2 (11.9-21.4)
Baseline L-T4 dose (µg/day)	88.3 ± 35.4 (0-200)

4.3.1.2 Groups formed according to dose

The women were again subdivided according to the change in dose. Majority of the women (n=59) had to increase their dose one or more times during the course of pregnancy (Table 4.41). Hallengren, *et al.* 2009 reported that of the 63 patients on thyroxine substitution for hypothyroidism forty two (67 percent) had to increase their thyroxine dose during pregnancy whereas according to Alexander, *et al.* 2004 serum thyrotropin level increased during the first 10 weeks of gestation, prompting an increase in the dose in 85 percent of the subjects.

In sixty three subjects out of ninety two (68.5 percent) the TSH test values shifted outside the reference range (>2.5 µIU/ml) whereas rest of them had TSH within the normal range.

Table 4.41 Distribution of group II A patients according to dose increment during pregnancy (n=92)

Groups	Status of dose	Number (%)
a	No increase in dose	30 (32.6)
b	Dose increased during pregnancy	59 (64.1)
c	Dose decreased during pregnancy	1 (1.1)
d	Dose increased then decreased during pregnancy	2 (2.2)

The baseline characteristics of group 'a' and group 'b' patients are shown in table 4.42. There was no significant differences between the pregnancies where the doses had to be increased (Group b) and those where it did not (Group a) with regard to mean age (p=0.124), mean parity (20.3 percent nulliparous versus 16.7 percent nulliparous; p=0.615), mean body mass index (p=0.354) and median duration of hypothyroidism (p=0.393). Approximately seventy percent of the subjects in both the groups had either grade 1 or 2 goiter (Fig. 4.28). Not surprisingly, pregnancies where the dose has to be altered had more thyroid function tests (TFT) performed than those who did not (median 3 tests [3-8] versus 3 tests [3-4] tests, respectively; p <0.05). Similarly, (Kothari and Girling 2008) showed no significant difference in age, parity, BMI and duration of hypothyroidism between the pregnancies where the doses had to be changed and those where it did not.

Table 4.42 Baseline characteristics of Group 'a' and Group 'b'

Group	Age (Mean ± SD)	Parity (Mean ± SD)	BMI (Median) (kg/m^2)	Median duration of hypothyroidism (year) (Range)
a (n=30)	27.6 ± 3.7	0.77 ± 0.57	26.09	3 (0-15)
b (n=59)	27.83 ± 4.4	0.9 ± 0.78	24.69	4 (0-25)

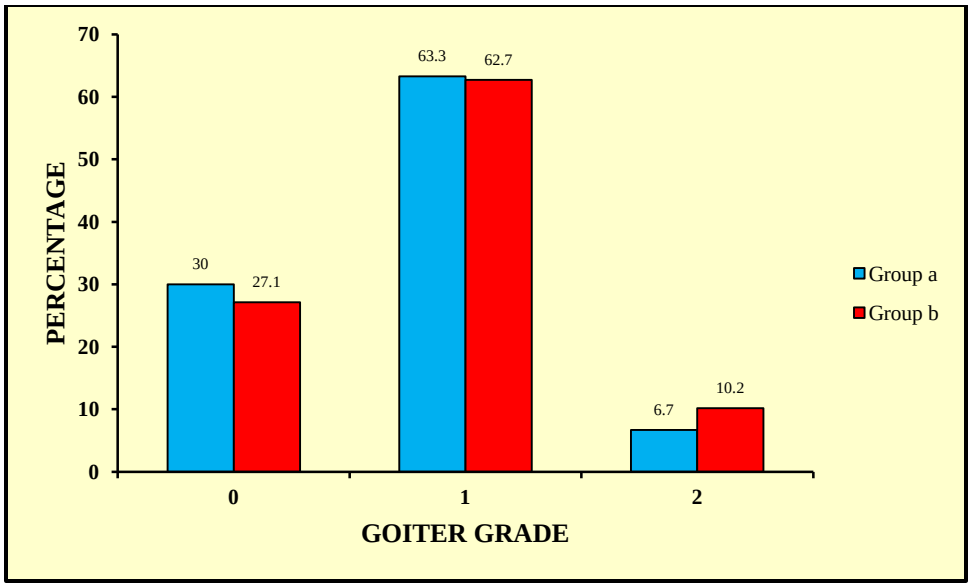


Figure 4.28 Prevalence of goiter grade in group 'a' and 'b'

Comparison of thyroid function tests and levothyroxine dose between groups 'a' & 'b'

In both group 'a' and 'b' the mean TSH, FT_4 and median L-T4 was compared pre-pregnancy, throughout pregnancy and postpartum, and it was found that pre-pregnancy values were almost similar. The mean TSH was significantly ($p<0.05$) higher during all the trimesters in the group whose dose had to be increased during pregnancy (Table 4.43 a) whereas postpartum the mean TSH significantly decreased (Table 4.43 b, Fig. 4.29). There was no significant difference found between FT_4 values (Fig. 4.30). The median L-T4 dose was significantly higher by 25 percent in group 'b' when compared with group 'a' (Fig. 4.31). Our results are supported by the results of Abalovich, Alcaraz, *et al.* 2010 that serum TSH was significantly higher ($p<0.005$) in group requiring dose increment during pregnancy as compared to group who need not increase the dose whereas no significant difference was found between FT_4.

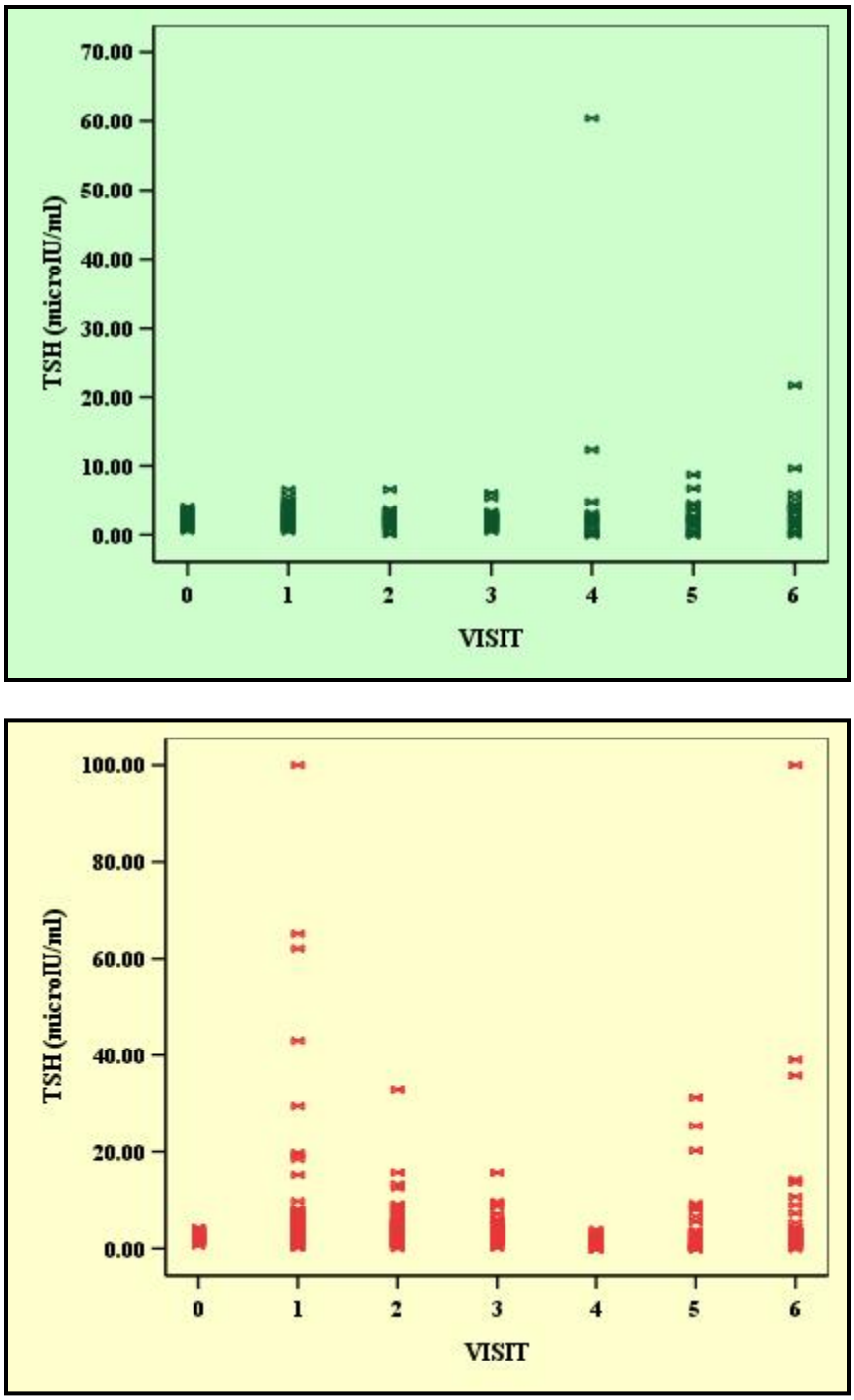
Table 4.43(a) Mean TSH (µIU/ml), FT_4 (pM/L) and median L-T4 (µg) pre-pregnancy and in each trimester in pregnancies of women with hypothyroidism according to whether dose alteration was done or not

Group	Pre-pregnancy			1st trimester			2nd trimester			3rd trimester		
	TSH	FT_4	L-T4	TSH	FT_4	L-T4	TSH	FT_4	L-T4	TSH	FT_4	L-T4
a (n=30)	2.31±1.01 (0.6-4.1)	17.02±2.4 (11.9-21.4)	100 (25-150)	3.56±4.8 (0.50-6.58)	16.35±3.2 (9.72-23.17)	100 (25-150)	2.18±1.3 (0.07-6.6)	15.71±2.3 (10.85-20.13)	100 (25-150)	2.23±1.2 (0.53-6.07)	14.52±4.4 (11.5-23.39)	100 (25-150)
b (n=59)	2.37±1.01 (0.66-4.2)	16.71±2.2 (12.34-21.10)	100 (25-150)	9.7±17.4* (0.23-65.1)	15.84±4.1 (0.97-25.15)	100 (25-150)	4.57±4.9* (0.03-32.85)	14.88±2.7 (10.75-24.15)	125* (37.5-175)	3.87±2.8* (0.20-15.67)	14.97±2.6 (10.05-23.5)	125* (50-200)

Table 4.43(b) Mean TSH (µIU/ml), FT_4 (pM/L) and median L-T4 (µg) postpartum in pregnancies of women with hypothyroidism according to whether dose alteration was done or not

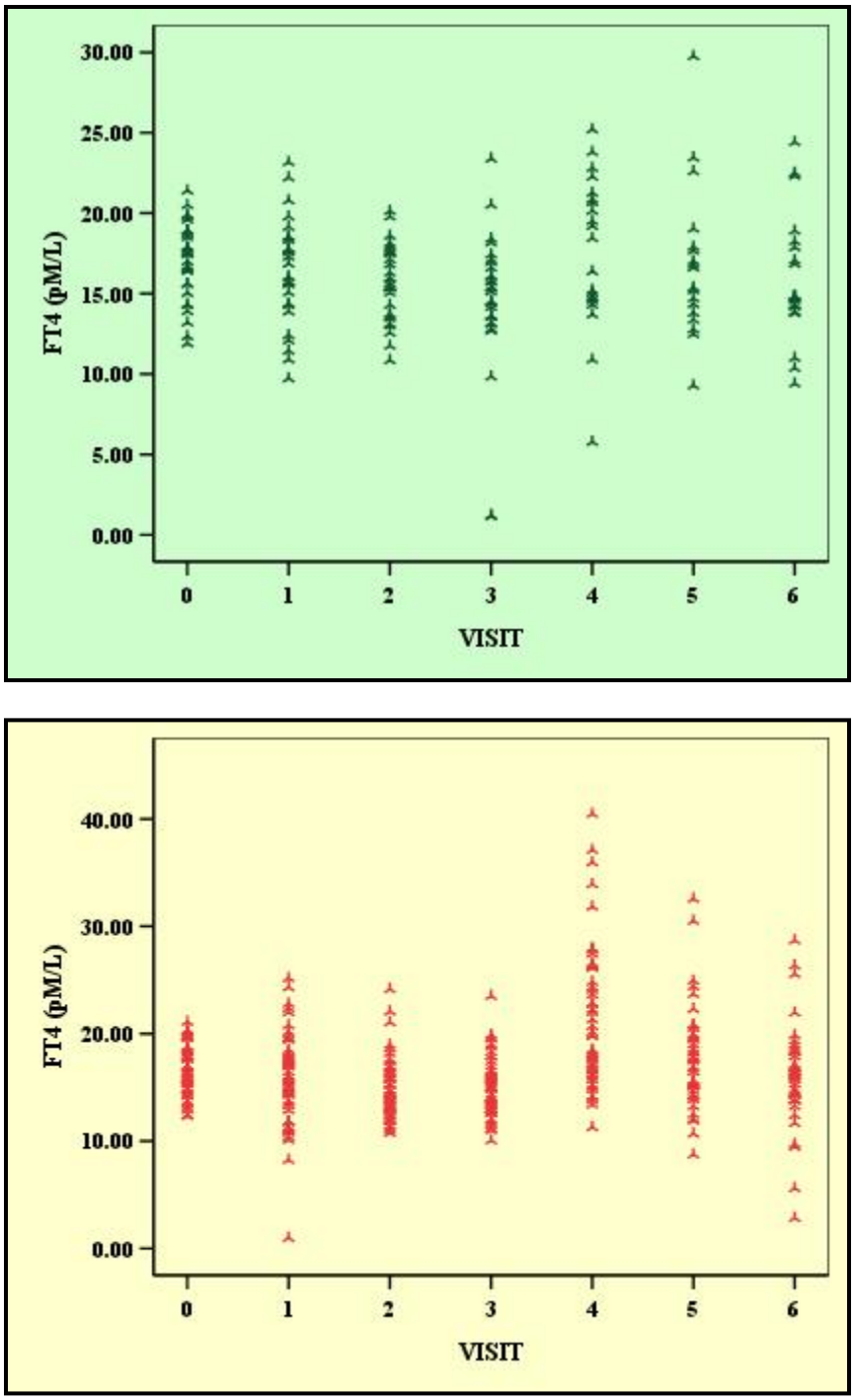
Group	1st postpartum			2nd postpartum			3rd postpartum		
	TSH	FT_4	L-T4	TSH	FT_4	L-T4	TSH	FT_4	L-T4
a (n=30)	4.37±12.8 (0.01-60.44)	17.59±4.7 (5.77-25.2)	75 (25-150)	7.92±19.41 (0.01-8.75)	16.78±4.7 (9.28-29.76)	75 (25-125)	5.59±10.1 (0.01-21.7)	15.81±3.8 (9.4-24.41)	86.85 (25-132.4)
b (n=59)	0.72±1.0* (0.01-3.87)	21.51±6.6* (11.28-40.46)	100* (25-200)	3.64±6.4* (0.01-31.2)	17.51±4.6 (8.74-32.55)	100* (25-200)	6.99±16.2 (0.01-39)	15.99±4.5 (2.83-28.68)	100 (25-150)

* $p<0.05$ when compared to group a; *Value in parentheses indicate range*



0=Before pregnancy; 1= 1st trimester; 2= 2nd trimester; 3= 3rd trimester; 4= 1st postpartum; 5= 2nd postpartum; 6= 3rd postpartum

Figure 4.29 Scatter plot depicting before pregnancy, trimester and postpartum variation of TSH levels in group 'a' (upper) and group 'b' (lower)



0=Before pregnancy; 1= 1st trimester; 2= 2nd trimester; 3= 3rd trimester; 4= 1st postpartum; 5= 2nd postpartum; 6= 3rd postpartum

Figure 4.30 Scatter plot depicting before pregnancy, trimester and postpartum variation of FT_4 levels in group 'a' (upper) and group 'b' (lower)

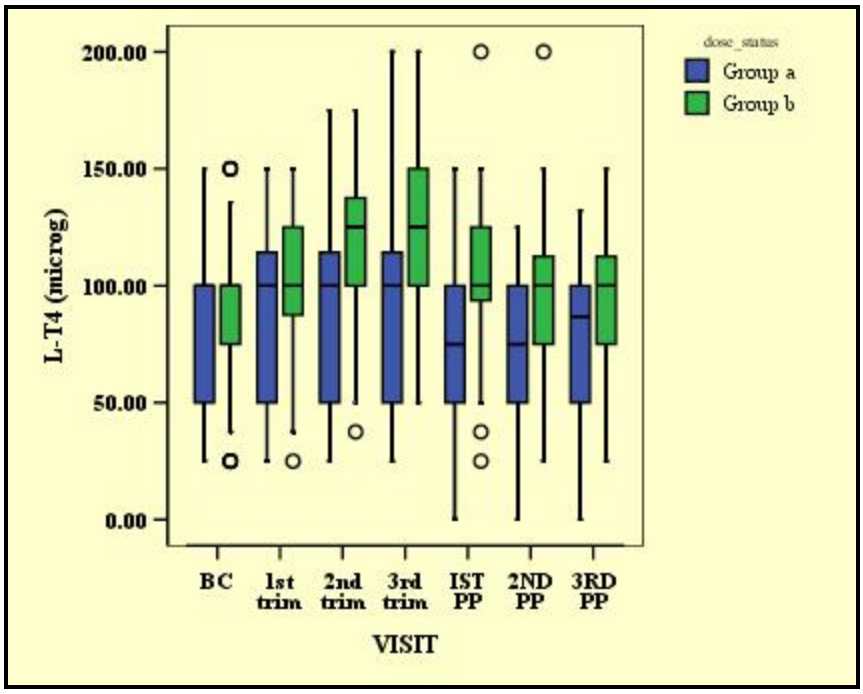


Figure 4.31 Median and interquartile ranges of L-T4 in group 'a' and 'b' subjects before pregnancy, trimesters and postpartum

Etiology of hypothyroidism

Twenty-three percent subjects of group 'a' were subclinical hypothyroidic while 76.2 percent in group 'b' were overtly hypothyroidic. Nearly half of the patients in both the subgroups were TPO-Ab positive (Table 4.44).

Table 4.44 Distribution of patients in group 'a' and group 'b' according to hypothyroidism etiology

Group	SCH		OH	
	TPO positive	TPO negative	TPO positive	TPO negative
a	4 (13.3)	3 (10)	11 (36.7)	12 (40)
b	4 (6.8)	10 (16.9)	34 (57.7)	11 (18.6)

Value in parenthesis indicate percentage

4.3.1.3 Adjustment of levothyroxine dose

Out of the 59 subjects, 40 subjects (67.8 percent) required dose increment in first trimester (Fig. 4.32). Dose of 16.9 percent had to be adjusted in all the three trimesters

while 49.2 percent required dose increment in either of the one trimester only (Table 4.45). In a study by Hallengren, *et al.* 2009 approximately 65 percent of the subjects required first dose change at gestational week 11 (median 3-32 weeks). The dose was increased on two or three occasions in 10 out of 52 (19.2 percent) subjects and the second and third changes made during gestational week 11-35.

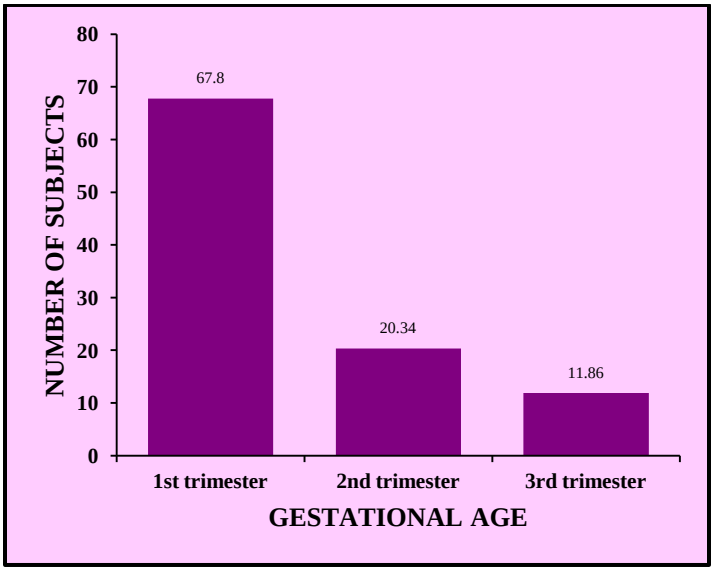


Figure 4.32 Distribution of group 'b' subjects according to initial increase in L-T4 dose

Table 4.45 Distribution of patients who required dose increment during pregnancy (group b)

Dose adjustment	No. of patients (Percentage)
First trimester only	14 (23.7)
First and second trimester	9 (15.3)
First and third trimester	7 (11.9)
All the three trimesters	10 (16.9)
Only second trimester	8 (13.6)
Second and third trimester	4 (6.8)
Third trimester only	7 (11.9)

Table 4.46 shows that the mean TSH and L-T4 dose significantly increased during gestation as compared to before pregnancy while FT_4 significantly decreased. The mean

TSH significantly increased but FT_4 non-significantly decreased in first trimester as compared to before pregnancy values. The mean TSH decreased significantly ($p<0.05$) from first to second trimester by 53 percent and then decreased non-significantly. There was non-significant change in FT_3 values. The mean L-T4 dose significantly ($p<0.05$) increased by 17.7 percent from the start of pregnancy till the end of pregnancy. Idris, *et al.* 2005 reported that the mean and median thyroxine hormone replacement dose of pregnant subjects increased by 25.8 percent and 50 percent respectively, from initial presentation to the beginning of the third trimester.

At 12 weeks of gestation, the levothyroxine dose had increased by 26±40 percent ($p<0.005$) when compared with the dose at baseline. At 20 weeks, the increase relative to baseline was 39 percent ($p<0.001$) and fifty three percent ($p<0.001$) by third trimester. Similar results were found by Alexander, *et al.* 2004 that at 10 weeks of gestation, the levothyroxine dose had increased by 29±25 percent ($p<0.005$) and at 20 weeks the increase was 48 percent ($p<0.001$) when compared with the dose at baseline. The dose remained stable thereafter.

On an average, the entire group 'b' required a cumulative increase in thyroid hormone dosage from baseline of 14.3 percent in the first trimester, 33 percent in the second trimester and 37.5 percent in the third trimester (Mann-Whitney U test, $p=0.02$, $p=0.00$, $p=0.00$, respectively) (Table 4.47). Loh JA, *et al.* 2009 showed that on an average, patients with hypothyroidism required a cumulative increase in thyroid hormone dosage from baseline of 13 percent, 26 percent and 26 percent in the first, second and third trimester, respectively ($p<0.001$, $p<0.001$, $p<0.001$, respectively).

In our study it was found that the median cumulative increase in L-T4 dose from baseline in OH subjects was statistically significant ($p<0.005$ in all the three trimesters) whereas in SCH subjects it was non-significant ($p=0.194$) in the first trimester and significant ($p=0.035$, $p=0.027$) in second and third trimester, respectively. The median cumulative percentage L-T4 dose increase in subclinical hypothyroidic patients was high in comparison to overt hypothyroidic subjects (Fig. 4.33). The difference in median L-T4 dose between both the groups was non-significant. Abalovich, Alcaraz, *et al.* 2010 also reported that during pregnancy within the group needing to increase their L-T4 dose,

Table 4.46 Serum values before and during gestation in all women who required an increase in the dose of L-T4 during gestation (Group b)

Variable	Week of Gestation				p-value	p-value by Scheffe post-hoc test					
	BC	12 week	20 week	31 week		BC *vs.* 12 wk	BC *vs.* 20 wk	BC *vs.* 31 wk	12 *vs.* 20 wk	12 *vs.* 31 wk	20 *vs.* 31wk
TSH (µIU/ml)	2.37±1.0	9.71±7.4	4.56±4.9	3.88±2.8	0.000	0.000*	0.642	0.852	0.028*	0.009*	0.983
FT_4 (pM/L)	16.7±2.2	15.84±4.1	14.88±2.7	14.97±2.6	0.003	0.481	0.013*	0.020*	0.381	0.469	0.999
FT_3(pM/L)	-	3.88±0.8	3.81±0.7	3.59±0.6	0.087	-	-	-	0.888	0.11	0.26
L-T4 dose	87.63±33.1	106.45±33.8	115.56±33.9	125.3±37.4	0.000	0.035*	0.000*	0.000*	0.563	0.035*	0.506
L-T4 dose (fraction of dose before pregnancy	1.00±0	1.26±0.4	1.39±0.4	1.53±0.47	0.000	0.002*	0.000*	0.000*	0.379	0.002*	0.230

* p<0.05; BC= Before conception

Table 4.47 Variation in L-T4 dose in Group 'b' patients according to whether they have subclinical or overt hypothyroidism

Type of hypothyroidism	L-T4 dose preconception	L-T4 dose 1st trimester	L-T4 dose 2nd trimester	L-T4 dose 3rd trimester
SCH (n=14)				
Median	87.5	100	113.4*	119.65*
Range	25-125	25-150	37.5-150	50-200
OH (n=45)				
Median	100	112.5†	125†	125†
Range	37.5-150	37.5-150	50-175	75-200

* p<0.05 *vs.* SCH preconception dose; † p<0.05 *vs.* OH preconception dose

patients with SCH required a greater increase than those with OH. This may be due to the fact that the mean value of pre-conception serum TSH on L-T4 in the patients with SCH was higher than in the patients with OH. However, the number of patients with OH was quite small. Therefore, initial TSH levels in the SCH and OH groups cannot be reliably compared, and the finding that patients with SCH required a greater increase in their L-T4 dose than those with OH may not be a reliable observation.

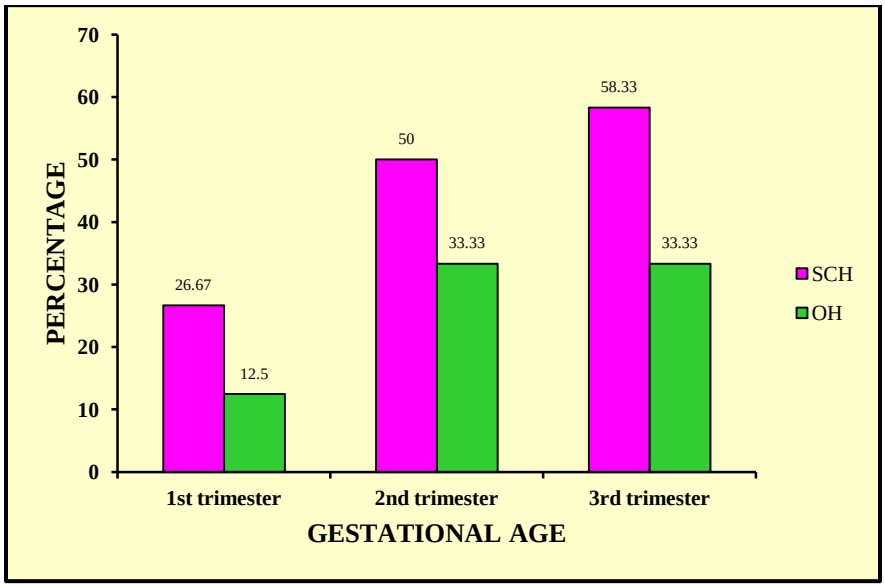


Figure 4.33 Median cumulative levothyroxine dose increase (percent) in trimesters calculated from baseline

Between the SCH and OH group comparison, the OH group before pregnancy and final L-T4 dose (µg/day) was significantly higher by 33.9 percent and 20.7 percent, respectively (Table 4.48) when compared to SCH group. The Δ% of absolute doses (expressed as µg/day or µg/kg day) was significantly different in the two groups of patients as compared to baseline dose. It was observed that the increment in Δ% of absolute doses was higher in SCH than OH group. The serum FT_4 and TSH values did not show any significant difference.

Similar results were reported by Verga, *et al.* 2009 that initial and final L-T4 doses (either as µg/day or µg/kg day), as well as Δ% of absolute doses, were significantly different in the three group of patients [SCH, OH and post-ablative (PH) hypothyroidism].

After dividing the subjects according to the time they reached definitive therapeutic dose, it was revealed that 23.7 percent reached definitive dose in first trimester itself while 47.5 percent reached by third trimester (Table 4.49). Verga, *et al.* 2009 showed that among patients who increased L-T4 doses, 6 percent reached a definitive dosage within the 12th week of gestation, 47.8 percent within the 20th and 46.2 percent within the 31st week of gestation.

Table 4.48 Initial and final L-T4 doses and FT_4 levels in patients with subclinical (SCH) and Overt (OH) hypothyroidism already treated before pregnancy who increased the substitutive therapy (Group b)

	SCH (n=14)	**OH (n=45)**	**t-test**
L-T4 dose before pregnancy (µg/day)	69.64 ± 38.2 (25-125)	93.23 ± 29.6* (37.5-150)	0.048
L-T4 dose at the end of pregnancy (µg/day)	108.2 ± 43.2 (50-200)	130.63 ± 34.2* (75-200)	0.049
Δ of L-T4 absolute dose (µg/day) (%)	84 ± 69.6 (14-200)	47.5 ± 39.1* (8-167)	0.05
L-T4 doses before pregnancy (µg/kg day)	1.18 ± 0.68 (0.31-2.36)	1.54 ± 0.49* (0.43-2.7)	0.03
L-T4 doses at the end of pregnancy (µg/kg day)	1.61 ± 0.69 (0.57-2.68)	1.95 ± 0.66* (0.78-3.88)	0.01
Δ of L-T4 doses (µg/kg day) (%)	63.57 ± 62.79 (0.19-178.98)	31.5 ± 36.5* (0.83 to 136.8)	0.021
Serum FT_4 levels at the first evaluation (pM/L)	15.87 ± 3.2 (8.23-22.36)	15.83 ± 4.4 (0.97-25.15)	0.977
Serum FT_4 levels at the end of pregnancy (pM/L)	14.5 ± 2.26 (11.01-18.79)	15.11 ± 2.6 (10.05-23.5)	0.438
Serum TSH levels at the first evaluation (µIU/ml)	8.72 ± 16.34 (0.31-65.1)	10.07 ± 17.94 (0.23-100)	0.813
Serum TSH levels at the end of pregnancy (µIU/ml)	3.54 ± 2.16 (0.41-8.74)	3.98 ± 2.9 (0.20-15.67)	0.616

Data are expressed as mean ± SD and (range); * $p < 0.05$ *vs.* SCH

No significant difference was found between the three groups when comparing initial and final doses of L-T4 expressed either as µg/day or µg/kg day (Table 4.49). No significant difference was found in Δ% of L-T4 doses among these three groups. However Verga, *et al.* 2009 reported that Δ% of L-T4 doses were statistically different among the group of patients ($p<0.0005$) who reached definitive therapeutic dosage in first, second and third trimester.

Table 4.49 Initial and final L-T4 doses and delta (%) in Group 'b' patients divided in three groups depending on the week of gestation at which the definitive dose was reached

	Week of Gestation			p-value (ANOVA)
	12 (n=14)	20 (n=17)	31 (n=28)	
L-T4 dose before pregnancy (µg/day)	83.92 ± 28.8 (25-125)	95.79 ± 35.4 (25-150)	84.5 ± 33.9 (25-150)	0.491
L-T4 dose at the end of pregnancy (µg/day)	110.84 ± 28.9 (50-150)	126.57 ± 28.1 (75-175)	131.76 ± 44.5 (50-200)	0.232
Δ of L-T4 absolute dose (µg/day) (%)	44.71 ± 50.2 (13-200)	44.78 ± 45.47 (8-200)	68.79 ± 51.1 (10-200)	0.183
L-T4 doses before pregnancy (µg/kg day)	1.31 ± 0.49 (0.34-1.83)	1.51 ± 0.5 (0.58-2.48)	1.49 ± 0.63 (0.31-2.7)	0.584
L-T4 doses at the end of pregnancy (µg/kg day)	1.58 ± 0.52 (0.66-2.68)	1.77 ± 0.32 (1.06-2.31)	2.07 ± 0.84 (0.57-3.88)	0.065
Δ of L-T4 absolute dose (µg/kg day) (%)	32.29 ± 49.24 (0.03 to 178.98)	27.06 ± 38.3 (0.83 to 151.27)	49.87 ± 47.4 (0.49 to 173.07)	0.225

Data are expressed as mean ± SD and (range)

This shows that there is a wide variability in L-T4 doses, both before and during gestation in women affected with hypothyroidism of various etiologies. In 67.8 percent of patients already adequately treated before conception, L-T4 dosage was increased at the first evaluation during pregnancy by 27.76 ± 17.8 µg/day and subsequently one or more times, thus confirming that strict evaluation during pregnancy is crucial. An increase of 22.9 ± 9.8 µg/day was found by Verga, *et al.* 2009.

It is worth noting that only a minority of subjects did not require any dose adjustment and consistent number of patients reached the definitive dosage only at the second and third trimester of pregnancy. These findings support the previously reported data from Kaplan 1992 that up to 26 percent of women with an initial normal TSH level in the first trimester and 37 percent of those with an initial normal TSH level in the second trimester may require dosage increase.

One-fourth of the subjects who needed two or more L-T4 increments showed normal TSH levels at least once between two modifications of therapy. These findings suggest that the first adjustment of therapy is useful in obtaining TSH level in the optimal therapeutic range, but this modification cannot be considered definitive and the patient must be followed until the end of pregnancy.

According to the week of gestation in which patients reached adequate treatment, it is surprising that L-T4 final dose was lower in patients who reached a definitive dosage within the 12^{th} and 20^{th} than that of patients who reached a definitive therapeutic dosage within the 31^{st} week, despite the body weight increase.

The increased demand for thyroxine seen in our patients is a result of several physiological changes that occur with pregnancy. There are several possible mechanisms for an increased requirement at different times during gestation. In early pregnancy T_4 binding globulin increases due to increased synthesis and altered sialylation of TBG moiety resulting in decreased clearance of protein, leading to increased serum hormone binding capacity and an expansion in the extra-thyroidal thyroid hormone pool. Additionally, there is an increase in maternal plasma volume that occurs early in pregnancy and continues up until the time of delivery. Thus, more thyroid hormones are required during pregnancy. This may be the reason why majority of the patients require dosage increment in the first trimester itself. Later, with placental growth, there is increased T_4 metabolism to its inactive metabolite rT_3 by high levels of placental de-iodinase type III (Glinoer 1997). In addition, there is some placental transfer of maternal T_4 and a different distribution of thyroid hormones in the fetal/ placental unit (Zigman, Cohen and Garber 2003).

Initial and final L-T4 doses were significantly different depending on the etiology of the hypothyroidism. Women with OH required an initial increase of L-T4 substitutive doses by 40 percent, as already reported by others (Chopra and Barber 2003, Alexander, *et al.* 2004, Lazarus 2005). Patients with SCH showed the highest increments of L-T4 dosage, though a wide variability was observed (14 to 200 percent), with a mean dosage increment of 55.4 percent over the preconception replacement dosage. These results show that a consistent number of SCH patients with normal serum TSH and FT_4 circulating levels were possibly undertreated in the nonpregnant state.

Achieving euthyroidism in the first trimester of pregnancy should be the goal of management in hypothyroid pregnancy as this may be important in optimizing fetal neurodevelopment. The optimal timing for increasing L-T4 therapy is the first trimester of pregnancy, but many patients require adjustment of therapy also during the second and third trimester. In our study approximately 30 percent of women did not need a dose increment and only one patient needed a reduction in dose. Alexander, *et al.* 2004 concluded that as 9 of their 12 pregnant women on replacement doses of thyroxine needed to increase thyroxine in the first trimester, all women should increase their dose by 30 percent as soon as possible after conception. Yassa, *et al.* 2010 concluded that a 29 percent increase in maternal L-T4 (two extra tablets weekly) initiated at confirmation of pregnancy significantly reduces the risk of maternal hypothyroidism throughout the first trimester. Others have made similar recommendations, with 20-75% women increasing their dose in pregnancy, although the gestational age at which testing or dose adjustment are made is not clearly specified (Girling 2006). In contrast, Kothari and Girling 2008 reported in their study that 63 percent of the subjects did not require any change in dose and remained on a stable dose.

These finding suggest that a global increase in thyroxine in early pregnancy is not appropriate. The follow-up of the hypothyroid pregnant women should be changed and serum TSH and FT_4 measurements should be performed every month until the end of pregnancy to prevent risks of adverse maternal and neonatal outcomes. Moreover, the aetiology of hypothyroidism may influence the adjustment of L-T4 therapy. Abalovich, Gutierrez, *et al.* 2002 demonstrated that adequate thyroxine treatment was very important for pregnancy outcome both in overt and subclinical hypothyroidism. With inadequate

treatment, pregnancy ended with abortion in 60-71 percent of women with overt and subclinical hypothyroidism, respectively, with an increased prevalence of preterm deliveries. Conversely, the frequency of abortions in hypothyroid pregnant women receiving adequate treatment was very low and in general they went to term without complications.

4.3.1.4 Urinary iodine status

The median urinary iodine concentration of the pregnant and lactating women (n=92) showed iodine sufficiency in the population (Fig. 4.34). Approximately 30 percent of the women during pregnancy had insufficient iodine intake whereas postpartum it reduced to 10 percent (Table 4.50 a & b).

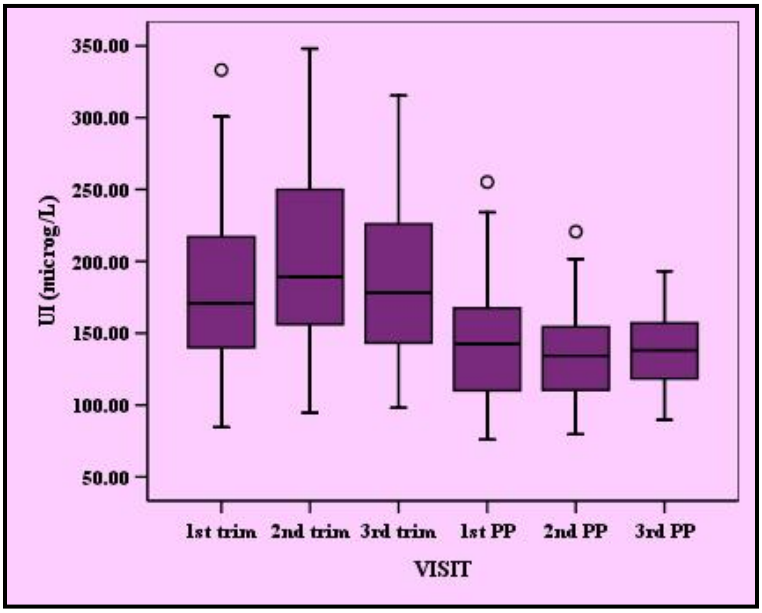


Figure 4.34 Median urinary iodine concentration of group II A subjects in different trimesters and postpartum

Table 4.50(a) Distribution of group II A pregnant women in different trimesters according to urinary iodine

UIC (µg/L)	1st trimester	2nd trimester	3rd trimester
<150	31 (33.7)	20 (21.7)	26 (28.3)
150-249	48 (52.2)	49 (53.3)	46 (50)
250-499	13 (14.1)	23 (25)	20 (21.7)

Table 4.50(b) Distribution of group II A lactating women according to urinary iodine concentration

UIC (µg/L)	1st postpartum	2nd postpartum	3rd postpartum
50-99.9	10 (10.9)	5 (5.4)	1 (1.1)
100-199.9	77 (83.7)	84 (93.3)	91 (98.9)
200-299.9	5 (5.4)	3 (3.3)	-

Value in parenthesis indicate percentage

4.3.1.5 Pregnancy Outcome of Group II A subjects

The pregnancy outcomes of ninety subjects of Group II A are presented in table 4.51. All details of only these neonates are available as some subjects had miscarriage or dropped out from the study or had home delivery. Out of 95 subjects, who reported of giving birth, 1.95% had home delivery. Five subjects had preterm delivery.

One still birth was reported and one baby had congenital hypothyroidism.

Table 4.51 Characteristics of group II A neonates (n=90)

	Frequency (Percentage)
Sex of the baby	
Male	52 (57.8)
Female	38 (42.2)
Type of delivery	
Normal	46 (51.1)
Cesarean	37 (41.1)
Other	7 (7.8)

The mean APGAR score of the neonates at 1, 5 and 10 min was 7.77 ± 1.3, 8.65 ± 1.2 and 8.89 ± 1.3, respectively which is normal. Table 4.52 shows mean parameters of the neonates after excluding preterm babies.

Table 4.52 Mean parameters of group II A neonates (n=85)

Parameters	Mean ± SD	Range
Gestational age at delivery (weeks)	39.32 ± 1.1	37-41
Birth weight (kg)	2.96 ± 0.45	2.09-4.00
Birth length (cms)	48.46 ± 2.88	42-58
Head circumference (cms)	32.72 ± 1.6	30-37

The data when classified according to Z-scores revealed that 15 percent of all the neonates were stunted and wasted whereas 10 percent were underweight (Table 4.53). Ten percent of the neonates did not have normal head circumference-for-age.

Thyroid function tests of neonates

Cord blood FT_4 and TSH levels showed a wide scatter or variability. Important characteristics of both FT_4 and TSH levels are shown in table 4.54. Cord TSH levels were asymmetrical and skewed towards right side (positively skewed) which means their trends shifted towards higher values. A study by Abbas, *et al.* 2003 also showed cord blood TSH values trend towards higher values.

Elevated serum TSH in the neonates indicates insufficient supply of thyroid hormones to the developing brain. WHO/UNICEF/ICCIDD has included neonatal TSH as one of the indicators for assessing IDD and their control in a population (Delange 1998). In the absence of iodine deficiency, the frequency of neonatal serum TSH above 10 µIU/ml is less than 3%. A frequency of 3%-19.9% indicates mild IDD. Frequencies of 20%-39.9% and above 40% indicates moderate and severe IDD respectively.

According to our data 23.5 percent neonates had TSH level above 10 µIU/ml which indicates moderate iodine deficiency (Fig. 4.35). It means substantial number of fetuses were iodine deficient and hence were victims of utero hypothyroxinemia.

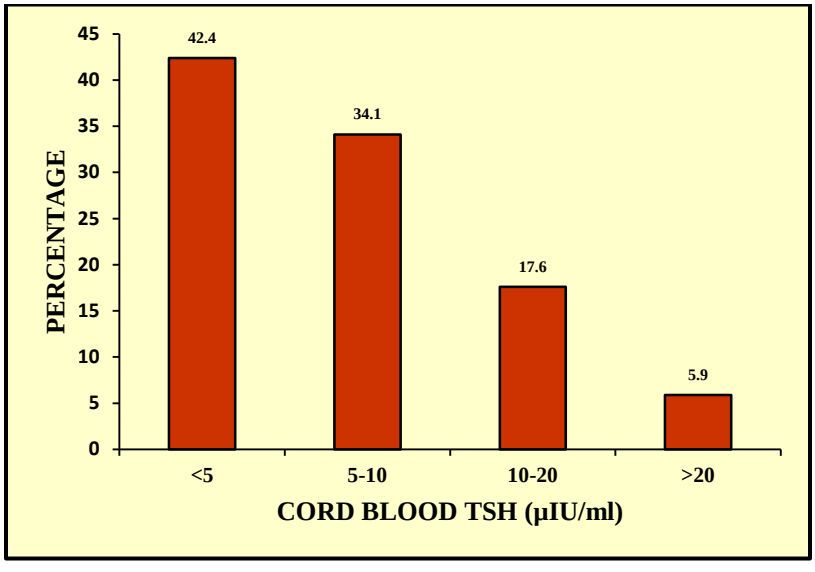


Figure 4.35 Distribution of group II A neonates according to cord blood TSH level

Table 4.53 Nutritional status of group II A neonates based on Z-scores

Sex	n	Height-for-age				Weight-for-height				Weight-for-age			
		<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD
M	50	1 (2)	4 (8)	43 (86)	2 (4)	6 (12)	5 (10)	38 (76)	1 (2)	-	6 (12)	44 (88)	-
F	35	3 (8.6)	5 (14.3)	27 (77.1)	-	-	1 (2.9)	33 (94.2)	1 (2.9)	-	2 (5.7)	33 (94.3)	-
T	85	4 (4.7)	9 (10.6)	70 (84.4)	2 (2.4)	6 (7.1)	6 (7.1)	71 (83.5)	2 (2.4)	-	8 (9.4)	77 (90.6)	-

Value in parenthesis indicate percentage

Table 4.54 Serum FT_4 and TSH levels in cord blood of group II A neonates

Parameters	Mean ± SD	Median	Range
Cord blood FT_4 (pM/L)	15.93 ± 3.2	15.45	4.3-27.9
Cord blood TSH (µIU/ml)	9.61 ± 7.44	7.25	1.00-39.6

Various neonatal parameters like head circumference (r=0.019; p=ns), length (r=0.063; p=ns) and weight (r=-0.113; p=ns) were found to have no correlation with cord blood TSH. Association between cord FT_4 with birth weight (r=0.604; p<0.05), birth length (r=0.574; p<0.05) and head circumference (r=0.545; p<0.05) suggest that fetal thyroid function may be important in regulating fetal growth. Similar results were reported by Shields, *et al.* 2011 that cord FT_4 is positively associated with birth weight, length, head circumference and sum of skinfolds while there were no associations between birth measurements and either cord TSH or cord FT_3.

Obstetric and neonatal outcomes based on maternal thyroid status at initial presentation and at the beginning of third trimester

Maternal thyroid status at initial presentation, defined as hypothyroidism, TSH >4.2 µIU/ml (n=34), and controlled thyroid status defined as TSH <4.2 µIU/ml (n=56), did not affect the rate of caesarean section (32.4% *vs.* 41.8%), median gestational age at term (40 for both the groups) or median neonatal birth weight [2.95 (2.12-3.8) *vs.* 3.00 (1.13-4.00); p=ns]. The prevalence of low birth weight infants (neonatal birth weight of <2.5 kg) in mothers with maternal hypothyroidism at initial presentation and in mothers with controlled thyroid status was 11.8 percent and 10.9 percent, respectively, not statistically significant. Low birth weight infants were due to premature delivery (delivery at or <37 weeks of gestation) in only one case in both the hypothyroid and controlled thyroid status group at the time of initial presentation. Similar results were reported by Idris, *et al.* 2005 that maternal thyroid status at initial presentation, did not affect the rate of caesarean section, median gestational age at term or median neonatal birth weight, prevalence of low birth weight infants.

At the beginning of the third trimester, maternal thyroid status, defined as suboptimal thyroid levels TSH >3 µIU/ml (n=34) and optimal thyroid levels TSH <3 µIU/ml (n=56), showed no association with the rate of caesarean section (41.2 % *vs.* 37.5 %), median gestational age at term (40 for both the groups) or mean neonatal birth weight [2.98 (2.09-4.00) *vs.* 2.94 (2.09-3.80); p=ns]. The prevalence of low birth weight infants in suboptimal and optimal group was 11.8 percent and 10.4 percent, respectively, not statistically significant. One of the low birth weight infant was due to prematurity in optimal thyroid group whereas suboptimal thyroid group had no premature baby.

There was very low incidence of still birth, preterm delivery and congenital and developmental anomalies were negligible and compares favorably with findings from other studies (Leung, *et al.* 1993, Haddow, Palomki, *et al.* 1999, Idris, *et al.* 2005). The favorable outcome documented here may be the manifestation of our increased knowledge about the potential adverse effects of maternal hypothyroidism on perinatal and long-term neurodevelopmental outcomes of the offspring (Pop, Brouwers, *et al.* 2003) and hence an improvement in antenatal care.

The correlation between first and third trimester maternal thyroid function tests and fetal cord blood TSH showed no significant relationship between the parameters. The cord blood FT_4 and first trimester FT_4 ($r=0.494$; $p<0.05$) and third trimester FT_4 ($r=0.652$; $p<0.05$) had significant positive correlation (Table 4.55 & 4.56). Also significant negative correlation was found between cord blood FT_4 and maternal third trimester TSH. The correlation between third trimester maternal FT_4 and cord FT_4 supports the belief that maternal T_4 crossed the placenta even in late gestation (Shields, *et al.* 2011).

Table 4.55 Correlation between maternal thyroid function tests (first trimester) and fetal thyroid function tests of group II A subjects

	FT_3	**FT_4**	**TSH**	**UIC**	**CFT_4**	**CTSH**
FT_3	1					
FT_4	0.424**	1				
TSH	-0.202	-0.281**	1			
UIC	-0.261*	-0.133	0.002	1		
CFT_4	0.003	0.494*	0.814	-0.027	1	
CTSH	-0.084	0.154	0.027	0.163	-0.116	1

** $p<0.01$; * $p<0.05$

Table 4.56 Correlation between maternal thyroid function tests (third trimester) and fetal thyroid function tests of group II A subjects

	FT_3	**FT_4**	**TSH**	**UIC**	**CFT_4**	**CTSH**
FT_3	1					
FT_4	-0.020	1				
TSH	-0.046	-0.138	1			
UIC	-0.126	-0.076	0.03	1		
CFT_4	-0.138	0.652*	-0.328*	-0.049	1	
CTSH	-0.116	-0.045	-0.03	0.196	-0.116	1

* $p<0.05$

4.3.2 GROUP II B: HYPERTHYROIDIC SUBJECTS (n=10)

4.3.2.1 Disease history

The median duration of hyperthyroidism before conception was 7.5 years (2 year to 12 year). Twenty percent of the subjects had history of repeated abortions and known pregnancy complications (Table 4.57). Only two of the subjects did not have goiter.

Table 4.57 Prior disease history of group II B subjects

Prior disease history	Frequency	Percentage
Any known Complication	2	20
History of repeated abortions	2	20
Any known autoimmune disorder	6	60
Family history of thyroid disorder	10	100

All the subjects were on anti-thyroid drug propylthiouracil (PTU) and had to decrease their dosage during pregnancy.

4.3.2.2 Urinary iodine status

The median urinary iodine concentration showed iodine sufficiency in the group (Table 4.58). During pregnancy 40 percent of the subjects and postpartum 10 percent of the subjects were iodine deficient whereas no subject remained iodine deficient after 3 months postpartum (Figure 4.36 a & b).

Table 4.58 Median urinary iodine concentration of group II B subjects in different trimesters and postpartum

Stage	Median UIC (µg/L)
1st trimester	176.4
2nd trimester	158.53
3rd trimester	187.3
1st postpartum	133.59
2nd postpartum	143.4
3rd postpartum	125.8

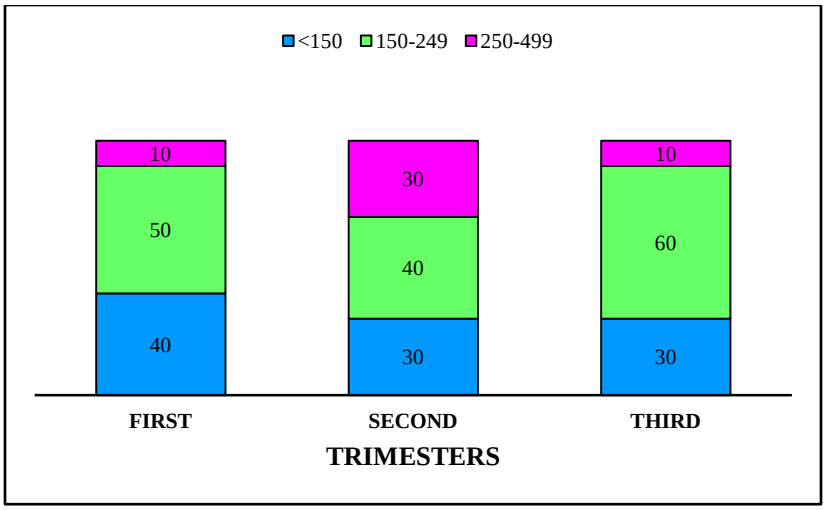


Figure 4.36(a) Distribution of group II B pregnant women in different trimesters according to urinary iodine concentration

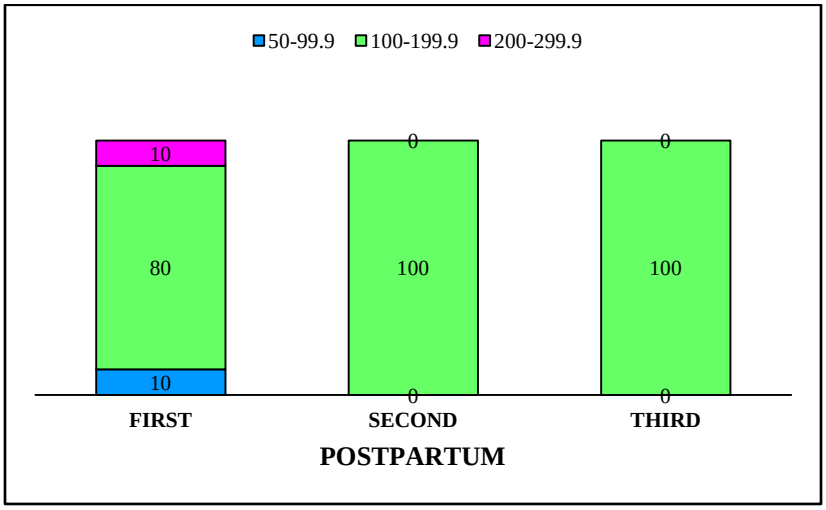


Figure 4.36(b) Distribution of group II B lactating women according to urinary iodine concentration

4.3.2.3. Pregnancy Outcome

One of the subjects had preterm delivery while rest of them delivered healthy babies. The characteristics of the neonates are shown in table 4.59.

The mean APGAR score of the babies at 1, 5 and 10 min was 7.88 ± 0.84, 8.63 ± 0.52 and 8.88 ± 0.64, respectively. The mean parameter of the neonates is shown in table 4.60.

Table 4.59 Characteristics of group II B neonates (n=9)

Characteristic	Frequency (Percentage)
Sex of the baby	
Male	7 (77.8)
Female	2 (22.2)
Type of delivery	
Normal	5 (55.6)
Cesarean	3 (33.3)
Other	1 (11.1)

Table 4.60 Mean parameters of group II B neonates (n=9)

Parameters	Mean ± SD	Range
Gestational age at delivery (weeks)	39.67 ± 1.41	37-41
Birth weight (kg)	3.19 ± 0.48	2.5-4.0
Birth length (cms)	48.23 ± 2.2	44-51
Head circumference (cms)	33.35 ± 0.83	32-34
Cord blood FT_4 (pM/L)	15.17 ± 3.7	9.9-19.8
Cord blood TSH (µIU/ml)	13.78 ± 15.6	3.3-40.2

Two of the neonates had cord blood TSH >20 µIU/ml but came to normal after repeat TSH test within 7 days (Fig. 4.37).

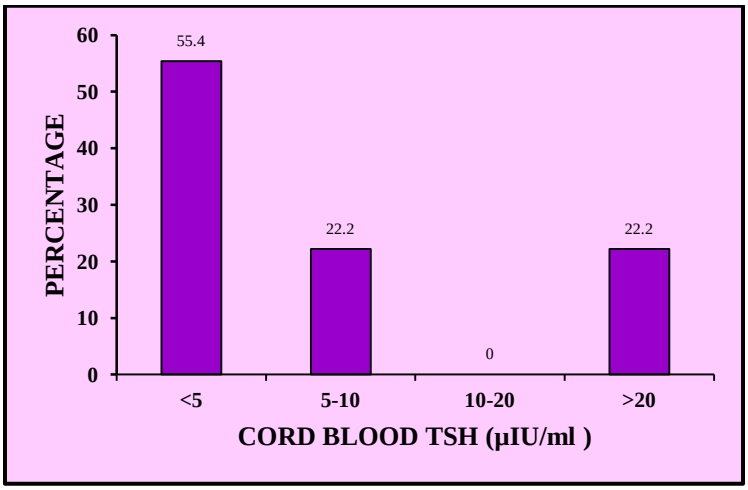


Figure 4.37 Distribution of group II B neonates according to cord blood TSH level

The data when classified according to Z-scores that except one female all the neonates were normal for weight-for-age, height-for-age and weight-for-height (Table 4.61). One female was moderately stunted. All the neonates had normal head circumference-for-age.

Table 4.61 Nutritional status of group II B neonates based on Z-scores

Sex	n	Height-for-age				Weight-for-height				Weight-for-age			
		<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	>+2SD
M	7	-	-	7 (100)	-	-	-	7 (100)	-	-	-	7 (100)	-
F	2	-	1 (50)	1 (50)	-	-	-	2 (100)	-	-	-	2 (100)	-
T	9	-	1 (11.1)	8 (88.9)	-	-	-	9 (100)	-	-	-	9 (100)	-

Value in parenthesis indicate percentage

4.4 GROUP III: SEAWEED GROUP

Thyroid deficiencies during pregnancies are always a risk and hormonal approach of treatment becomes essential. Alongwith the hormonal induction dietary supplements can also help in sustaining the circulating levels of iodine which is the major element in thyroxine. Various seaweeds and algae are reported to have rich iodine content. A few were reported by McClendon 1933. The role of iodine in goiter control with a population of Far East was studied by the author on Japanese coast containing good quantity of iodine. The author further opines that the Japanese diet contains thousand times much iodine compared to other foods and is the only non-goitrous country of the World. The average iodine intake from seaweeds in Japan is 1.2 mg/day (Nagataki 2008). The author further reported ingestion of 10 mg of iodine in one week increases response of thyrotropin in normal subjects. Studies with 20-30 mg iodine was carried out for 4 weeks, significant changes were observed in 30 mg intake which reported normality of thyroid by echogram. The abnormalities disappeared after 2 weeks of withdrawal of the dose, due to adaptive changes of autoregulation of pitutary-thyroid axis.

Variability of iodine content available in commercially available edible seaweeds was reported by Teas, *et al.* 2004. Bioavailability of iodine from seaweeds for human beings was studied by Aquaron, *et al.* 2002. The study was on two species *Laminaria hyperborea*; *Gracilaria verrucosa* due to high level of iodine in a population from France. One group of subjects from Marseille (iodine sufficient area) had median UIC at 137 µg/day and the other population from Brussels had 73 µg/day (the subjects were in mild deficient areas). The results revealed that, bioavailability for *Gracilaria verrucosa* was better than *Laminaria hyperborea*, 101 percent *vs.* 90 percent in Marseille and 85 percent *vs.* 61.5 percent in Brussels, though urinary excretion of iodine was lower in Brussels when compared to Marseille population.

It was of great interest to review the reports on marine algae and its iodine content in coastal India. Few reports by Ganga Devi, Sobha and Nair 1996, Sobha, *et al.* 2008, Manivanna, *et al.* 2008, Manivannan, *et al.* 2009 revealed the species, content,

composition etc. Recipes with these algae were reported by Sobha, *et al.* 2008 (Ulva toffy, Ulva squash, mixed algae pickle, algae cutlet, algae biryani, algae thoran). Thus it was of interest in our study to incorporate the species availed in Gujarat.

4.4.1 Baseline characteristics

Total of thirty pregnant women irrespective of either trimester of pregnancy were enrolled for this part of the study. The mean age and height of the study group was 23.9 ± 3.2 yr and 155.8 ± 5.5 cm, respectively. The mean parity and abortion rate was 1.23 ± 1.1 and 0.17 ± 0.46. Majority of the subjects (68 percent) enrolled had normal BMI while rest of them were underweight.

The women were subdivided into two groups based on their urinary iodine concentration and thyroid function tests. It was found that out of thirty subjects ten of them had below normal (<150µg/L) UIC. These women were supplemented for one month with seaweed (Group III B). Other 20 subjects (Group III A) were not provided with any supplementation.

4.4.2 Proximate composition and mineral content of seaweed

Four species of seaweeds namely *Caulerpa scalpelliformis, Caulerpa racemosa*, *Caulerpa veravelense* and *Padina tretastromatica* were collected from Gujarat coast and were analyzed at National Institute of Nutrition for proximate and mineral composition. After analysis it was found that *Caulerpa racemosa* has the highest content of iodine as well as iron when compared to other seaweeds. The moisture, protein and total dietary fiber content of all the species was almost similar (Table 4.62). Hence, it was decided to supplement *Caulerpa racemosa* to iodine deficient pregnant women for one month.

The study was planned as per experimental design and the subjects were supplemented accordingly. Constituency of the *ladoos* (20 g) was taken into consideration when the seaweed was incorporated to provide 50µg/day of iodine and 0.343 mg/day of iron from 0.17g of the seaweed.

Table 4.62 Proximate composition and mineral content of the seaweeds

S.No.	Parameters	*C.scalpelliformis*	*C.racemosa*	*C.veravelense*	*P. tretastromatica*
1.	Moisture (g/ 100g)	7.37	6.47	7.25	12.44
2.	Protein (g/100g)	23.12	22.32	25.39	12.57
3.	Total Ash (g/100g)	5.6	12.24	9.18	13.21
4.	Fat (g/100g)	2.32	1.41	2.94	1.21
5.	Total dietary fiber (g/100g)	46.08	47.85	49.27	48.28
	Insoluble dietary fiber (g/100g)	36.77	37.52	39.63	39.33
	Soluble dietary fiber (g/100g)	9.31	9.33	9.64	8.95
6.	Carbohydrates (g/100g)	15.51	9.70	5.97	12.29
7.	Energy (Kcal)	194	159	171	128
8.	Minerals (mg/100g)				
	Iron	56.45	201.89	53.18	89.76
	Iodine	2.69	28.31	3.58	4.26

Daily 0.17g of the seaweed was supplemented to these subjects so as to provide 50µg/day of iodine and 0.343 mg/day of iron.

4.4.3 Urinary iodine status

The median UIC of the non-supplemented group (Group III A) before and after one month was 171.5 (153.8 – 207.8 µg/L) and 167.8 (154.1 – 198.7 µg/L), respectively. The median UIC of the supplemented group (Group III B) before and after one month of supplementation was 104.75 (87.9 – 134.8 µg/L) and 121.05 (90.7 - 154.4 µg/L), respectively. Though the median UIC was still below the normal levels after supplementation in Group III B, slight improvement was observed (Table 4.63). This is

suggestive that prolonged period of supplementation may bring a positive change in iodine status of the subjects.

Table 4.63 Distribution of group III pregnant women according to urinary iodine

UIC (µg/L)	Group III A		Group III B	
	Before	After	Before	After
<150	-	-	10 (100)	9 (90)
150-249.9	20 (100)	20 (100)	-	1 (10)

Value in parentheses indicate percentage

4.4.4 Thyroid function parameters

The TSH of supplemented group before supplementation was significantly higher than that of non-supplemented group. No significant effect of supplementation was observed in the thyroid function parameters of the subjects (Table 4.64). This could be due to the short period of supplementation and the low dose by which it was supplemented.

Table 4.64 Thyroid function parameters of the group III sub-groups before and after one month

Parameters	Group III A		Group III B	
	Initially	After 1 month without supplementation	Initially	After 1 month with supplementation
FT_3 (pM/L)	3.98 ± 0.57	4.01 ± 0.49	4.11 ± 0.77	4.25 ± 0.68
FT_4 (pM/L)	14.32 ± 1.5	13.58 ± 0.69*	13.67 ± 2.25	13.8 ± 1.4
TSH (µIU/ml)	1.71 ± 0.57	1.82 ± 0.49	2.4 ± 0.87*	2.32 ± 0.71†

* $p<0.05$ when compared to Group III A initially

† $p<0.05$ when compared to Group III A after 1 month without supplementation

4.4.5 Hemoglobin profile

The hemoglobin profile of supplemented group was better than that of non-supplemented group both before and after supplementation (Fig. 4.38). The mean and median hemoglobin concentration of supplemented group was better than that of non-supplemented group after one month of supplementation (Table 4.65).

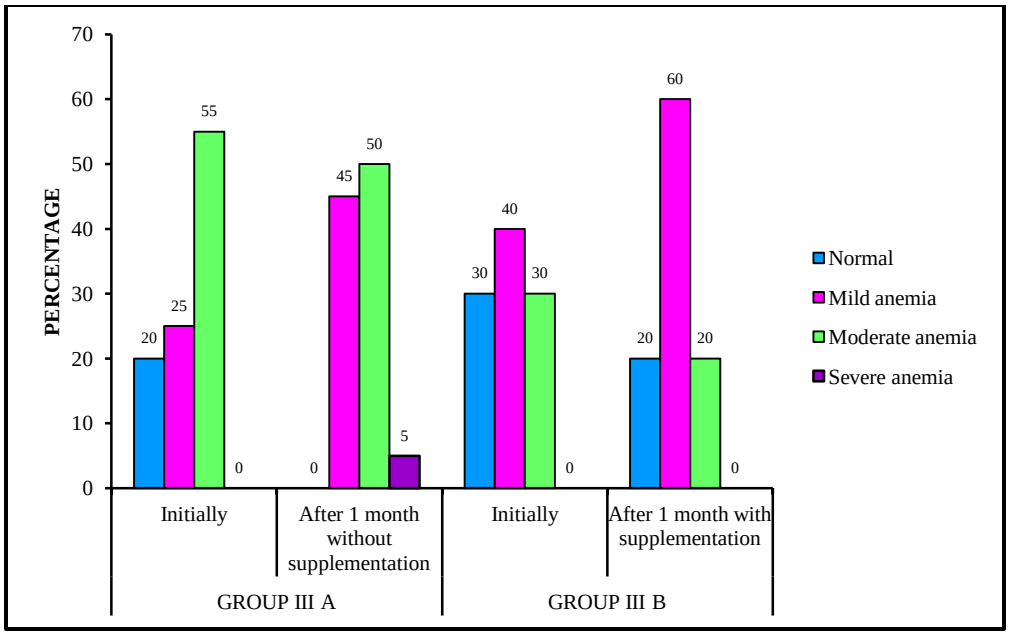


Figure 4.38 Distribution of group III pregnant women according to hemoglobin level

Table 4.65 Mean and median hemoglobin concentration of the group III sub-groups before and after one month

Hemoglobin (g/dl)	Group III A		Group III B	
	Initially	After 1 month without supplementation	Initially	After 1 month with supplementation
Mean	9.68 ± 1.3	9.53 ± 1.14	10.23 ± 1.2	10.39 ± 0.8†
Median	9.86	9.75	10.44	10.58†

† $p<0.05$ when compared to Group III A after one month without supplementation

Though the increase in iron level is very little and the change was significant when hemoglobin concentration of group III B after supplementation was compared to group III A after one month without supplementation. This could be due to other minerals present in the algae along with Iron and Iodine. Thus, it can be stated that supplementing these seaweeds can bring a positive shift in iodine and iron status, thereby combating micronutrient deficiency. Prolonged supplementation needs to be carried out further to opine on the impact of algae.

4.5 GROUP IV : CONTROL GROUP

4.5.1 Baseline characteristics

One hundred and fifty pregnant women in the same reproductive age of 18-45 years were enrolled randomly from the area. Out of one hundred and fifty women, thirteen had thyroid dysfunction so were excluded from the study. The mean age and height of these included women was 30.01 ± 6.6 yr and 151.56 ± 5.7 cm, respectively.

Majority of the women (66.4%) were normal according to body mass index. The prevalence of goiter in the population was 6.6% while no women had positivity for TPO-Ab.

4.5.2 Hemoglobin status

Majority of the women were normal. Almost thirty four percent of women had anemia but none of them were severe anemic (Fig. 4.39). Anemia is a major public health problem in Delhi especially among women and children. According to National family health survey (NFHS)-3 data, 44 percent of the women in Delhi are anemic (Ministry of Health and Family Welfare, Government of India 2009).

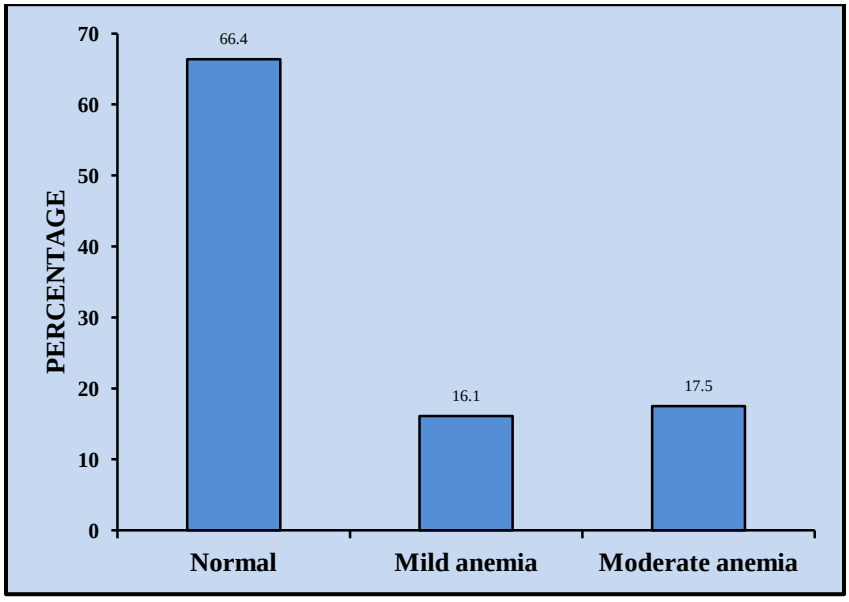


Figure 4.39 Hemoglobin concentration of Group IV subjects

4.5.3 Urinary iodine status

The median urinary iodine of 141.54 µg/L showed iodine sufficiency in the population. Some women (19%) had below sufficient (<100 µg/L) iodine levels (Table 4.82).

Table 4.66 Distribution of group IV women according to urinary iodine concentration

UIC (µg/L)	Frequency (Percentage)
<20	1 (0.7)
20-49	6 (4.4)
50-99	19 (13.9)
100-199	108 (78.3)
200-299	3 (2.2)

4.5.4 Thyroid function parameters

After excluding subjects with goiter and abnormal thyroid function tests, the reference non-pregnant population comprised of 125 subjects. Table 4.67 shows mean thyroid function tests of this population.

Table 4.67 Mean thyroid function tests of reference non-pregnant subjects (n=125)

Parameters	Mean ±SD	Median
FT_3 (pM/L)	4.61±0.86	4.56
FT_4 (pM/L)	15.64±2.68	15.32
TSH (µIU/ml)	2.51±1.47	2.43

This shows that the general population of Delhi is iodine sufficient and their thyroid parameters are also within the normal reference range.

4.6 ESTABLISHMENT OF THYROID HORMONES TRIMESTER-SPECIFIC REFERENCE INTERVALS

Determination of reference intervals for TSH, FT_4 and FT_3 according to trimester is the first step for screening of thyroid disease in pregnancy. A set of pregnant and non-pregnant women were analyzed to establish trimester-specific reference intervals for our population. Group I A and Group IV were included in this analysis.

The U.S. National Academy of Clinical Biochemistry (NACB) recommends that 'trimester-specific reference intervals should be used when reporting thyroid test values in pregnant women' (Baloch, *et al.* 2003). In order for these reference intervals to be relevant, they must be determined using well characterized specimens. According to results from National Health and Nutrition Examination survey (NHANES) studies on regular thyroid function (Hollowell, Staehling and Flanders 2002), the NACB proposed that for the establishment of new TSH reference intervals, only euthyroid healthy volunteers be included, who should be free from detectable autoantibodies against thyroid peroxidase (TPO-Abs) or thyroglobulin (TgAbs) and any personal or family history of thyroid dysfunction. In addition, no visible or palpable goiter and no medication except estrogens are allowed.

Out of sixty eight women from Group I A with complete three trimesters data, women were excluded on the basis of laboratory results that revealed positive serum thyroid peroxidase antibody (TPO-Ab > 35 IU/ml). All the women having goiter, overt hypothyroidism or overt hyperthyroidism were excluded.

The values of serum FT_3, FT_4 and TSH from this pregnant women group (n= 46) and nonpregnant women (n=125) who were considered as 'normal subjects' were used to derive thyroid function test reference intervals. These subjects constituted the normal reference study population.

The characteristics of the women under study is shown in table 4.68.

Table 4.68 Characteristics of the normal reference population

Women	n	Age	Gestational age
Nonpregnant	125	29.92 ± 6.5	-
Pregnant	46	24.96 ± 2.7	11.78 ± 1.6 (4-12)

The median urinary iodine concentration of the subjects showed iodine sufficiency (Fig. 4.40) both in pregnant and nonpregnant women. Urinary iodine levels of pregnant women were higher than that of the nonpregnant women. There was not much difference between the three trimesters. All levels fell within the range (150-249 µg/L) recommended by WHO/ UNICEF/ ICCIDD 2007 for pregnant women. A study by Yan, *et al.* 2011 showed that urinary iodine levels of pregnant women in all the three trimesters (182.8, 164, 189.3 µg/L in first, second and third trimester, respectively) were consistently lower than those of nonpregnant women (195.9 µg/L). Our study showed lowest level during first trimester whereas their study demonstrated lowest level during the second trimester.

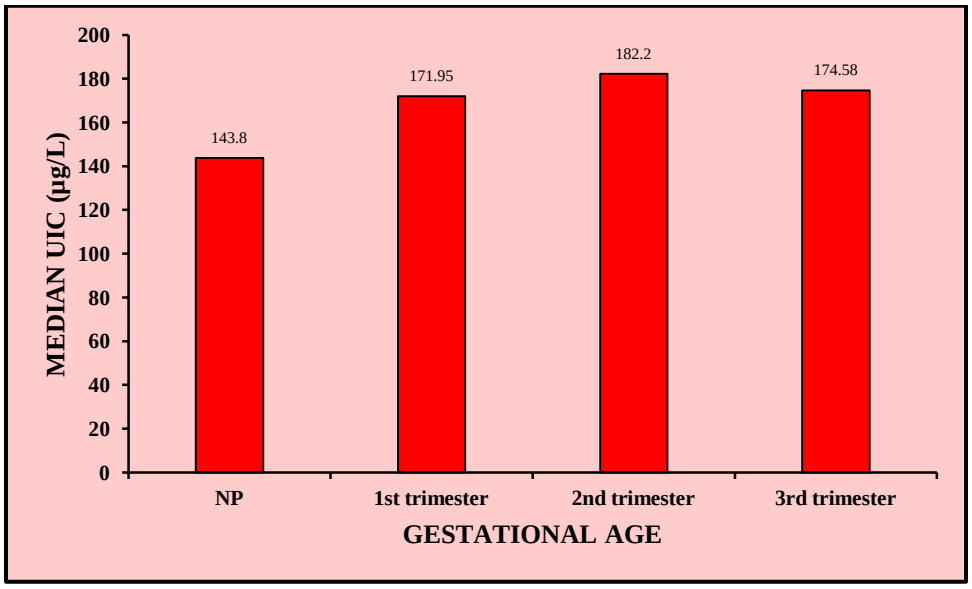


Figure 4.40 Median urinary iodine concentrations of the pregnant and nonpregnant women

The trimester-specific-reference intervals for thyroid hormones could be calculated. Percentile points 2.5, 5, 25, 75, 95 and 97.5 and medians of TSH, FT_4 and FT_3 concentrations categorized using different trimesters are presented in table 4.69.

***Free triiodothyronine*:** Table 4.69 and fig. 4.41 presents trimester- specific values for FT_3 obtained from our study. The reference intervals for FT_3 decreased from first to second trimester and then remained almost constant in third trimester in all the percentile categories except 2.5. The upper FT_3 reference interval was lower than the non-pregnancy value and manufacturer's range. The multiples of medians (MoMs) were

calculated to allow comparison between different populations and methods. The 95th percentile was approximately 1.3 MoM in all the trimesters and the 97.5th percentile was approximately 1.4 MoM during first trimester (Fig. 4.42). A study by Mannisto, *et al.* 2011 reported that 95th percentile was approximately 1.25 MoM and the 97.5th percentile was approximately 1.3 MoM during gestational week 2-20. According to them there was no clear rise or fall in FT_3 values during pregnancy in any of the studied percentile categories whereas Ashoor, *et al.* 2010, Yan, *et al.* 2011 showed that FT_3 decreased with gestational age. Panesar, Li and Rogers 2001 study revealed that FT_3 decreased over the first trimester from 3.9 to 3.3 pM/L and then remained unchanged till term. Our study also showed the same trend.

Table 4.69 Trimester- and method- specific reference intervals for thyroid tests of pregnancy in comparison with non pregnancy

Gestational trimesters	n	Percentiles						
		2.5	5	25	50	75	95	97.5
FT_3								
NP	125	2.58	3.34	4.18	4.56	5.05	6.11	6.73
1st trim	46	1.7	3.14	3.87	4.15	4.71	5.39	5.83
2nd trim	46	2.38	2.68	3.44	4.05	4.58	5.12	5.15
3rd trim	46	2.21	2.49	3.35	3.85	4.30	5.18	5.20
Manufacturer's nonpregnant adult reference interval is 3.7 to 7.2 pM/L								
FT_4								
NP	125	10.93	11.27	13.78	15.32	17.51	20.58	22.06
1st trim	46	10.12	10.81	12.74	13.53	15.4	18.56	19.13
2nd trim	46	8.29	9.28	11.36	12.84	14.31	19.02	19.43
3rd trim	46	9.27	9.39	10.87	12.8	14.57	17.69	18.63
Manufacturer's nonpregnant adult reference interval is 12.0 to 23.0 pM/L								
TSH								
NP	125	0.16	0.72	1.37	2.43	3.62	4.38	4.58
1st trim	46	0.03	0.21	1.1	2.02	2.69	4.31	4.59
2nd trim	46	0.54	1.07	1.61	2.38	3.10	4.02	5.45
3rd trim	46	0.78	1.06	1.85	2.29	3.10	4.29	4.83
Manufacturer's nonpregnant adult reference interval is 0.27 to 4.2 μIU/ml								

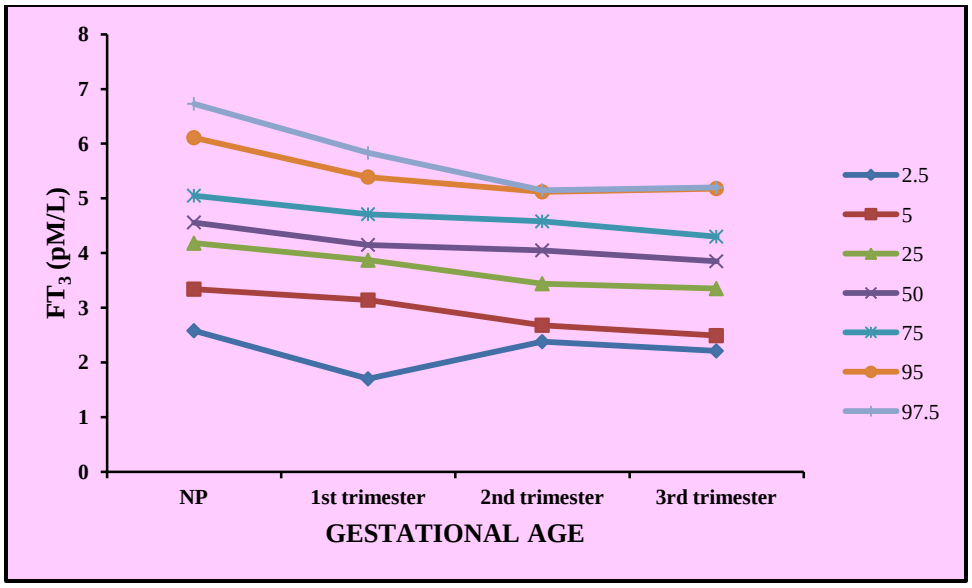


Figure 4.41 Percentile categories of FT_3 by trimester and non-pregnancy

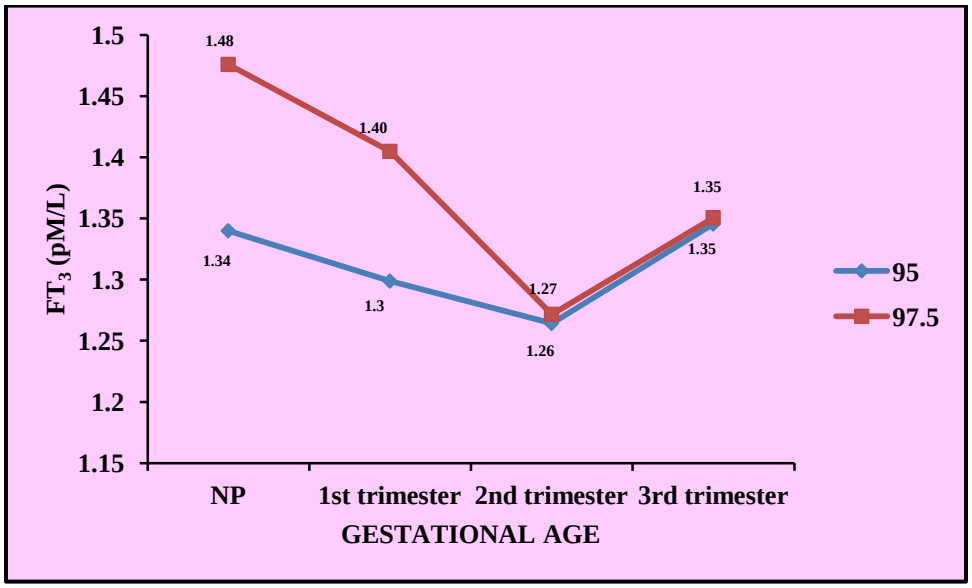


Figure 4.42 Percentile points 95 and 97.5 of FT_3 as multiples of medians

Free thyroxine: The lower reference interval of FT_4 decreased maximum in second trimester but the upper reference limit remained almost same in all the three trimesters. The highest upper limit of FT_4 was lower than the non-pregnancy and manufacturer's reference range. FT_4 concentrations showed higher variability in high- percentile categories (Table 4.69) (Fig. 4.43). The 95^{th} percentile was approximately 1.4 MoM and the 97.5^{th} percentile was approximately 1.5 MoM during the three trimesters (Fig. 4.44).

Similar results were reported by Ashoor, *et al.* 2010, Mannisto, *et al.* 2011, Yan, *et al.* 2011 that FT_4 decreased with increasing gestational age. In a study by Mannisto *et al.* 95th percentile was approximately 1.3 MoM and the 97.5th percentile was approximately 1.4 MoM during gestational week 2-20.

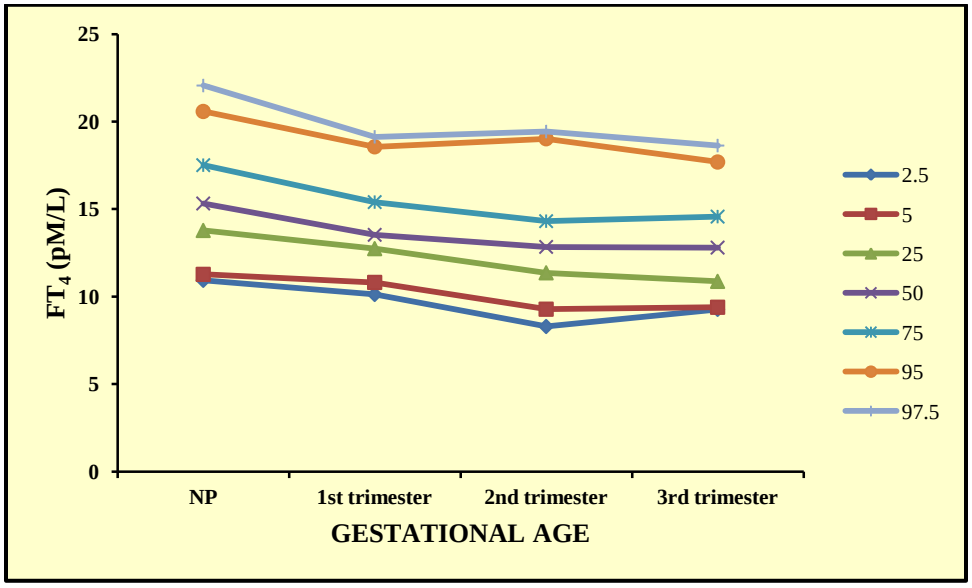


Figure 4.43 Percentile categories of FT_4 by trimesters and non-pregnancy

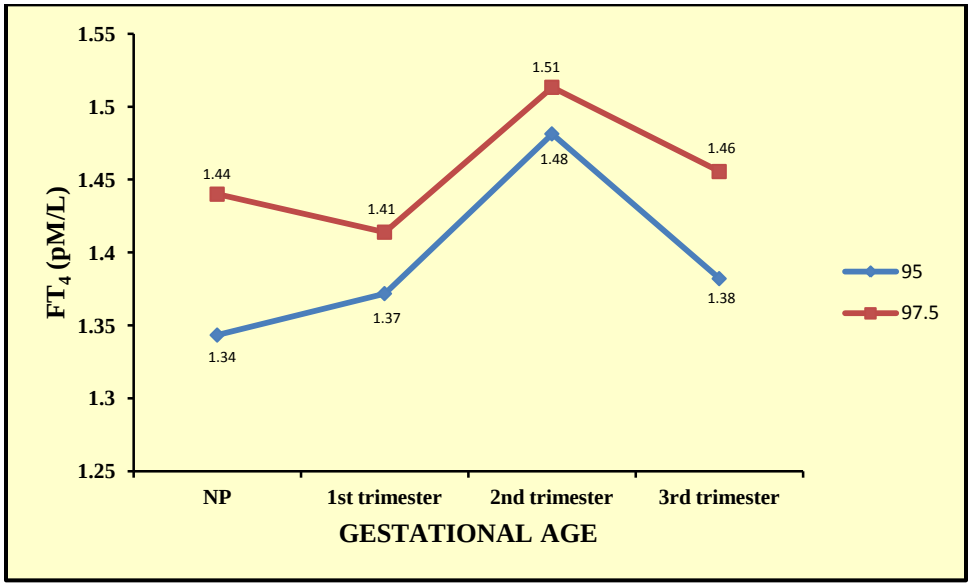


Figure 4.44 Percentile points 95 and 97.5 of FT_4 as multiples of median

***Thyrotropin*:** TSH values were suppressed in the first trimester. The TSH reference interval for pregnant women (Table 4.69) showed that in first trimester, lower TSH

reference limit (0.03 µIU/ml) was quite lower than the non-pregnant reference interval (0.16 µIU/ml). This would lead to misclassification of normal pregnant women with such low TSH results. Median concentrations of serum TSH was lowest in first trimester and then increased (Fig. 4.45). The upper TSH reference interval increased in second trimester and then decreased slightly. The variation in TSH concentration was greater in high percentile categories. Our results are in line with those reported by Ashoor, *et al.* 2010, Mannisto, *et al.* 2011. Figure 4.46 demonstrates the MoMs of percentile points 95 and 97.5 of TSH concentrations. In our data, 95th percentile was 2 MoM and 97.5th percentile was 2.2 MoM during the three trimesters.

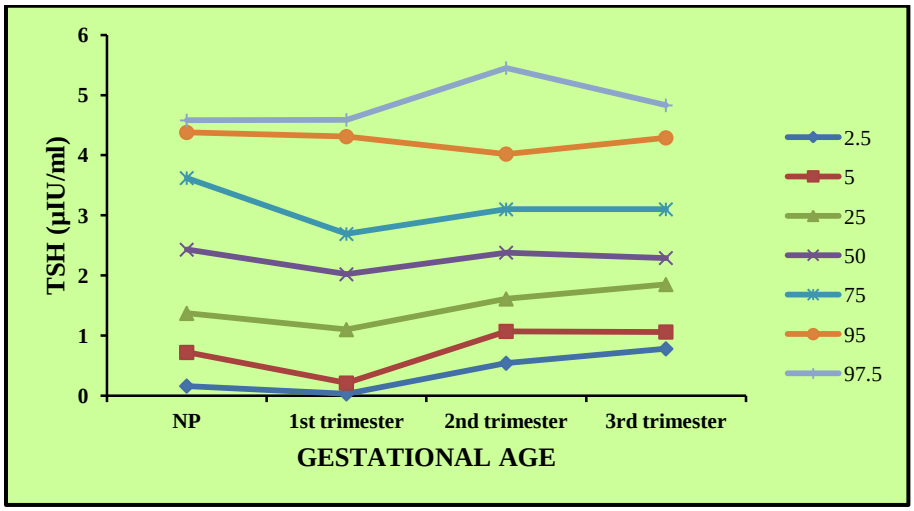


Figure 4.45 Percentile categories of TSH by trimesters and non-pregnancy

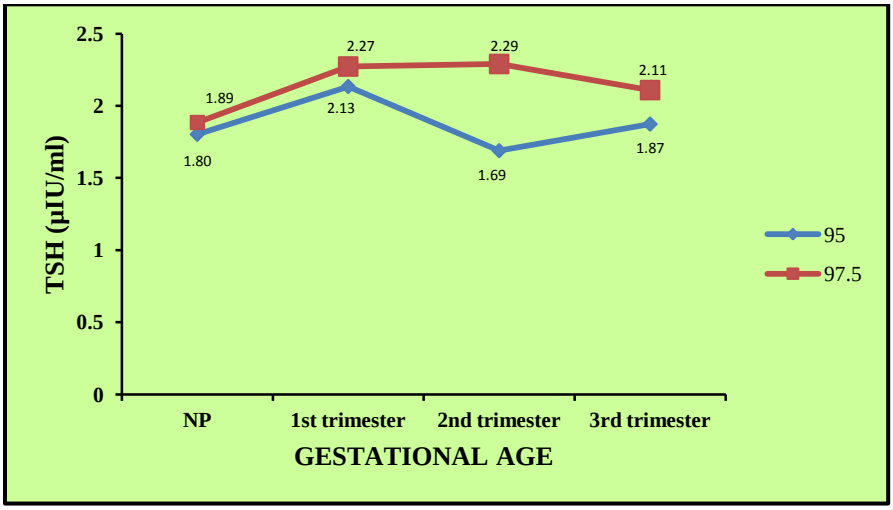


Figure 4.46 Percentile points 95 and 97.5 of TSH as multiples of medians

The 95th percentile was selected to represent the upper reference limit so as not to omit any cases of hypothyroidism. The 2.5th percentile was used to represent the lower reference limits.

Trimester changes in thyroid hormone concentration during pregnancy

The mean concentration of each hormone during each trimester is shown in table 4.70. When comparing each hormone between pregnancy and non-pregnancy and among gestational stages, TSH concentration showed statistically significant decline ($p<0.05$), followed by a rise which almost reached the non-pregnancy level in the third trimester ($p<0.05$). The mean concentration of FT_4 gradually decreased throughout pregnancy ($p<0.05$) with the lowest levels present during the third trimester (16.94% below non-pregnancy levels). The mean concentrations of FT_3 decreased significantly ($p<0.05$) by 13.45% below the non-pregnancy level in the second trimester and by 16.92% in the third trimester.

Similarly, Dhatt, *et al.* 2006 reported a significant difference in TSH levels between trimesters 1 and 2, and 1 and 3 ($p<0.0005$), but no significant difference between trimesters 2 and 3. There was a decrease in the mean FT_4 levels with each progressive trimester. There were significant differences in FT_4 between all the trimesters ($p<0.005$) with maximum difference between the first and second trimesters.

Table 4.70 Changes in thyroid hormone concentrations during gestation *vs.* non-pregnancy

Groups	n	TSH (µIU/ml)		FT_3 (pM/L)	FT_4 (pM/L)
		Geometric mean	Mean ± SD		
NP	125	2.05	2.52 ± 1.48	4.61 ± 0.87	15.64 ± 2.68
1st trim	46	1.51	2.00 ± 1.08*	4.19 ± 0.75*	13.95 ± 2.14*
2nd trim	46	2.19	2.39 ± 0.98†	3.99 ± 0.71*†	13.04 ± 2.5*†
3rd trim	46	2.27	2.45 ± 0.91†	3.83 ± 0.72*†	12.99 ± 2.39*†

* $p<0.05$ *vs.* NP; † $p<0.05$ *vs.* 1st trimester

The findings of the present study are in line with those of other authors that change in thyroid hormone levels during pregnancy differ between stages of gestation and

compared with non-pregnancy levels. Ethnic background could play a part in the prevalence of thyroid disease as well as the establishment of reference intervals for TSH, FT_4 and FT_3. Boucai and Surks 2009, Surks and Boucai 2010 reported that reference intervals of TSH and FT_4 were significantly influenced by race and age and suggested that age and race specific TSH reference intervals should be employed to provide accurate intervals for a specific population. This would minimize the misclassification of patients with thyroid disease. Yan, *et al.* 2011 compared reference intervals for TSH and FT_4 between different authors and different manufacturer methods used for analysis and found that the major discrepancy exists in the upper not the lower TSH reference limits.

Our data showed relatively higher cut-off values for the upper TSH reference limits in comparison to other studies (Panesar, Li and Rogers 2001, Laulu and Roberts 2007, Stricker, *et al.* 2007, Gong and Hoffimann 2008, Lambert-Messerlian, *et al.* 2008, Yan, *et al.* 2011, Mannisto, *et al.* 2011). This may be attributed to ethnic variance. A high prevalence of raised TSH was found in Whites as compared to Asians and Blacks when second trimester thyroid function was done (Laulu and Roberts 2007). Another study showed that Asian women have lower TSH than that of Caucasian women during the second trimester of pregnancy (Price, *et al.* 1996). However, no difference was found by Price, Owen, *et al.* 2001 in the thyroid hormone changes between Asian and Caucasian pregnant women. Hollowell, Staehling and Flanders 2002 reported that the serum concentration of both TSH and the thyroid hormones is lower than in white women in both pregnant and nonpregnant women. Serum TSH concentrations in our population appeared to follow a pattern similar to that seen in other populations: concentrations decrease during first trimester and rise thereafter, especially in the second trimester (Haddow, *et al.* 2004, Dashe, *et al.* 2005).

To evaluate upper reference limits in our pregnant population and to enable comparison with other-population based data and different methods, we calculated MoMs from our data. It appeared that a MoM of approximately 2 represented the upper reference limit for TSH during the trimesters. Percentile point 95 and 97.5 in our population both represented approximately the same MoM and the lower percentile point was chosen as the upper reference limit to not exclude any cases of hypothyroidism when using these reference intervals, because hypothyroidism during pregnancy is thought to be associated

with adverse outcomes (Allan, Haddow and Palomaki 2000, Casey, *et al.* 2005). This translates to 4.31 to 4.59 µIU/ml in the first trimester, 4.02 to 5.45 µIU/ml in the second trimester and 4.29 to 4.83 µIU/ml in the third trimester, a result lower than the upper reference limit reported for pregnant population by Marwaha, *et al.* 2008. Mannisto, *et al.* 2011 reported that both of these 95 and 97.5 percentile points were approximately 2.5 MoM during gestational week 2-20. Dashe, *et al.* 2005 have reported MoMs with regard to TSH in singleton pregnancies and found that upper reference limit of TSH concentrations was 4.0 MoM in the first and 2.5 MoM in the second trimester. However, this study comprised of population with different ethinicity compared with ours, theirs being mainly Hispanic and Black, which could have an effect on TSH values.

Yan, *et al.* 2011 compared the TSH reference intervals for nonpregnant adults provided by different manufacturers and concluded that manufacturer's methodology can significantly influence the setting of reference intervals, and such methodology should be standardized to enable comparison of data between countries. Moreover, each laboratory should determine reference intervals for the population it serves.

Reference intervals for FT_4 and TSH varied according to different manufacturer methods and between authors even when the same manufacturer method was applied. The difference in trimester-specific reference intervals also depends on the calculation method (i.e. a central 95% interval with the 2.5^{th} and 97.5^{th} percentile values or 5^{th} and 95^{th} percentile value or 5^{th} and 98^{th} percentile values) used. As compared to another Indian study Marwaha, *et al.* 2008 our reference interval has slightly lower upper TSH limit while the lower FT_4 reference limit decreased. This upper reference limit is lower than the upper reference limit for normal reference nonpregnant population but almost equal to manufacturer's upper reference limit for the general population. Such high upper reference limit during early gestational week (9 week: 4.9 mU/L; 10 week: 4.7 mU/l; 11 week: 4.4 mU/L) has also been reported by (Cotzias, et al. 2008) using Advia Centaur method. Similarly, Cleary-Goldman, *et al.* 2008, Berbel, *et al.* 2009 also showed TSH upper reference limit in first trimester to be 4.28 and 4.8 mU/L, respectively.

Our result showed lower first trimester FT_4 reference limit to be 10.12 pM/L. Similar lower limit of 10.6 pM/L has been reported by Vaidya, *et al.* 2007 using 2.5^{th} percentile.

Pop, *et al.* 1999 and Berbel, *et al.* 2009 showed the lower limit to be 10.4 and 10.5 pM/L using 10^{th} percentile.

Our results concerning trimester- specific TSH concentrations are comparatively higher than those in other studies from various countries (Panesar, Li and Rogers 2001, Bocos-Terraz, *et al.* 2009, Springer, Zima and Limanova 2009, Mannisto, *et al.* 2011). To some extent, the differences are probably due to sample size, sample processing, assay methodology, population-specific characteristics and variation in iodine intake.

Several studies have been conducted on pregnant subjects among which, some are cross sectional while others are longitudinal in the same study group. They also vary on the assay method used for the estimation of thyroid hormones.

Analysis of our longitudinal data on mean TSH showed that it significantly increased in the second and third trimester as compared to first whereas mean FT_4 and FT_3 significantly decreased with advancing pregnancy. However, Marwaha, *et al.* 2008 reported no significant difference in values of FT_3 and TSH between the trimesters though same assay method was used for evaluation of thyroid hormones. This difference may be due to the fact that we serially followed the women during gestation. Another study from India by Kumar, *et al.* 2003 used radioimmunoassay (RIA) to evaluate pregnant women and showed that TSH increased progressively with each trimester while serum T_3 and T_4 values increased from 1^{st} to 2^{nd} trimester, but declined from 2^{nd} to 3^{rd} trimester.

A study on group of pregnant women using immunoassay and mass spectrometry (MS) to measure FT_4 and TSH reported that mean FT_4 decreased by about 15 percent from the first to second trimester and then remained stable during the remainder of pregnancy. On the other hand, TSH concentration increased from first to second and then remained stable throughout pregnancy (Soldin, Tractenberg, *et al.* 2004).

Another longitudinal study was carried out by Price, Owen, *et al.* 2001 on paired samples from 1^{st} and 2^{nd} trimesters of 20 Asian pregnant women to establish trimester specific reference range of thyroid hormones used immunoassay technique. They found that TSH increased from 1^{st} to 2^{nd}, while FT_4 decreased with progress in pregnancy. FT_3 did not change between first and second trimesters.

In a cross-sectional study on pregnant Chinese women, Yan, *et al.* 2011 found an obvious decline in median TSH concentration during the first trimester compared with non-pregnancy ($p<0.01$) followed by a rise in the second trimester. The mean concentrations of FT_4 and FT_3 gradually decreased throughout pregnancy with the lowest levels present during the third trimester (21.2 percent and 14.4 percent below non-pregnancy levels, respectively).

A cross-sectional study of 522 pregnant subjects from Japan using ECL kits showed significant decrease in both FT_3 and FT_4 and increase in TSH with advancing pregnancy (Kurioka, Takahashi and Miyazaki 2005).

The rigor applied for selection of normal subjects may account for variations in reference intervals by different authors, despite use of same methodology for testing. The reference population should be as exclusive as possible.

BMI and thyroid hormone levels

The subjects were categorized as underweight, normal weight, overweight and obese according to BMI at the time of registration and data is presented in fig. 4.47. None of our subjects were obese.

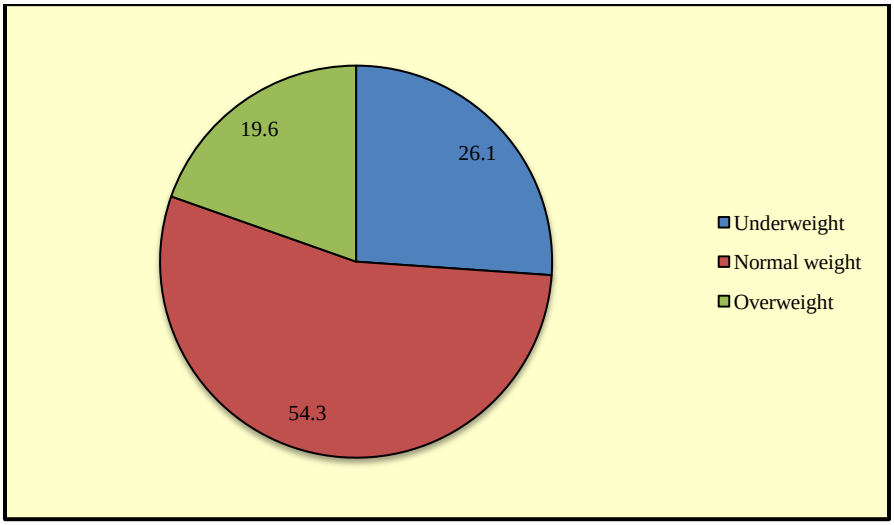


Figure 4.47 Classification of subjects according to body mass index

Serum TSH increased with BMI whereas FT_4 decreased significantly ($p<0.05$) with BMI. Similar results were reported by Ashoor, *et al.* 2010, Mannisto, Surcel, *et al.* 2011.

Higher TSH concentrations were observed in high BMI categories but it was non-significant (Fig. 4.48). When the percentage of FT_4 in different BMI categories were compared, a significant inverse association was seen between FT_4 concentration and BMI categories (Kruskal-Wallis test, $p<0.05$) in first and third trimester but the association was non-significant in second trimester (Table 4.71). Comparison of FT_4 concentrations in different BMI categories revealed that FT_4 levels were significantly lower in normal subjects as compared to underweight subjects (Mann Whitney U test, $p=0.038$). No significant difference was seen when comparing normal weight and overweight women (Fig. 4.49).

Serum FT_3 levels decreased non-significantly with increasing BMI. Comparison of FT_3 levels in different BMI categories revealed that FT_3 concentrations decreased with increasing BMI but non-significantly (Fig. 4.50). This was in contrast to the result reported by Ashoor, *et al.* 2010, Mannisto, Surcel, *et al.* 2011 that FT_3 concentration rose with BMI and statistically significant higher FT_3 concentrations were seen in overweight and obese women versus normal weight women ($p<0.001$).

Though our results did not show significant increase in TSH with increasing BMI but studies have reported that TSH concentrations increase significantly with increasing BMI. Obese euthyroid subjects have been found to have higher serum TSH concentrations than lean subjects (Bastemir, *et al.* 2007). In another study, subjects with morbid obesity were found to have higher serum TSH, FT_4 and FT_3 concentrations than normal weight subjects (Michalaki, *et al.* 2006). The associations between BMI and levels of TSH have been studied in pregnant women by Haddow, McClain, *et al.* 2008 and reported that BMI was higher when FT_4 levels were lower.

The results show that overweight women are at higher risk for having thyroid dysfunction during pregnancy and, therefore, need to be considered as at-risk mothers for adverse pregnancy outcome. Overweight and obesity have been associated independently with adverse outcomes and are probably intervening factors in studies evaluating the

association between thyroid dysfunction and adverse perinatal outcomes (Raatikainen, Heiskanen and Heinonen 2006).

Table 4.71 Thyrotropin, free thyroxine and free triiodothyronine medians, quartiles, and 5 and 95 percentile points presented by BMI categories at the time of registration

BMI (kg/m^2)	n	TSH percentile				
		5th	25th	50th	75th	95th
<18.5	12	0.005	0.49	1.99	3.11	4.61
18.5-24.9	25	0.72	1.19	2.00	2.41	3.74
25-29.9	9	0.48	1.11	2.63	3.13	4.5
p-value	NS					
		FT_4 percentile				
		5th	25th	50th	75th	95th
<18.5	12	12.7	13.31	15.22	17.78	29.25
18.5-24.9	25	10.36	12.31	13.4	14.53	17.94
25-29.9	9	10.67	11.70	12.98	14.54	16.19
p-value	<0.05					
		FT_3 percentile				
		5th	25th	50th	75th	95th
<18.5	12	3.25	3.89	4.21	4.86	5.91
18.5-24.9	25	3.22	3.79	4.07	4.79	5.4
25-29.9	9	1.4	3.28	4.19	4.49	4.7
p-value	NS					

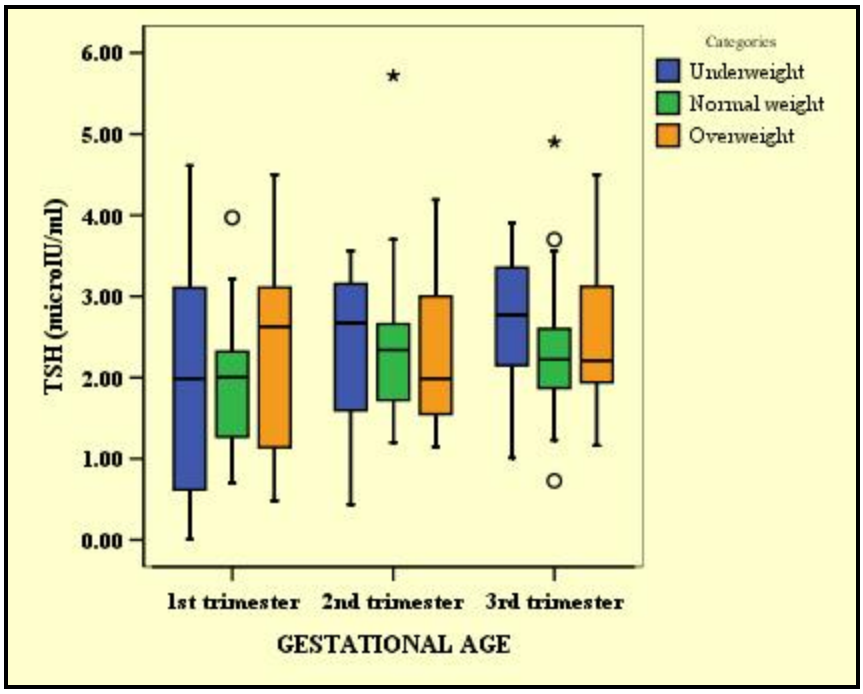


Figure 4.48 Trimester-wise median and interquartile ranges of TSH according to BMI category

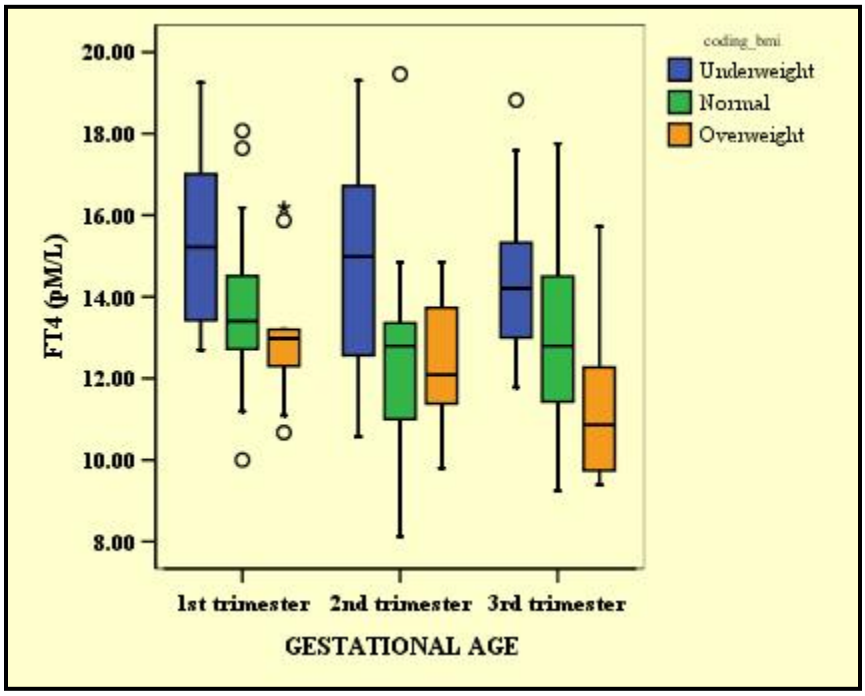


Figure 4.49 Trimester-wise median and interquartile ranges of FT_4 according to BMI

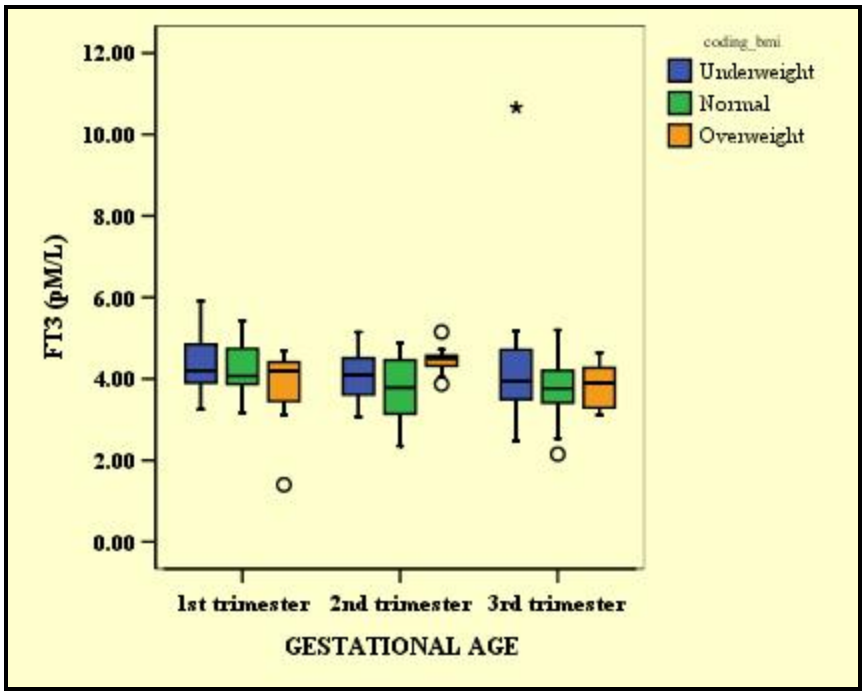


Figure 4.50 Trimester-wise median and interquartile ranges of FT_3 according to BMI

The results of this study have provided information on the reference intervals of thyroid hormones during pregnancy from thyroid antibody-negative, iodine-sufficient population. These reference intervals for FT_3, FT_4 and TSH determined for each trimester of pregnancy are recommended for evaluation of pregnant Indian women using IA, Roche Elecsys 1010 analyzer method or similar methods. The reference intervals for first, second and third trimester, respectively are:

	1st TRIMESTER	2nd TRIMESTER	3rd TRIMESTER
FT_3 (pM/L)	1.7-5.39	2.38-5.12	2.21-5.18
FT_4 (pM/L)	10.12-18.56	8.29-19.02	9.27-17.69
TSH (µIU/ml)	0.03-4.31	0.54-4.02	0.78-4.29

4.7 PREVALENCE OF THYROID DYSFUNCTION IN GROUP I SUBJECTS

The prevalence of thyroid dysfunction was assessed and compared using four different classification criteria:

- Trimester- specific reference intervals established by our study (Method I)
- Manufacturer's reference interval (Method II),
- Trimester- specific reference interval established by previous Indian study (Marwaha, *et al.* 2008) (Method III),
- TSH reference limit given by Endocrine Society Clinical Practice Guideline (Abalovich, Amino, *et al.* 2007) (Method IV).

Group I subjects i.e. general with non-specific thyroid status were analyzed to find the prevalence of thyroid dysfunction [overt hypothyroidism (OH), subclinical hypothyroidism (SCH), isolated hypothyroxinemia (IH), overt hyperthyroidism (OHyper) and subclinical hyperthyroidism (SCHyper)]. Total number of first, second and third trimester data of 189, 143 and 106 subjects, respectively was present.

Table 4.72 shows the cut-offs used for defining various thyroid dysfunctions viz OH, SCH, IH, OHyper and SCHyper.

4.7.1 Prevalence of thyroid dysfunction according to Method I

Table 4.73 shows that 19 percent of the women had raised TSH values in first trimester which increased to 23.1 percent in second trimester. Low serum FT_4 values were observed in 16 subjects in first trimester which decreased to 2 and 3 in second and third trimester, respectively.

The data showed that in the first trimester 17.45 percent subjects had hypothyroidism while 1.59 percent had hyperthyroidism (Table 4.74). In the second trimester none of the subject had either overt hypothyroidism or overt hyperthyroidism. The prevalence of isolated hypothyroxinemia reduced as pregnancy progressed (Fig. 4.51 a-c).

Table 4.72 Definition of OH, SCH and HT by different classification criteria

Method	Trimesters	OH		SCH		IH		OHyper		SCHyper	
		TSH	FT_4	TSH	FT_4	TSH	FT_4	TSH	FT_4	TSH	FT_4
I	1st trim	>4.32	<10.12	>4.32	N	N	<10.12	<0.03	>18.56	<0.03	N
	2nd trim	>4.02	<8.29	>4.02	N	N	<8.29	<0.54	>19.02	<0.54	N
	3rd trim	>4.29	<9.27	>4.29	N	N	<9.27	<0.78	>17.69	<0.78	N
II	All trim	>4.2	<12	>4.2	N	N	<12	<0.27	>22	<0.27	N
III	1st trim	>5	<12	>5	N	N	<12	<0.6	>19.45	<0.6	N
	2nd trim	>5.78	<9.48	>5.78	N	N	<9.48	<0.44	>19.58	<0.44	N
	3rd trim	>5.7	<11.32	>5.7	N	N	<11.32	<0.74	>17.7	<0.74	N
IV	1st trim	>2.5	<12	>2.5	N	N	<12	<0.10	>22	<0.10	N
	2nd trim	>3	<12	>3	N	N	<12	<0.10	>22	<0.10	N
	3rd trim	>3	<12	>3	N	N	<12	<0.10	>22	<0.10	N

N= Normal; Trim= trimesters; FT_4 (pM/L); TSH (µIU/ml)

Table 4.73 Categorization of subjects according to Method I FT_4 and TSH levels

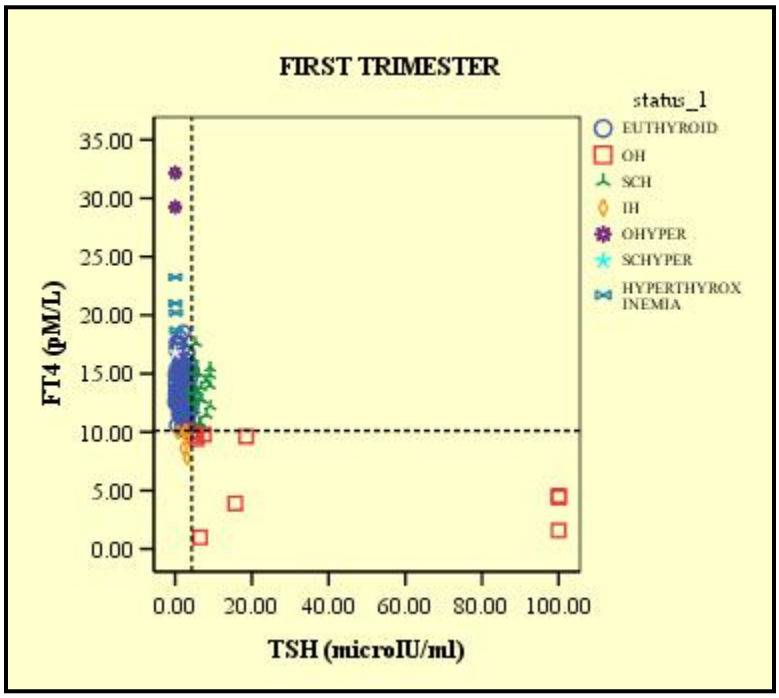
Method	Trimester	FT_4			TSH		
		Low	Normal	High	Low	Normal	High
I	1st trim	16	166	7	3	150	36
		(8.5)	(87.8)	(3.7)	(1.6)	(79.4)	(19)
	2nd trim	2	138	3	6	104	33
		(1.4)	(96.5)	(2.1)	(4.2)	(72.7)	(23.1)
	3rd trim	3	98	5	3	88	15
		(2.8)	(92.5)	(4.7)	(2.8)	(83)	(14.2)

Value in parenthesis indicate percentage

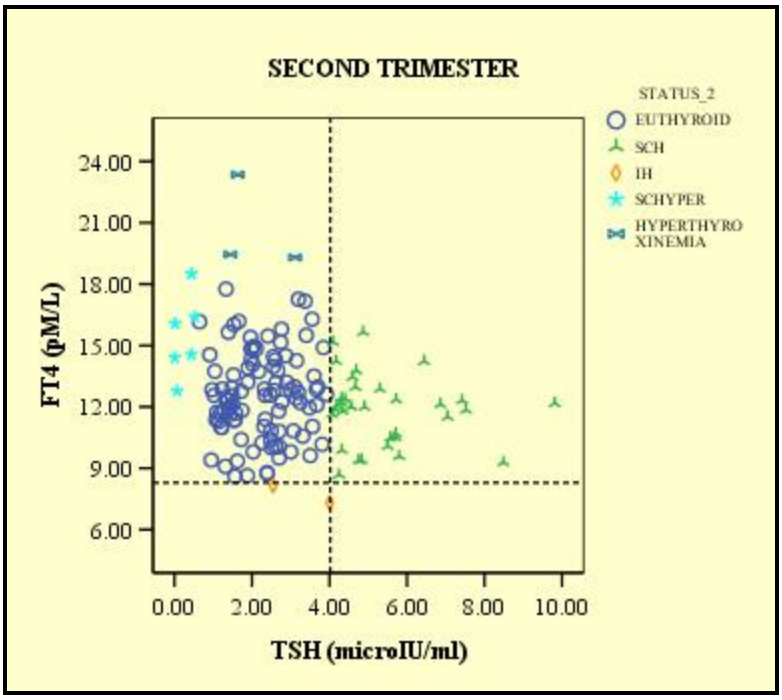
Table 4.74 Prevalence of thyroid dysfunction according to Method I

Method	Trimesters	OH	SCH	IH	OHyper	SCHyper
I	1st trim	11 (5.82)	22 (11.64)	5 (2.65)	2 (1.06)	1 (0.53)
	2nd trim	-	33 (23.08)	2 (1.40)	-	6 (4.20)
	3rd trim	1 (0.94)	14 (13.21)	1 (0.94)	-	2 (1.89)

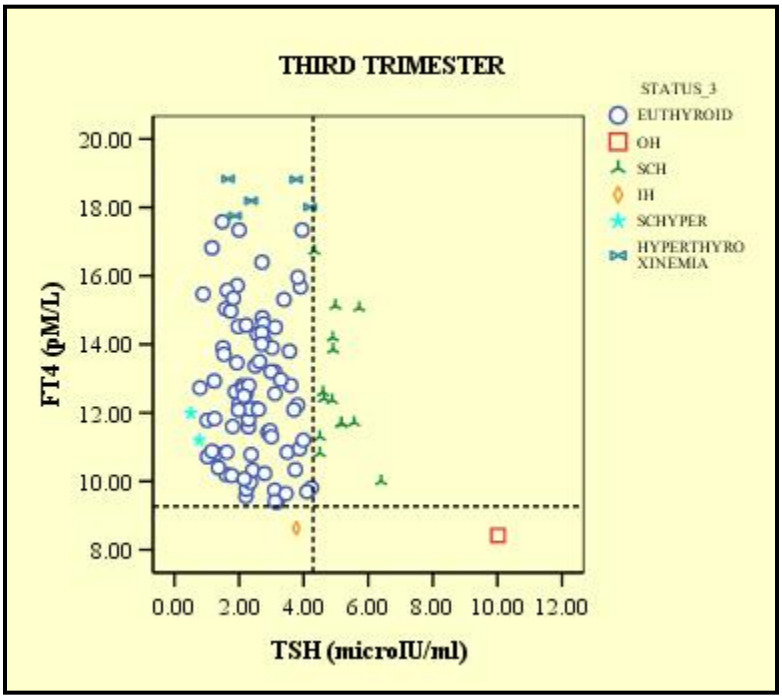
Value in parenthesis indicate percentage



(a)



(b)



(c)

Figure 4.51(a-c) show individual serum FT_4 and TSH values at first, second and third trimester according to method I. The vertical dotted lines indicate the highest normal trimester-specific TSH value (4.31, 4.02 and 4.29 μIU/ml at first, second and third trimester, respectively). The horizontal dotted lines indicate the lowest normal trimester-specific FT_4 value (10.12, 8.29, 9.27 pM/L at first, second and third trimester, respectively)

4.7.2 Prevalence of thyroid dysfunction according to Method II

One-fourth of the subjects in first trimester while two-fifth of the subjects in the second and third trimester had low FT_4. One-fifth of the subjects had high or supra-normal TSH in the first and second trimester (Table 4.75).

Table 4.75 Categorization of subjects according to method II FT_4 and TSH levels

Method	Trimester	FT_4			TSH		
		Low	Normal	High	Low	Normal	High
II	1st	46 (24.3)	140 (74.1)	3 (1.6)	14 (7.4)	139 (73.5)	36 (19.1)
	2nd	56 (39.2)	86 (60.1)	1 (0.7)	3 (2.1)	112 (78.3)	28 (19.6)
	3rd	42 (39.6)	64 (60.4)	-	-	90 (84.9)	16 (15.1)

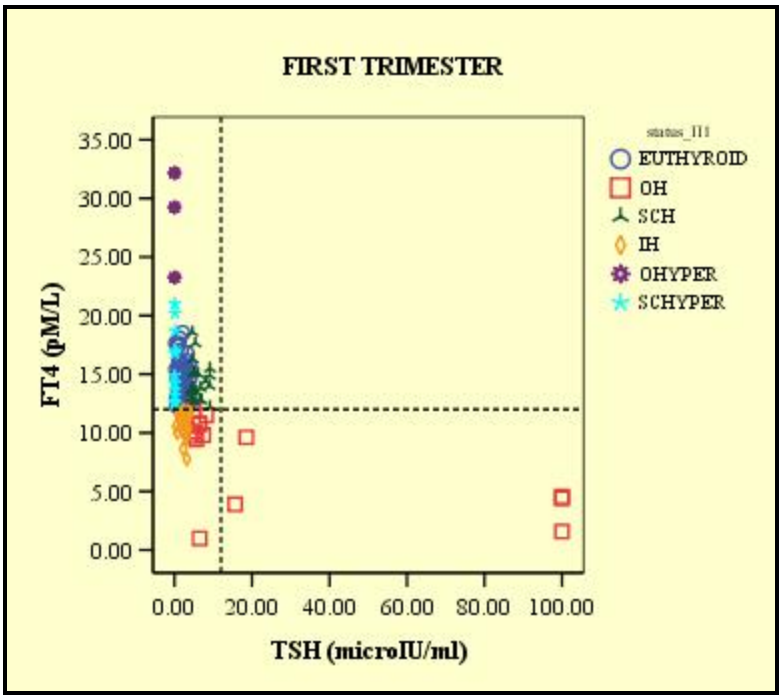
Value in parenthesis indicate percentage

In all the three trimesters the prevalence of IH was more as compared to other thyroid dysfunctions (Table 4.76). Three subjects had overt hyperthyroidism in first trimester but none had it in second and third trimester (Fig. 4.52 a-c).

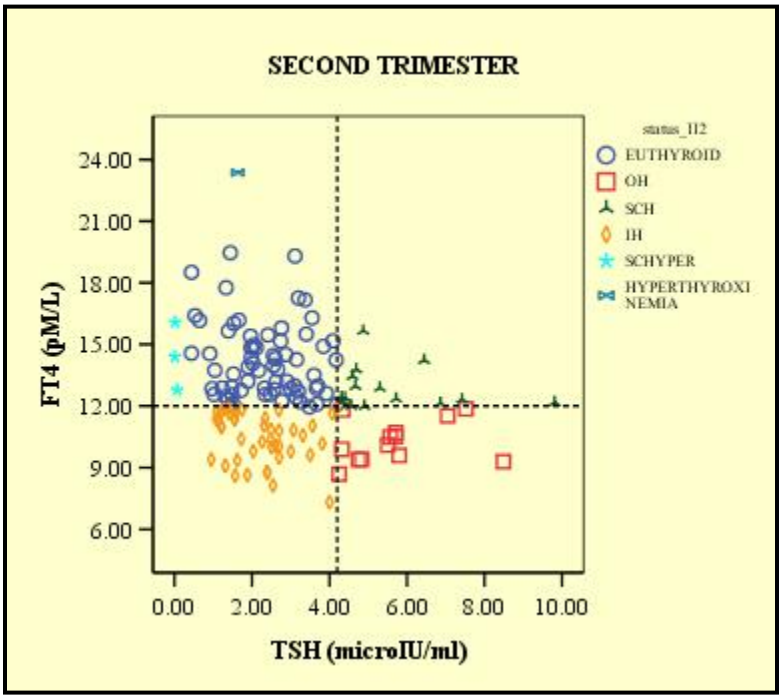
Table 4.76 Prevalence of thyroid dysfunction according to method II

Method	Trimesters	OH	SCH	IH	OHyper	SCHyper
II	1st	15 (7.93)	21 (11.11)	31 (16.4)	3 (1.59)	11 (5.82)
	2nd	13 (9.09)	15 (10.49)	43 (30.07)	-	3 (2.1)
	3rd	8 (7.55)	8 (7.55)	34 (32.08)	-	-

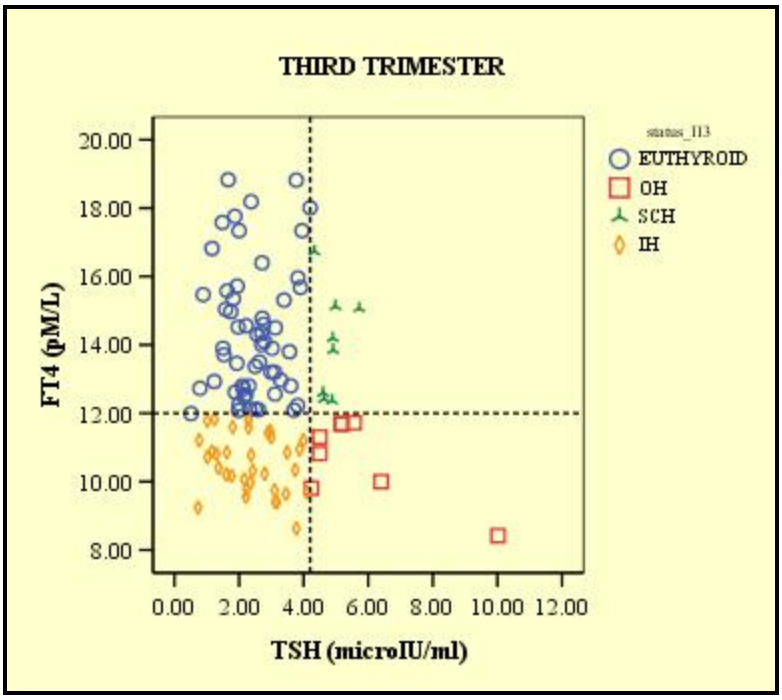
Value in parenthesis indicate percentage


FIRST TRIMESTER
FT4 (pM/L)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
20.00
40.00
60.00
80.00
100.00
TSH (microIU/ml)
status_I11
EUTHYROID
OH
SCH
IH
OHYPER
SCHYPER

(a)


SECOND TRIMESTER
FT4 (pM/L)
24.00
21.00
18.00
15.00
12.00
9.00
6.00
0.00
2.00
4.00
6.00
8.00
10.00
TSH (microIU/ml)
status_I12
EUTHYROID
OH
SCH
IH
SCHYPER
HYPERTHYROXINEMIA

(b)



(c)

Figure 4.52(a-c) show individual serum FT_4 and TSH values at first, second and third trimester according to method II. The vertical dotted lines indicate the highest normal trimester-specific TSH value (4.20µIU/ml in all the trimesters). The horizontal dotted lines indicate the lowest normal trimester-specific FT_4 value (12 pM/L in all the trimesters)

4.7.3 Prevalence of thyroid dysfunction according to Method III

When the trimester-specific reference intervals developed by other Indian study were used, it was found that in first trimester 46 subjects had low FT_4 and 28 had high TSH. Approximately 90 percent of the subjects had normal FT_4 and TSH values in second trimester (Table 4.77).

The prevalence of thyroid dysfunction in first trimester was found to be 40 percent which reduced to 16 percent in second trimester and 31 percent in third trimester (Table 4.78) (Fig. 4.53 a-c).

Table 4.77 Categorization of subjects according to method III FT_4 and TSH levels

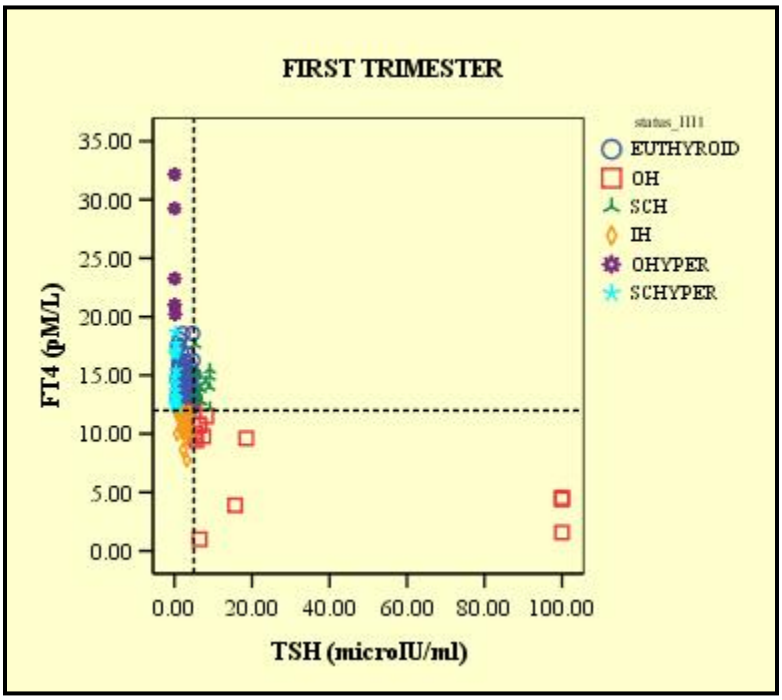
Method	Trimester	FT_4			TSH		
		Low	Normal	High	Low	Normal	High
III	1st	46	138	5	19	142	28
		(24.3)	(73)	(2.7)	(10)	(75.2)	(14.8)
	2nd	13	129	1	3	132	8
		(9.1)	(90.2)	(0.7)	(2.1)	(92.3)	(5.6)
	3rd	32	70	4	2	101	3
		(30.2)	(66)	(3.8)	(1.9)	(95.3)	(2.8)

Value in parenthesis indicate percentage

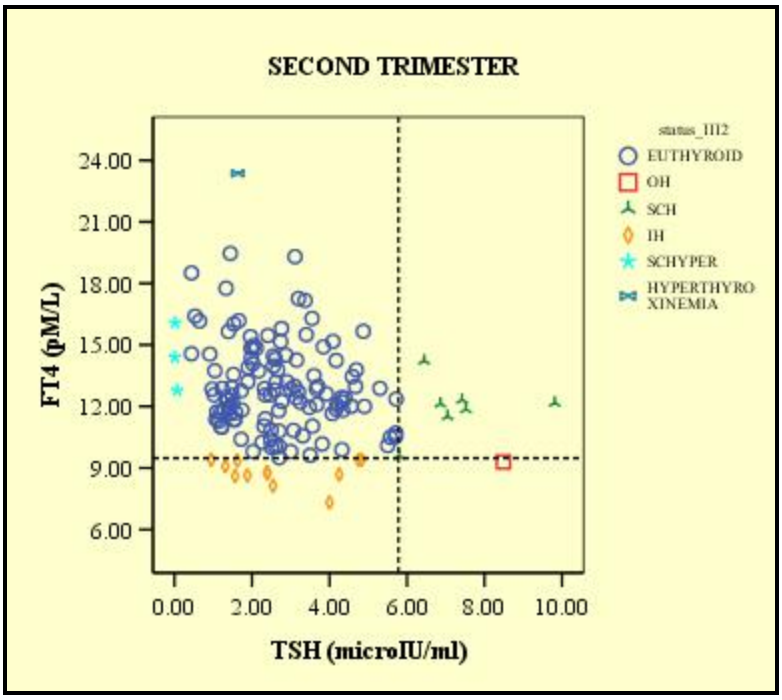
Table 4.78 Prevalence of thyroid dysfunction according to method III

Method	Trimesters	OH	SCH	IH	OHyper	SCHyper
III	1st	14 (7.41)	12 (6.35)	31 (16.4)	5 (2.65)	13 (6.88)
	2nd	1 (0.7)	7 (4.90)	12 (8.39)	-	3 (2.1)
	3rd	2 (1.89)	1 (0.94)	29 (27.36)	-	1 (0.94)

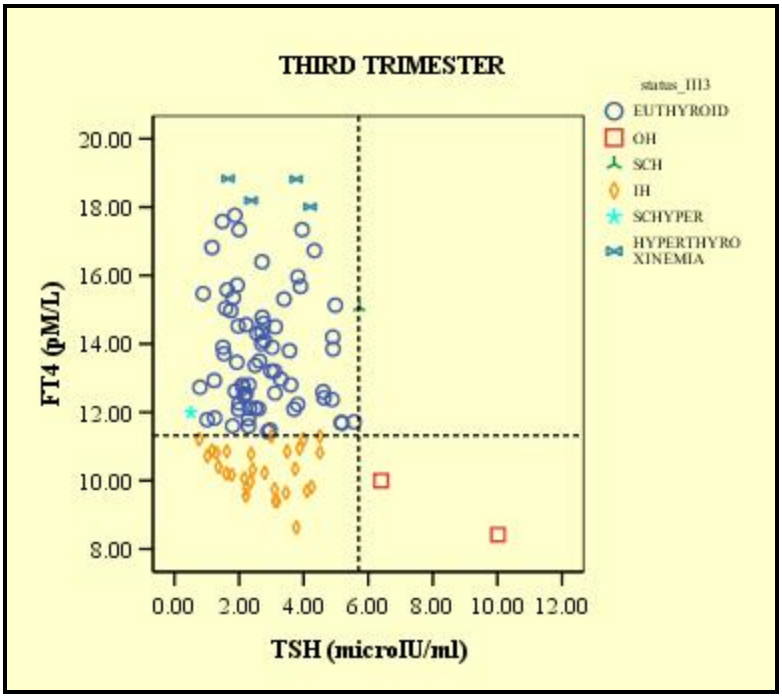
Value in parenthesis indicate percentage



(a)



(b)



(c)

Figure 4.53(a-c) show individual serum FT_4 and TSH values at first, second and third trimester according to method III. The vertical dotted lines indicate the highest normal trimester-specific TSH value (5.0, 5.78 and 5.7 μIU/ml at first, second and third trimester, respectively). The horizontal dotted lines indicate the lowest normal trimester-specific FT_4 value (12, 9.48 & 11.32 pM/L at first, second and third trimester, respectively)

4.7.4 Prevalence of thyroid dysfunction according to Method IV

When Endocrine society guidelines were followed, it was found that only 50 percent of the subjects in first trimester and 60 percent in second and third trimester had normal TSH. The lower FT_4 levels were found in 25 percent to 40 percent subjects from first to third trimester, respectively (Table 4.79).

Table 4.79 Categorization of subjects according to method IV FT_4 and TSH levels

Method	Trimester	FT_4			TSH		
		Low	Normal	High	Low	Normal	High
IV	1st trim	46 (24.3)	140 (74.1)	3 (1.6)	10 (5.3)	92 (48.7)	87 (46)
	2nd trim	56 (39.2)	86 (60.1)	1 (0.7)	3 (2.1)	82 (57.3)	58 (40.6)
	3rd trim	42 (39.6)	64 (60.4)	-	-	64 (60.4)	42 (39.6)

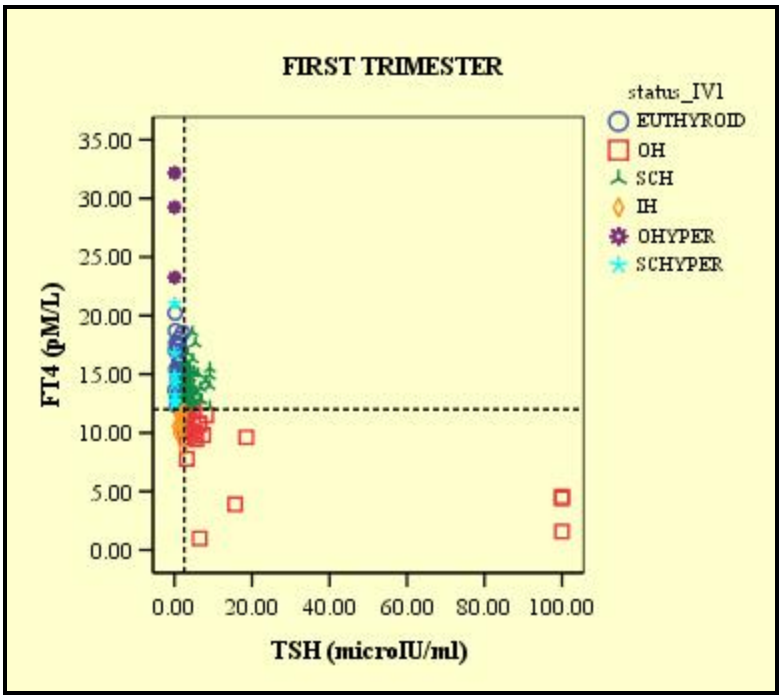
Value in parenthesis indicate percentage

High prevalence of SCH was found in first trimester. Approximately 20 percent of the subjects had SCH and IH in both second and third trimester (Fig. 4.54 a-c). Three of the subjects had overt hyperthyroidism in the first trimester (Table 4.80).

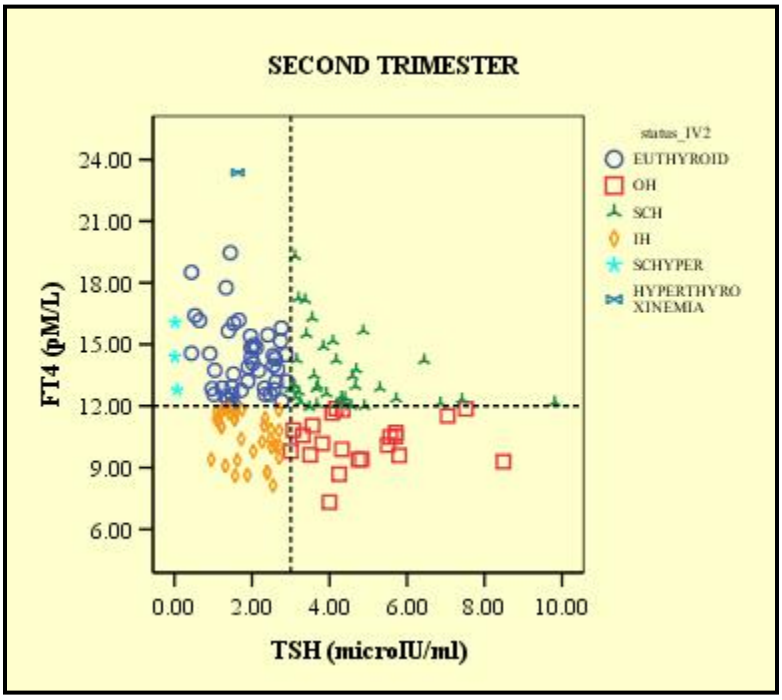
Table 4.80 Prevalence of thyroid dysfunction according to method IV

Method	Trimesters	OH	SCH	IH	OHyper	SCHyper
IV	1st trim	28 (14.81)	58 (30.69)	18 (9.52)	3 (1.59)	7 (3.7)
	2nd trim	22 (15.38)	36 (25.17)	34 (23.78)	-	3 (2.1)
	3rd trim	19 (17.92)	23 (21.69)	23 (21.70)	-	-

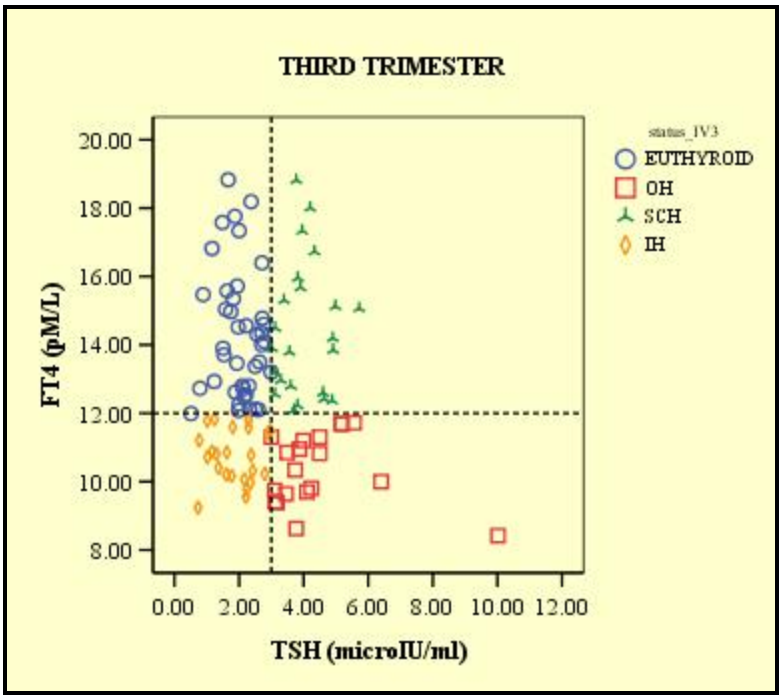
Value in parenthesis indicate percentage


FIRST TRIMESTER
status_IV1
EUTHYROID
OH
SCH
IH
OHYPER
SCHYPER
FT4 (pM/L)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0.00
20.00
40.00
60.00
80.00
100.00
TSH (microIU/ml)

(a)


SECOND TRIMESTER
status_IV2
EUTHYROID
OH
SCH
IH
SCHYPER
HYPERTHYRO XINEMIA
FT4 (pM/L)
24.00
21.00
18.00
15.00
12.00
9.00
6.00
0.00
2.00
4.00
6.00
8.00
10.00
TSH (microIU/ml)

(b)



(c)

Figure 4.54(a-c) show individual serum FT_4 and TSH values at first, second and third trimester according to method IV. The vertical dotted lines indicate the highest normal trimester-specific TSH value (2.0, 3.0 and 3.0 µIU/ml at first, second and third trimester, respectively). The horizontal dotted lines indicate the lowest normal trimester-specific FT_4 value (12 pM/L in all the three trimesters)

4.7.5 Comparison of prevalence according to different methods

Trimester- specific reference intervals were established for thyroid function tests (TFT). These reference intervals are significantly different from those reported by the assay manufacturer and guidelines given by other authors. Utilization of non-pregnant reference intervals to interpret TFT in pregnant women can result in a large number of misclassified results, and could contribute to suboptimal patient care. Direct comparison of our reference intervals to other published data is problematic due to several reasons. As there is no internationally recognized method for standardization of free thyroid hormone tests, assay result, as well as influence of pregnancy on assay performance varies among different assay manufacturers. This shows that method-specific reference intervals are required for free thyroid hormone assays (Stricker, *et al.* 2007).

When compared it was revealed that prevalence of IH by our established reference intervals (Method I) is much lower (2.6 percent in first trimester to 0.9 percent in third trimester) than found by other three methods in all the three trimesters (Fig. 4.55a- c). This is due to the fact that the lower reference limit of FT_4 in our method is lower than the other three methods. Similarly, Vaidya, *et al.* 2007 found that prevalence of hypothyroxinemia was 1.6 percent according to their own internal FT_4 1^{st} trimester – specific reference range, and as high as 7.8 percent when they used the manufacturer's general population reference range, the lower FT_4 limit in the latter (12 pM/L) being higher than those found in the pregnant population (10.6 pM/L).

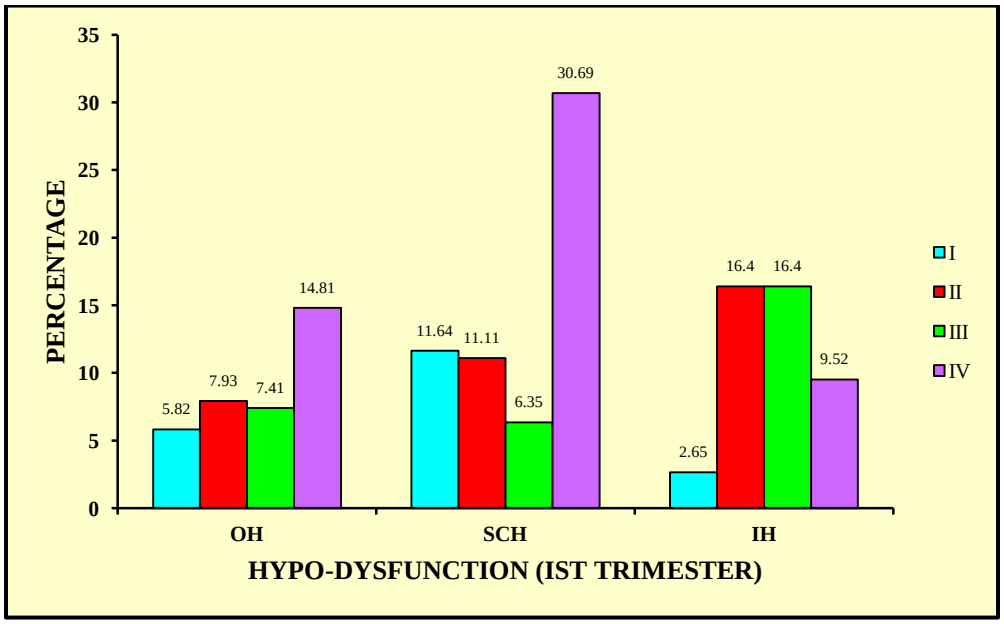
In regions where iodine intake is sufficient, as in US, the prevalence of IH ranges between 1.3 percent (Casey, Dashe and Spong, *et al.* 2007) and 2.3 percent (Cleary-Goldman, *et al.* 2008). In contrast, in mildly to moderately iodine deficient regions, IH affects a much higher percentage of women, reaching values upto 25-30 percent (Moleti 2008, Berbel, *et al.* 2009). A recent study by Henrichs, *et al.* 2010 reported the prevalence of mild hypothyroxinemia (FT_4 $<10^{th}$ percentile) 8.5 percent and that of severe hypothyroxinemia (FT_4 $<5^{th}$ percentile) 4.3 percent, which is significantly higher than those reported by previous studies conducted in iodine sufficient regions.

Our reference intervals showed that the prevalence of OH (5.8 percent) in first trimester was lower than found by other methods as the FT_4 lower reference limit decreased and upper TSH limit was high (Fig. 4.55a). Sahu, *et al.* 2010 showed that the prevalence of thyroid dysfunction was high among pregnant women of Delhi with OH in 4.58 percent and SCH in 6.47 percent.

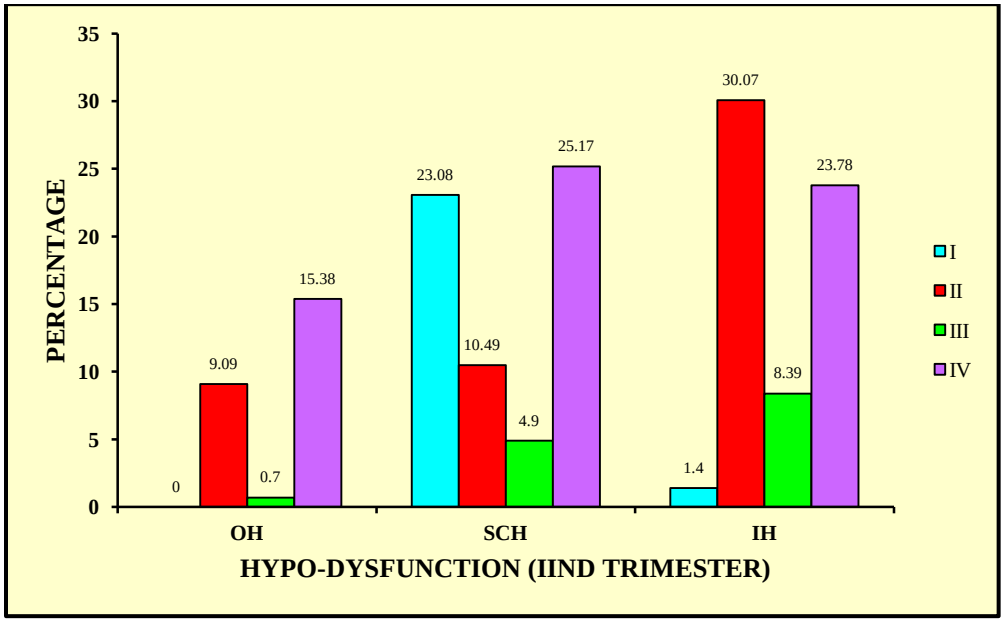
The prevalence of SCH in all the three trimesters (Fig. 4.55a-c) by method I increased in comparison to method II & III because of the decreased lower FT_4 reference limit whereas it decreased in comparison to method IV due to higher upper TSH reference limit. Marwaha, *et al.* 2008 reported that the prevalence of SCH decreased from 14.4 percent using kit reference interval to 6.6 percent when they used their reference interval.

Stricker, *et al.* 2007 reported that a total of 3.6 percent women with elevated TSH would not have been identified, and 3.7 percent women would have been incorrectly classified as having a low TSH if non-pregnant reference intervals were used. Potential for

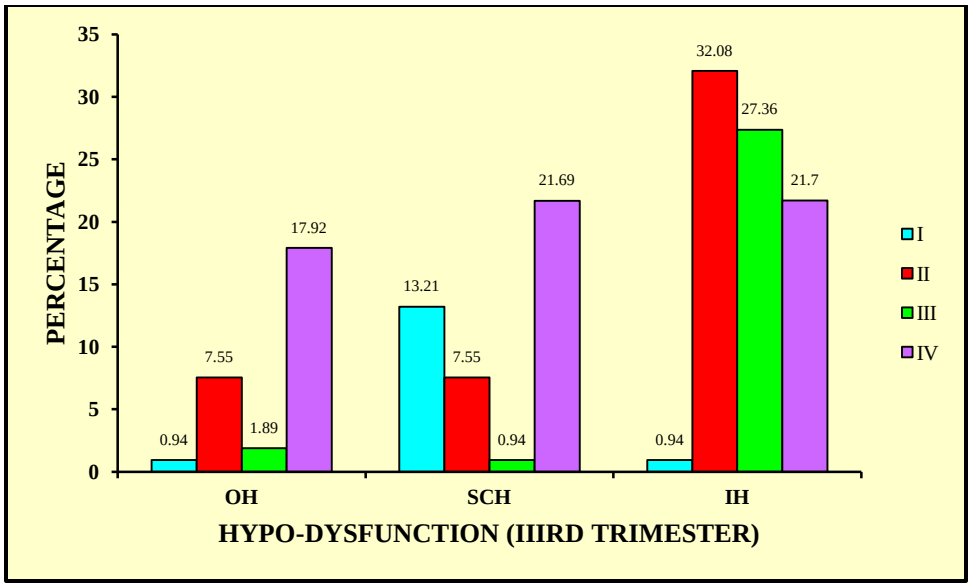
misclassification of TSH results was greatest in the first trimester (10.4 percent). For FT_4, 1.9 percent women with elevated results would not have been identified. In the first and second trimesters, 2.3 percent with low FT_4 would not have been identified.



(a)



(b)



(c)

Figure 4.55 Comparative prevalence of hypo-dysfunction in (a) first trimester (b) second trimester (c) third trimester using different classification criteria

According to all the methods the prevalence of overt hyperthyroidism was approximately 1.5 percent in first trimester (Fig. 4.56). No case of overt hyperthyroidism was observed in second and third trimester by any of the methods. Subnormal TSH levels in the first half of pregnancy cannot be interpreted as diagnostic of hyperthyroidism as it is most likely to be hCG mediated.

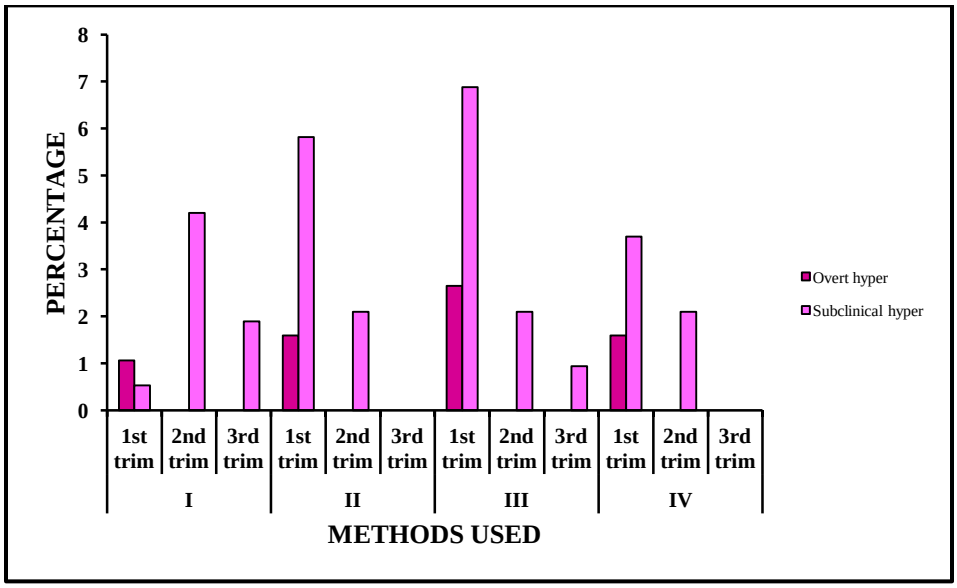


Figure 4.56 Comparative prevalence of hyper-dysfunction in different trimesters using different classification criteria

4.8 NUTRITIONAL STATUS OF THE RESPONDENTS

The diet history of the Group I and Group II subjects was recorded by 24-hour recall method at the time of initial visit i.e. first trimester and last visit i.e. third trimester whereas in Group III it was recorded at the time of registration and after one month. Nutrition health education (Annexure III) was provided to the subjects regarding foods to be consumed which are good sources of iron, calcium and vitamin-C. Also the subjects were given information about the food items containing goitrogens.

The mean energy, protein and fat intake were compared with recommended dietary allowance (RDA) (Gopalan, Sastri and Balasubramanian 2004).

In groups I, II and III the pre and post nutrition health education (NHE) data of 126, 118 and 30 subjects is present.

4.8.1 Nutrient intake of the respondents

Figure 4.57 depicts that majority of the respondents in group I and II were vegetarians whereas in group III 60 percent of the respondents were non-vegetarian.

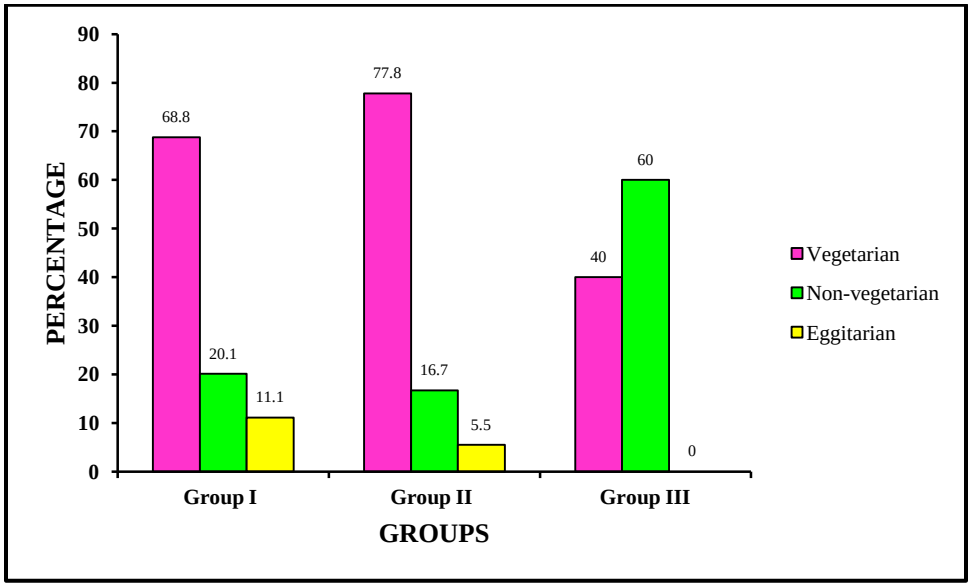


Figure 4.57 Eating habits of the respondents

Table 4.81 and 4.82 indicates the average intake of energy and protein in comparison to RDA. Recommended dietary allowance (RDA) is the intake of nutrients derived from the diet that keeps nearly all people in good health. It takes into account the individual

variation in nutrient needs and also availability of nutrients, which may vary from diet to diet (Gopalan, Sastri and Balasubramanian 2004). As per RDA, the energy consumption should have been 2525 kcal/day. The average energy consumption of group I, II and III pregnant women at the time of registration was considerably low 34 percent, 27 percent and 39 percent, respectively when compared to RDA (Table 4.81). The less intake of calories lead to various problems. Even though the pregnant women took more cereal foods, still a deficit intake was observed. In third trimester, an energy deficit of 17 percent and 16 percent was found in group I and II women, respectively whereas a deficit of 33 percent was found in group III subjects after one month (Table 4.82). In group I and II number of subjects with >10% deficient energy intake reduced from 93 percent to 70 percent as the pregnancy progressed. No change in energy intake was found in group III subjects (Fig. 4.58).

During pregnancy, protein rich diet promotes optimum fetal growth. RDA for protein for pregnant women is 65 g/day. In this study, a deficit of 25 percent, 12 percent and 35 percent was found in group I, II and III subjects, respectively at the time of initial presentation (Table 4.81) when compared to RDA. In third trimester, an excess intake of 10 and 20 percent was found in group I and II subjects, respectively (Table 4.82). The percentage of women with adequate RDA ± 10% increased by about 20 % in group I and II respondents, respectively (Fig. 4.59).

Epidemiological studies in India and studies in experimental animals indicate that maternal macro and micronutrient deficiencies can have short and long term impact on the nutrition and health status of the offspring. Fetal growth can be readily restricted in experimental animals by reducing maternal intakes of energy and protein during pregnancy (Harding 2001). Energy and protein deficiency in the mother is also associated with intrauterine growth retardation in humans; size at birth is strongly related to maternal body mass index and during acute famine birth weight falls by several hundred grams. Randomized clinical trials of energy and protein supplements have shown small effects on birth weight. There is evidence that supplementing undernourished mothers with micronutrients, or improving their diet quality, increases fetal growth (Fall 2009).

Table 4.81 Mean energy and protein intake in various groups at the time of initial presentation

Groups	n	Energy (Kcal)			Protein (g)		
		Mean ± SD	RDA	% Deficit	Mean±SD	RDA	% Deficit
I	189	1653.6 ± 343.1	2525	-34.51	48.52 ± 13.3	65	-25.35
II	144	1833.5 ± 351.1	2525	-27.38	56.87 ± 14.9	65	-12.5
III	30	1542.4 ± 231.5	2525	-38.9	42 ± 9.1	65	-35.38

Table 4.82 Mean energy and protein intake in third trimester (Group I & II) and after one month (Group III)

Groups	n	Energy (Kcal)			Protein (g)		
		Mean ± SD	RDA	%Deficit	Mean ± SD	RDA	%Excess/deficit
I	126	2092.85 ± 353.2	2525	-17.11	71.85 ± 28.4	65	+10.54
II	118	2124.5 ± 363.6	2525	-15.86	77.65 ± 25.6	65	+19.46
III	30	1681.2 ± 265.1	2525	-33.42	44.7 ± 10.1	65	-31.23

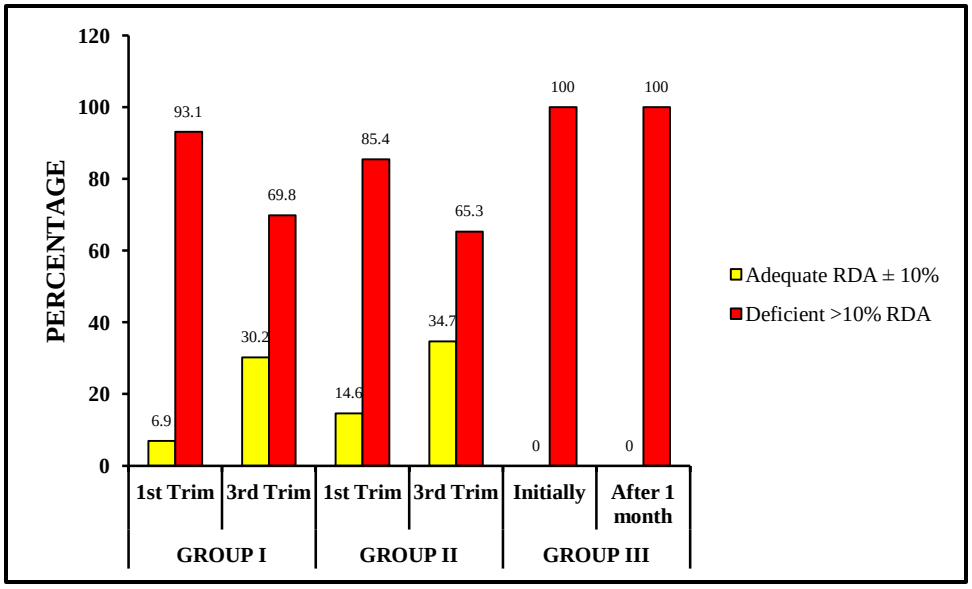


Figure 4.58 Distribution of pregnant women according to their status of energy intake

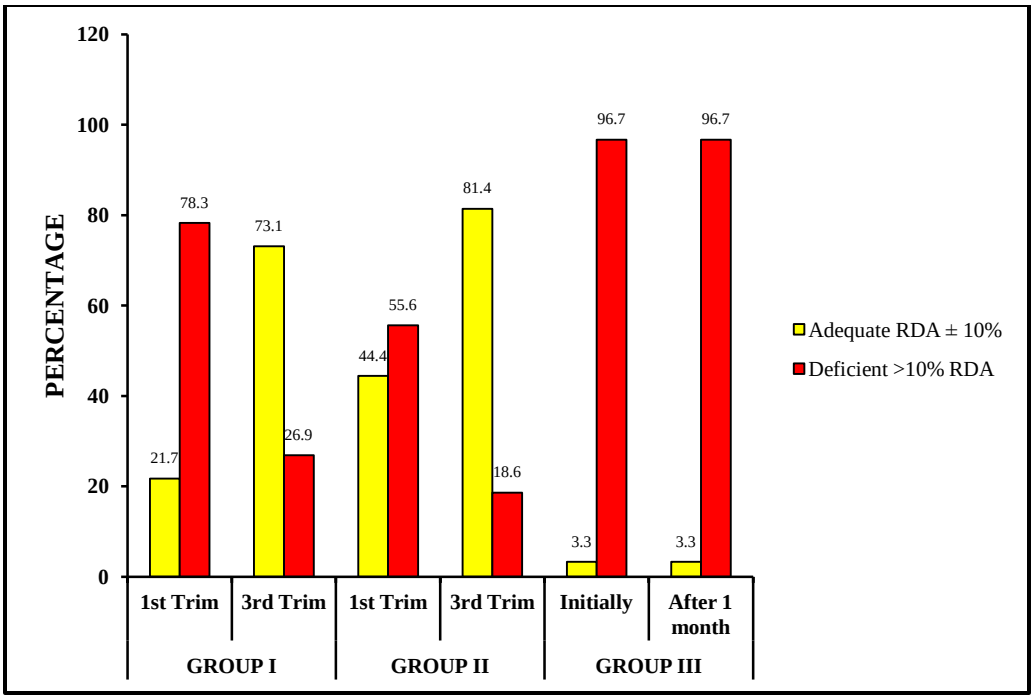


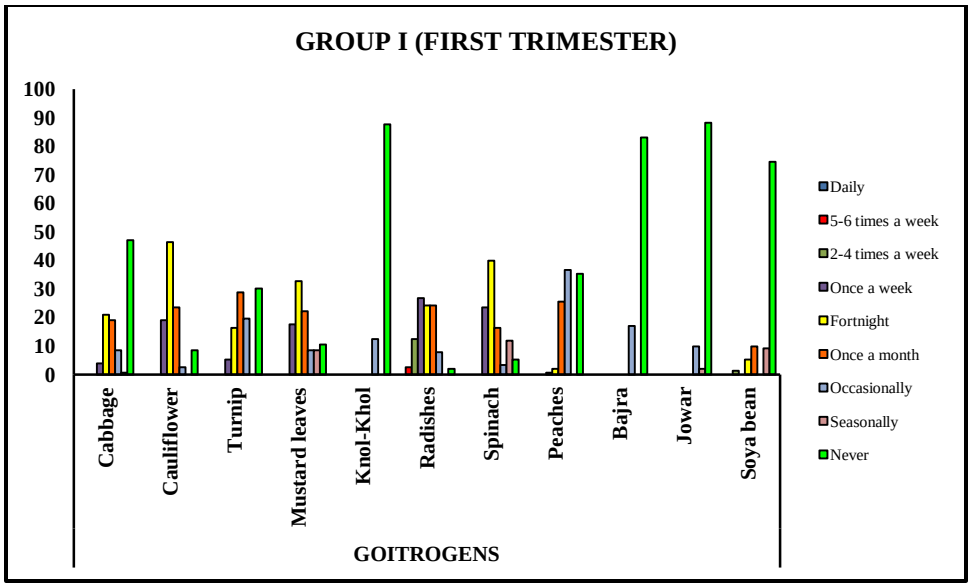
Figure 4.59 Distribution of pregnant women according to their status of protein intake

4.8.2 Food Frequency

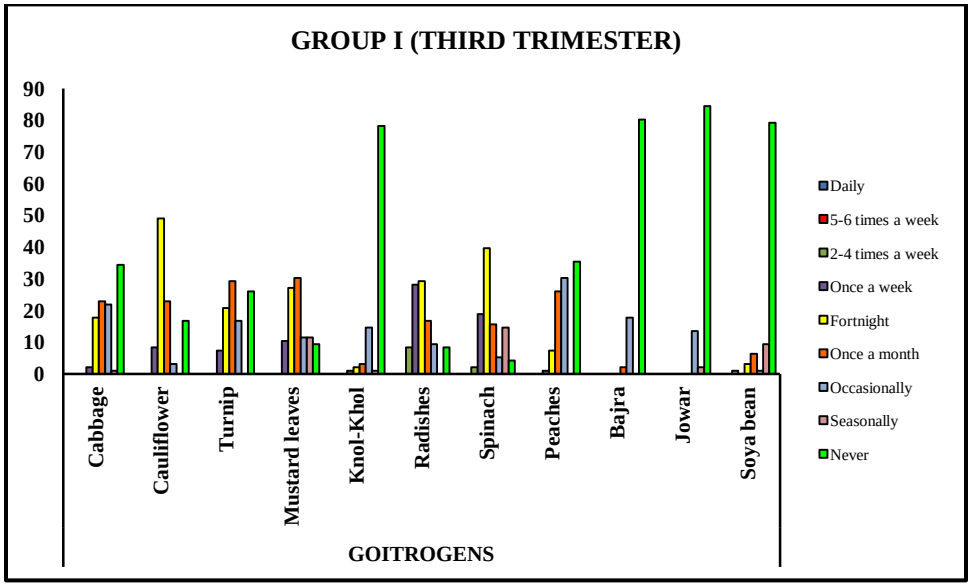
Majority of the subjects in all the three groups consumed full cream milk.

Group I:

***Goitrogens*-** Majority of the subjects (27 percent) consumed radish once a week while 40 percent consumed spinach fortnightly. Almost half of the subjects did not consume cabbage at all while consumed cauliflower once in 15 days. Seventy to 80 percent of the subjects did not consume *bajra*, *jowar* or soyabean (Fig. 4.60a). Though NHE was provided no much difference in goitrogens consumption was observed among the subjects (Fig. 4.60b).



(a)

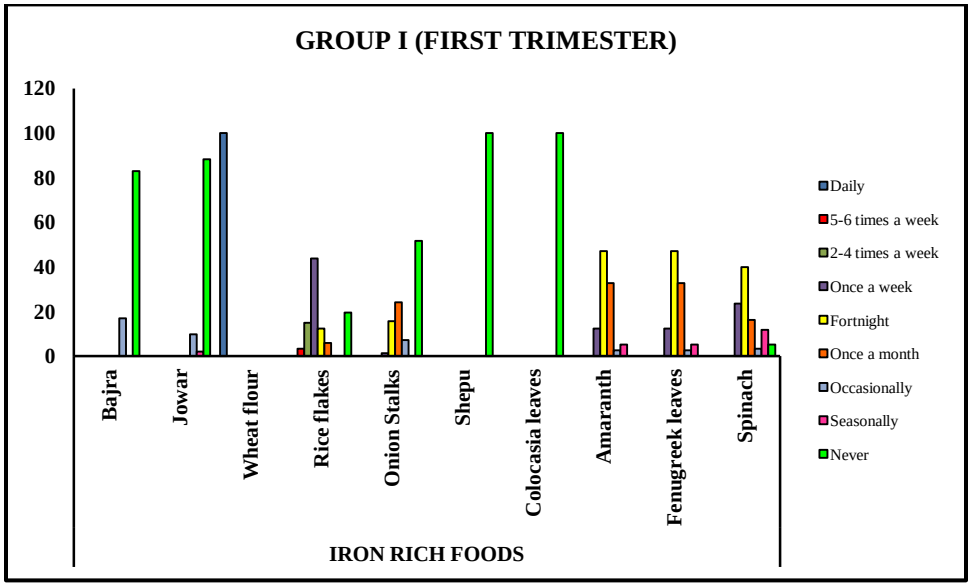


(b)

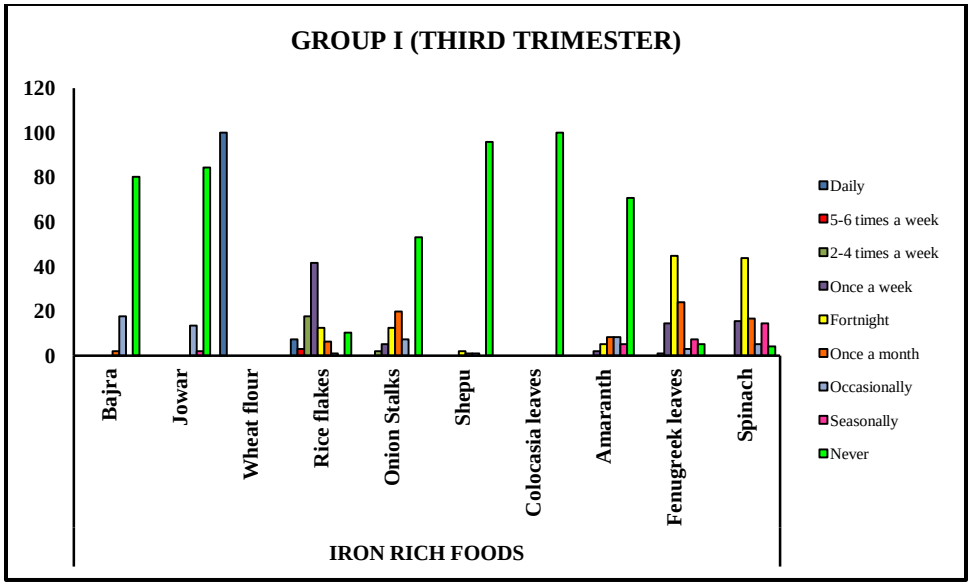
Figure 4.60 Consumption of goitrogens in Group I subjects (a) before and (b) after NHE

Iron rich foods- Wheat flour was consumed by the subjects daily while half of the subjects consumed rice flakes once a week. One-half of the subjects consumed onion stalks either fortnightly or once a week. Half of the subjects had amaranth, fenugreek

leaves and spinach once in fifteen days (Fig. 4.61a). No much difference in consumption pattern of iron rich foods was observed in third trimester (Fig. 4.61b).



(a)

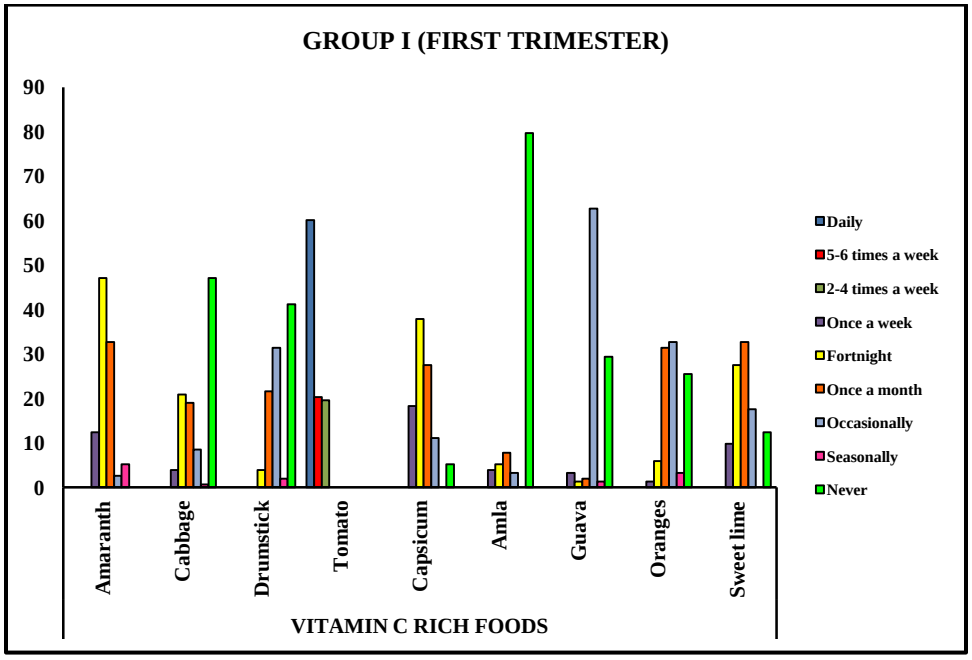


(b)

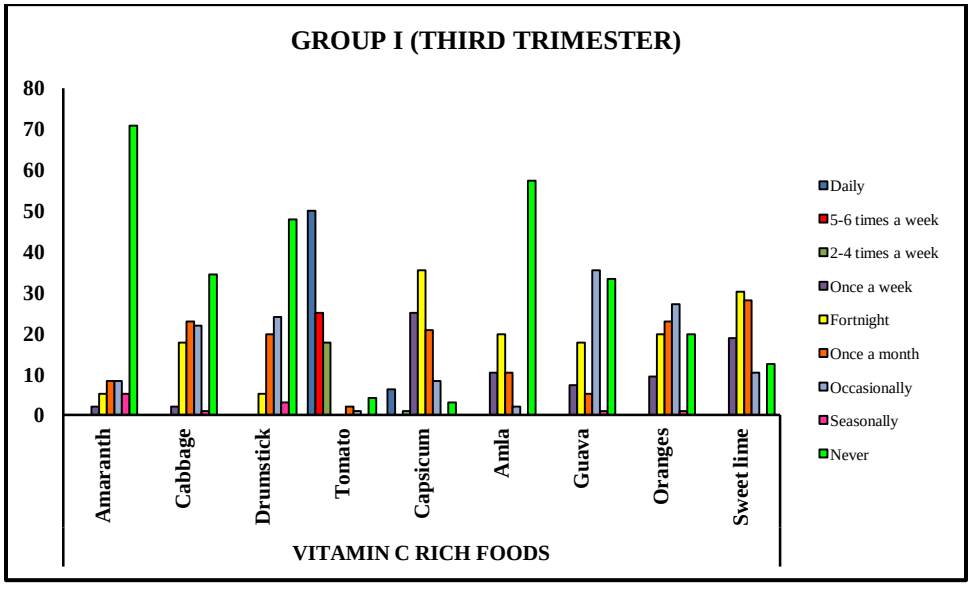
Figure 4.61 Consumption of iron rich foods in Group I subjects (a) before and (b) after NHE

Vitamin-C rich foods- About 20 percent of the subjects consumed amla either once a month or occasionally. Majority of the subjects took guava and oranges occasionally

while sweet lime once a month (Fig. 4.62a). After NHE half of the respondents started consuming amla and frequency of consumption of fruits like oranges and sweet lime also increased (Fig. 4.62b).



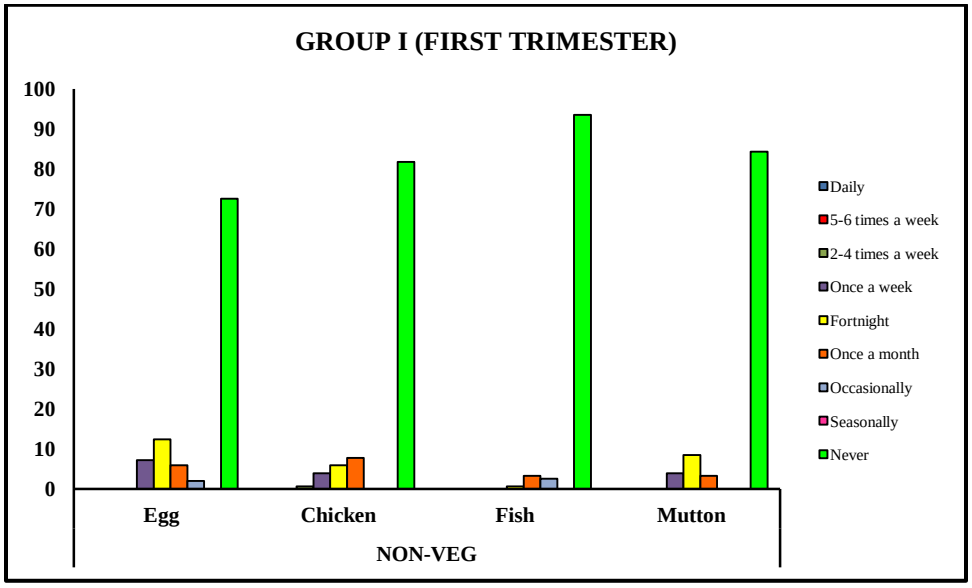
(a)



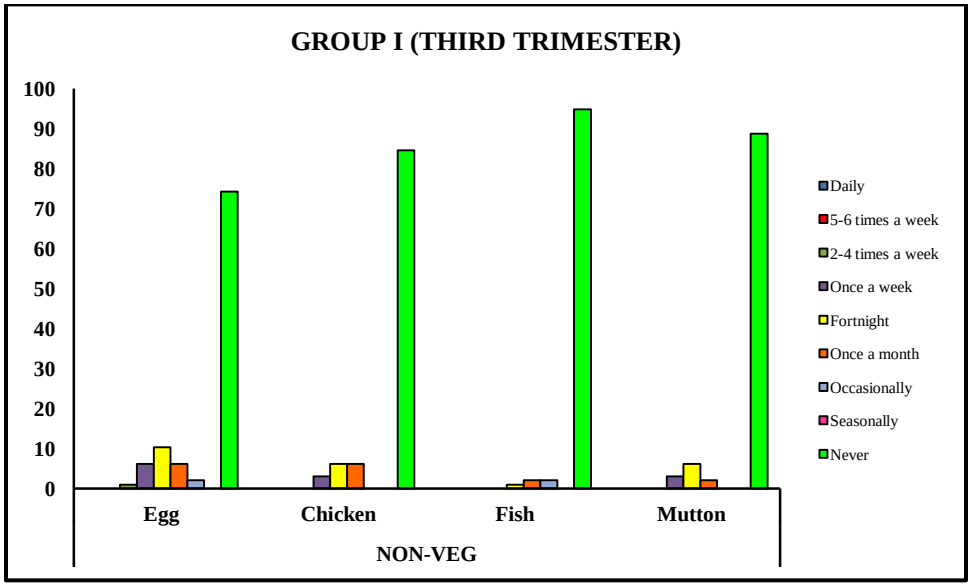
(b)

Figure 4.62 Consumption of vitamin-C rich foods in Group I subjects (a) before and (b) after NHE

Non-vegetarian food consumption- The percentage of people who were non-vegetarian consumed mostly chicken either once in 15 days or once a month. Very less percentage of subjects consumed fish (Fig. 4.63a). Same pattern was observed in third trimester also (Fig. 4.63b).



(a)

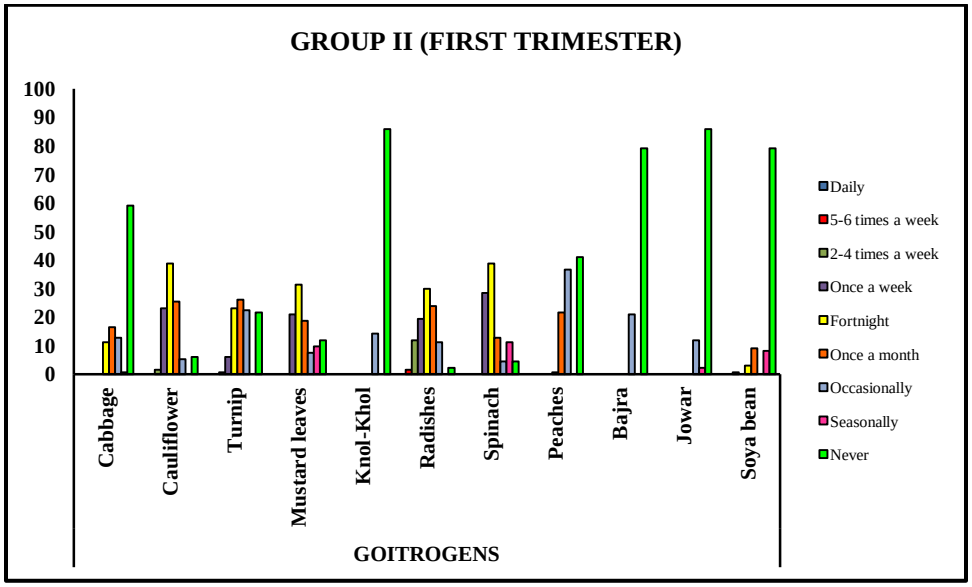


(b)

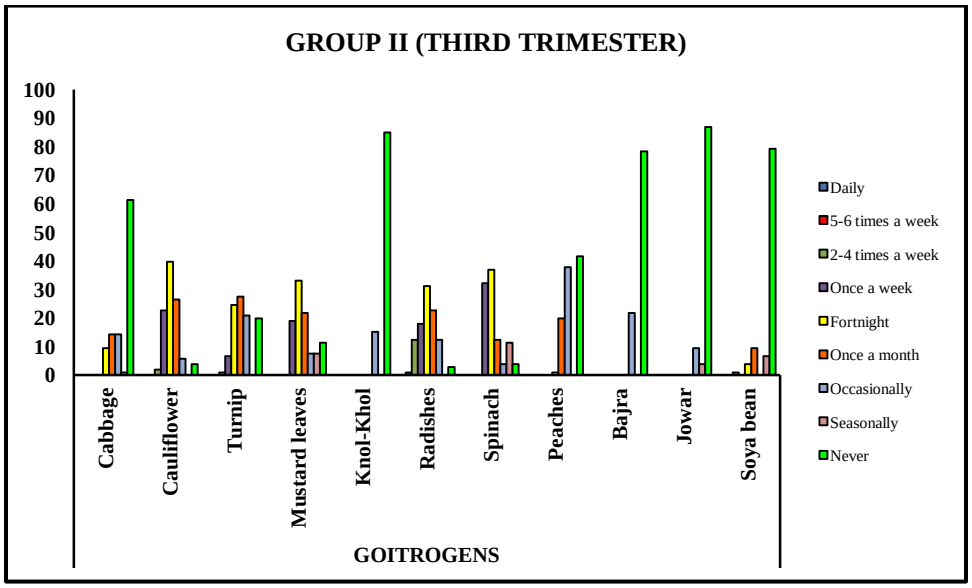
Figure 4.63 Consumption of non-vegetarian foods in Group I subjects (a) before and (b) after NHE

Group II:

Goitrogens- Sixty percent of the subjects never consumed cabbage while 40 percent consumed cauliflower fortnightly. Thirty to 35 percent of the respondents took mustard leaves, spinach and radishes once in 15 days. Approximately 80 percent of the subjects never had jowar, bajra or soyabean (Fig. 4.64a). No much difference in consumption pattern of goitrogens was observed in third trimester (Fig. 4.64b).



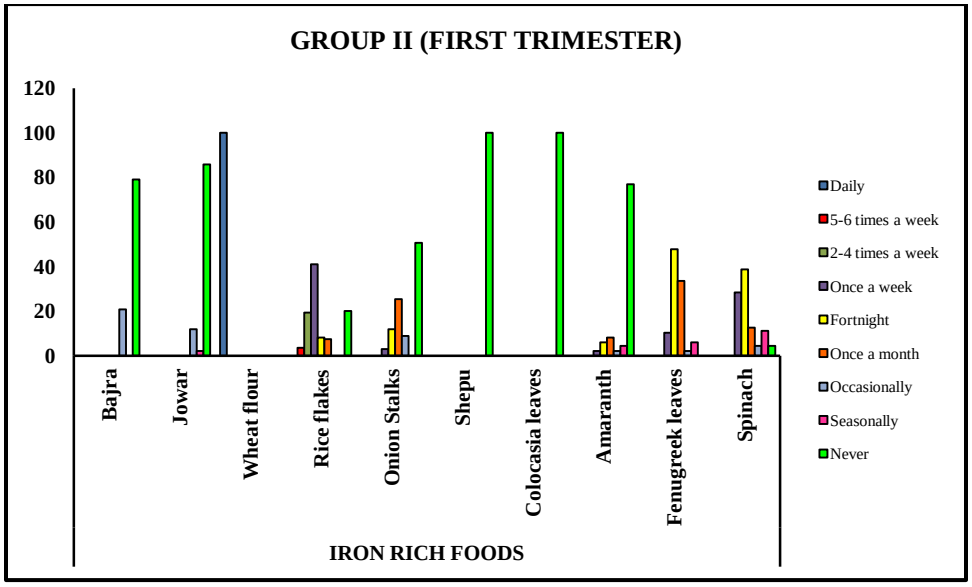
(a)



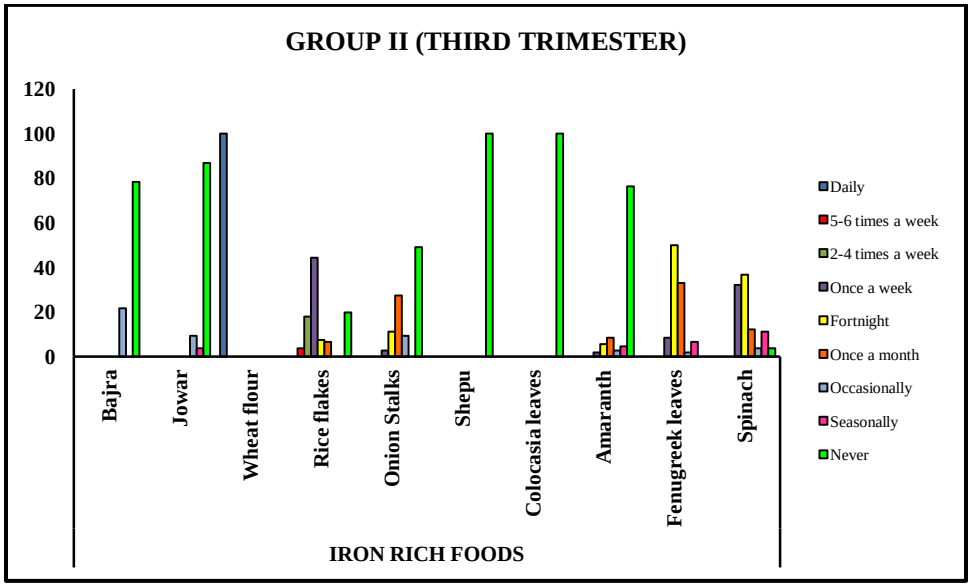
(b)

Figure 4.64 Consumption of goitrogens in Group II subjects (a) before and (b) after NHE

Iron rich foods- All the respondents consumed wheat flour daily while majority (80 percent) never consumed jowar and bajra. Twenty percent of the respondents had rice flakes 2-4 times a week and half of the subjects took fenugreek leaves once in 15 days. Almost 30 percent consumed spinach once a week (Fig. 4.65a). After NHE half of the subjects started consuming rice flakes once a week. No much difference in other foodstuffs was observed (Fig. 4.65b).



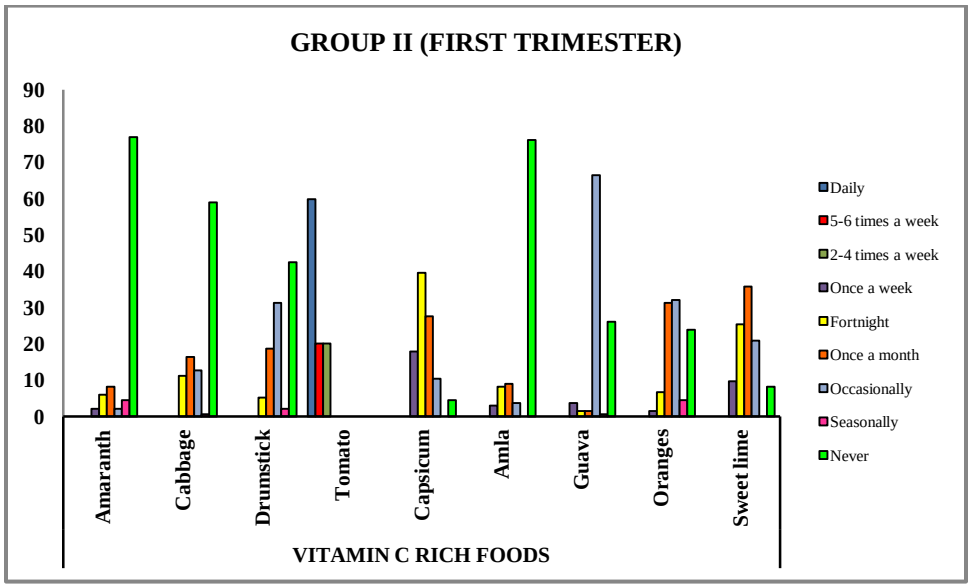
(a)



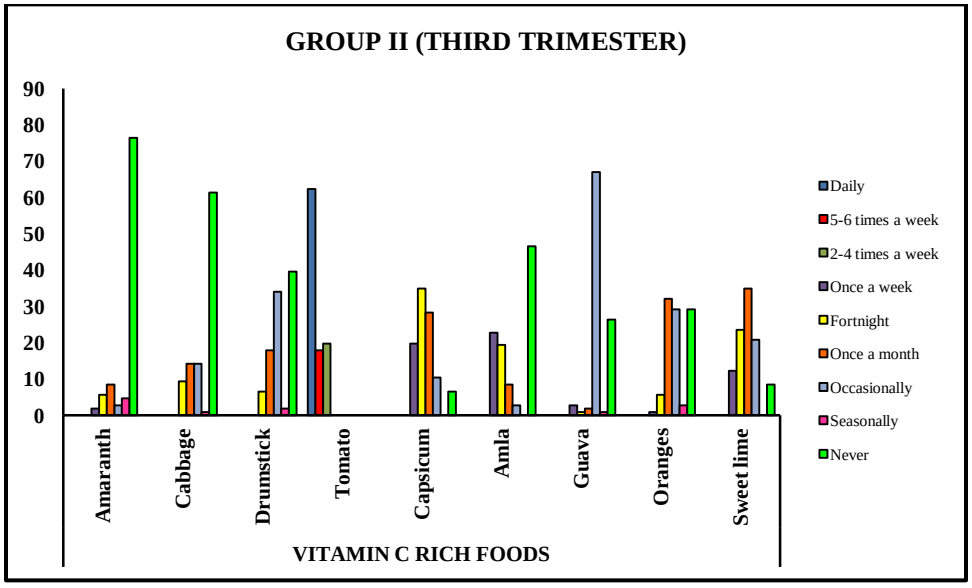
(b)

Figure 4.65 Consumption of iron rich foods in Group II subjects (a) before and (b) after NHE

Vitamin-C rich foods- Majority of the subjects (80 percent) never consumed amla and one-fourth never consumed oranges and sweet lime. Tomato was consumed daily by 60 percent of the respondents (Fig. 4.66a). After NHE an increase in amla consumption was observed. Almost 20 percent started consuming amla once a week. An increase in consumption of sweet lime was also observed (Fig. 4.66b).



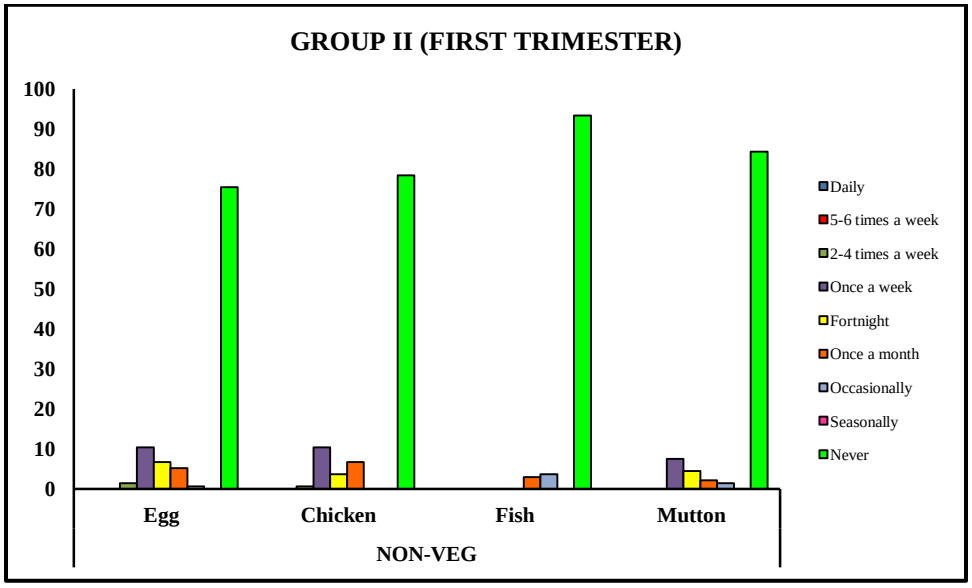
(a)



(b)

Figure 4.66 Consumption of vitamin C rich foods in Group II subjects (a) before and (b) after NHE

Non-vegetarian foods- Fish was consumed by 2-3 percent of the respondents and that too occasionally. Mostly respondents consumed egg and chicken once a week. Mutton was consumed by 7 percent once a week (Fig. 4.67a). Same consumption pattern was observed after NHE (Fig. 4.67b).



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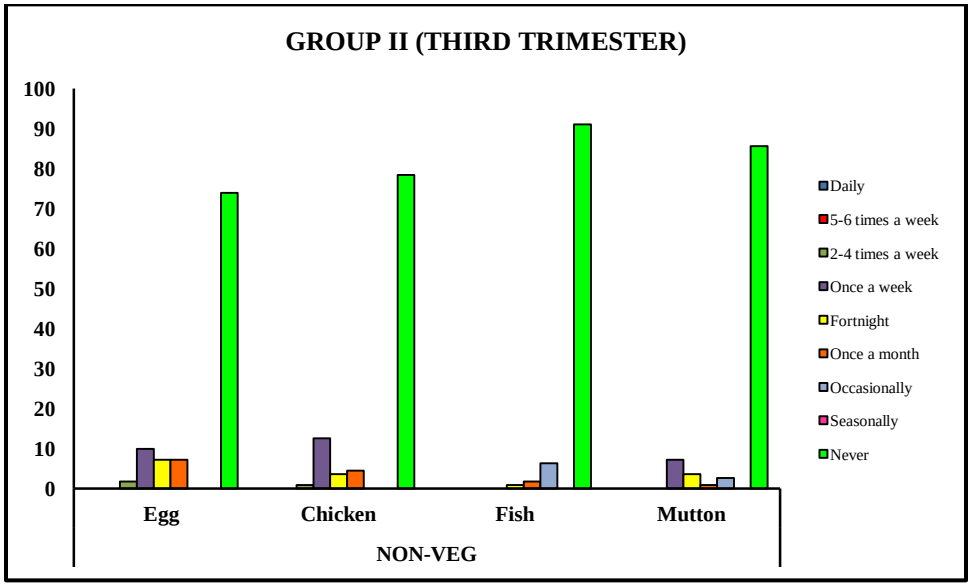

(b)

Figure 4.67 Consumption of non-vegetarian foods in Group II subjects (a) before and (b) after NHE

Group III:

Goitrogens- Half of the subjects consumed cabbage 2-4 times a week and 60 percent had cauliflower once a week. 20 percent of the respondents consumed jowar and bajra once a week. 30 percent consumed soyabean once a month or once in 15 days (Fig. 4.68).

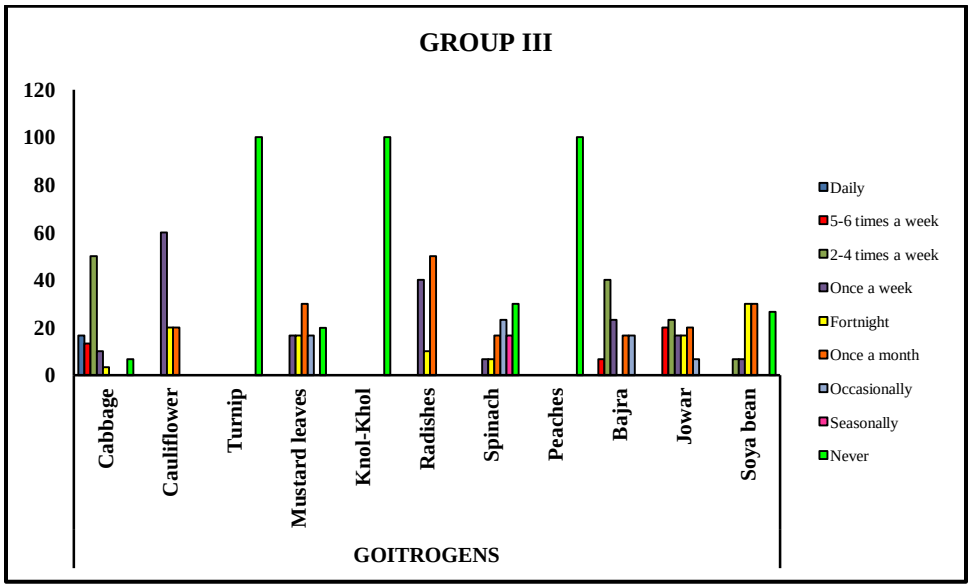

Figure 4.68 Consumption of goitrogens in Group III subjects

Iron rich foods- Very few (20 percent) consumed amaranth. High consumption of rice flakes was found among the subjects. Sixty percent never had onion stalks (Fig. 4.69).

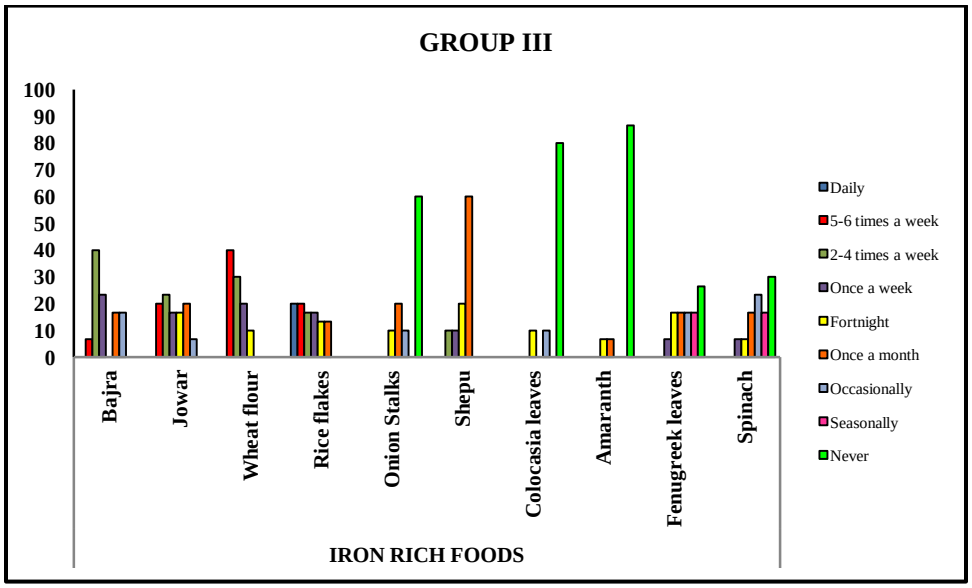

Figure 4.69 Consumption of iron rich foods in Group III subjects before and after NHE

Vitamin –C rich foods- Less consumption of these foods was found among the subjects. Approximately 80-90 percent of the subjects never had amaranth, drumstick, amla, oranges and sweet lime (Fig. 4.70).

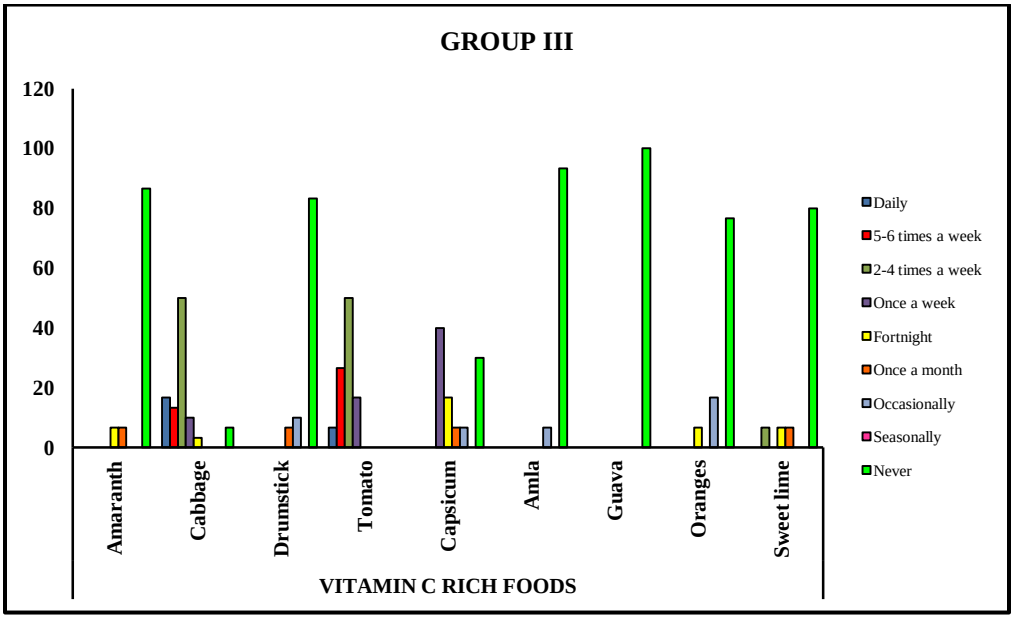

Figure 4.70 Consumption of vitamin C rich foods in Group III subjects before and after NHE

Non-vegetarian foods- High consumption of egg, chicken and mutton was observed. Approximately 30 percent of the subjects had egg and chicken once a week. 20 percent consumed mutton 2-4 times a week (Fig. 4.71).

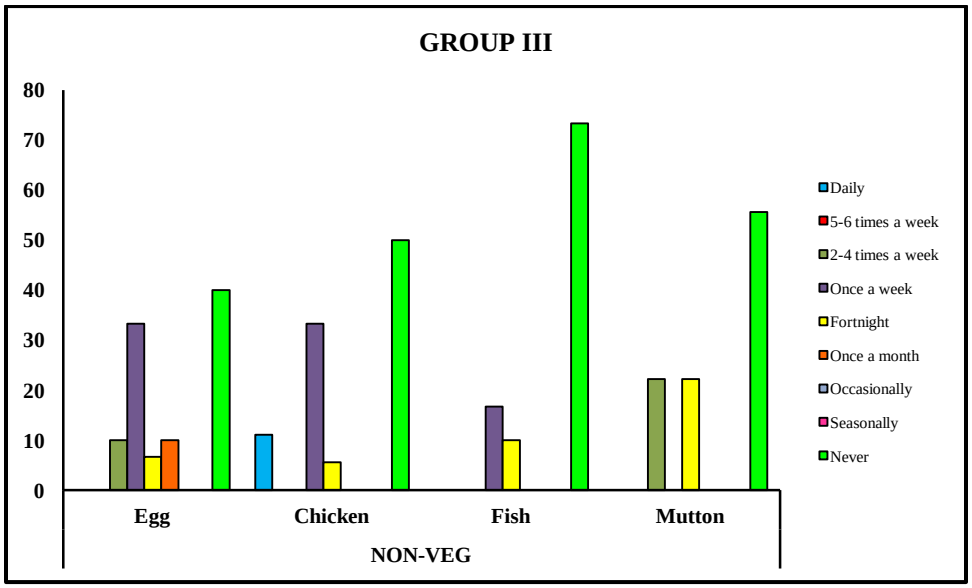

Figure 4.71 Consumption of non-vegetarian foods in Group III subjects

Thus, it can be stated that nutrition health education proved beneficial to improve consumption patterns regarding vitamin-C rich foods like amla and citrus fruits. Also, slight improvement was seen in consumption of iron rich foods. The consumption of protein rich foods increased which can be seen by excess of mean protein by third trimester in group I and II subjects whereas in first trimester mean protein deficit was observed.

4.9 Knowledge, attitude and practices (KAP) regarding iodized salt

Table 4.83 shows the knowledge of respondents about iodine, attitude regarding iodized salt and practices used for its consumption and storage. Majority of the subjects in group I (59 percent) and III (83 percent) did not have any knowledge about the importance of iodine for pregnant women and children. After NHE there was a 15- 20 percent increase in knowledge about importance of iodine in group I and II subjects. About 24 percent and 7 percent of the subjects in group I and III knew about the effects of iodine deficiency whereas 68 percent of group II subjects had knowledge regarding this. About 30 percent increase in knowledge about it was found in both group I and II subjects. Majority of the respondents purchased packed salt. Inspite of this almost 60 percent of group I and III subjects did not know that the salt which they consume is iodized or not. Seventy percent of group III subjects knew about the salt they consumed. Half of the subjects in group II were able to recognize iodized salt whereas in group I and III 20-25 percent were able to recognize. After NHE almost 65 percent of the respondent in group I and II were able to identify iodized salt. Tata salt was consumed by majority of the subjects in all the groups as it is readily available. About 85-90 percent subjects in all the groups added salt in the beginning while cooking but after NHE half of the subjects started adding salt at the end of cooking. Half of the subjects felt that iodized salt is costlier than non-iodized salt.

After nutrition health education, an improvement in consumption of salt, importance of iodine for pregnant women and how to recognize iodized salt was observed. This approach might have helped the population to sustain iodine levels in their circulation.

Table 4.83 Comparison of KAP regarding iodized salt between groups before and after NHE

	Group I		Group II		Group III
	Pre	**Post**	**Pre**	**Post**	**Pre**
Is adequate iodine intake important for pregnant women and children					
No	58.8	37.3	25.4	10.4	83.3
Yes	41.2	62.7	74.6	89.6	16.7
Do you know what happens due to iodine deficiency					
No	71.9	40	31.7	7.2	93.3
Yes	23.6	60	68.3	92.8	6.7
Which type of salt do you use					
Loose	2.6	0	0	0	33.3
Packed	97.4	100	100	100	66.7
Do you consume iodized salt					
No	54.2	20	29.1	0	60
Yes	45.8	80	70.9	100	40
Have you heard about iodized salt					
No	56.2	0	28.8	0	60
Yes	45.8	100	71.2	100	40
Can you recognize iodized salt					
No	75.2	37.3	53	33.8	80
Yes	24.8	62.7	47	66.2	20
Which brand of salt are you using					
Local	22.2	15.7	3.6	0	30
Tata	77.8	84.3	96.4	100	70
Reasons for using that particular brand of salt					
Iodized	20.9	30	32.4	35	6.7
Free flowing	3.3	10	2.1	0	0
Less price	9.3	10	2.1	0	33.3
Readily available	66.2	50	63.4	65	60
How frequently do you purchase salt					
Weekly	0	0	0	0	0
Fortnightly	4.6	5	5.9	0	40
Monthly	95.4	95	94	100	60

	Group I		Group II		Group III
	Pre	**Post**	**Pre**	**Post**	**Pre**
How much salt do you consume per month					
≤0.5 kg	34.6	20.9	0	0	0
0.5-1	36	45.4	55.2	50	30
1-1.5	10.5	3.3	0	0	70
1.5-2	12.4	30.4	29.9	50	0
>2	6.5	0	14.9	0	0
When do you add salt while cooking					
Beginning	97.4	33.4	82.8	20	100
In- between	2.6	16.7	14.9	35	0
End	0	49.9	2.2	45	0
Does loss of iodine occur during cooking & storing					
No	98	60	91	75	100
Yes	2	40	9	25	0
How do you store salt					
In jar with cap	89.5	95	94.8	100	80
In packet	10.5	5	4.5	0	20
In open container	0	0	0.7	0	0
Do you feel iodized salt is costlier than non-iodized salt					
No	54.2	45.5	67.6	82.8	40
Yes	45.8	54.5	32.4	17.2	60

4.10 GENERAL DISCUSSION

Iodine is an essential component of the hormones produced by the thyroid gland. Thyroid hormones, and therefore iodine, are essential for mammalian life. The native iodine content of most foods and beverages is low. In general, commonly consumed foods provide 3 to 80 µg/ serving. Foods of marine origin have higher iodine content because marine plants and animals concentrate iodine from seawater. Iodine in organic form occurs in high amounts of seaweeds. Inhabitants of the coastal regions of Japan, whose diets contain large amount of seaweed, have remarkably high iodine intakes amounting to 50 to 80 mg/d.

In healthy adults, the absorption of iodide is greater than 90 percent. Iodine is cleared from the circulation mainly by the thyroid and kidney, and whereas renal iodine clearance

is fairly constant, thyroid clearance varies with iodine intake. In conditions of adequate iodine supply, no more than 10 percent of absorbed iodine is taken up by the thyroid. In chronic iodine deficiency, this fraction can exceed 80 percent. The body of healthy adult contains 15-20 mg of iodine, of which 70-80 percent is in the thyroid. In chronic iodine deficiency, the iodine content of the thyroid may fall below 20 µg. In iodine-sufficient areas, the adult thyroid traps approximately 60 µg of iodine per day to balance losses and maintain thyroid hormone synthesis (Zimmermann 2009).

The thyroid adapts to low intakes of dietary iodine by marked modifications of its activity, triggered by increased secretion of TSH by the pitutary. In most individuals, if iodine intake falls below approximately 100 µg/ d, TSH secretion is augmented, which increases plasma inorganic iodide clearance by the thyroid through stimulation of sodium/ iodine symporter (NIS) expression. As a greater fraction of circulating iodide is cleared by the thyroid, there is a progressive reduction in renal iodide excretion. TSH also stimulates breakdown of TG and preferential synthesis and release of T_3 into the blood.

The iodine requirement during pregnancy is increased due to (i) an increase in maternal T_4 production to maintain maternal euthyroidism and transfer thyroid hormone to the fetus early in the first trimester, before the fetal thyroid is functioning; (ii) iodine transfer to the fetus, particularly in later gestation; and (iii) an increase in renal iodine clearance.

Balance studies have found that the average iodine retention of full-term infants is 7.3 µg/ kg˙d; the mean retention of a healthy fetus with a weight of 3 kg would be approximately 22 µg/d. WHO has recommended a daily iodine intake of 250 µg/d for pregnant women, a value approximately 10% higher than the RDA. The recommended daily iodine intake of lactating women is same as pregnant women (WHO/ UNICEF/ ICCIDD 2007).

Thus, the recommended daily intakes for pregnancy of 220-250 µg would correspond to a UIC of approximately 135-150 µg/L. Considering this, a recent WHO expert group recommended the median UIC that indicates adequate iodine intake during pregnancy to be 150-249 µg/L. For the mother, although the iodine requirement is high, after accounting for iodine losses into breast milk, the median UI in lactating women that indicates adequate iodine nutrition is the same as that of non-pregnant, nonlactating women.

In our study we found that the population was iodine sufficient and the median urinary iodine concentration increased during pregnancy, remained high throughout the pregnancy and decreased postpartum to non pregnancy levels.

Hormonal changes and metabolic demands during pregnancy result in profound alterations in the biochemical parameters of thyroid function. For thyroid economy, the main events occurring during pregnancy are a marked increase in serum thyroxin-binding globulin levels; a marginal decrease in free hormone concentrations (in iodine-sufficient areas) that is significantly amplifies when there is iodine restriction or overt iodine deficiency; a frequent trend toward a slight rise in basal thyrotropin (TSH) values between the first trimester and term; a transient stimulation of the maternal thyroid gland by elevated levels of human chorionic gonadotropin (hCG) resulting in a rise in free thyroid hormones and decrement in serum TSH concentrations during the first trimester; and finally, modifications of the peripheral metabolism of maternal thyroid hormones (Glinoer, Pregnancy and iodine 2001).

Our study also showed similar trend of decrease in TSH value below the non pregnancy value in the first trimester that increased in the second and third trimester but remained below the reference limit. This may be attributed to iodine sufficiency in the study population. The FT_4 and FT_3 values increased in the first trimester and then decreased significantly thereafter till third trimester. Postpartum the values were similar to non pregnant values.

Pregnancy represents a strong goitrogenic stimulus for both the mother and fetus, even in areas with only a moderate iodine restriction or deficiency. Although goiter formation was not noticeable in pregnant women residing in iodine-sufficient areas, several European studies showed that significant changes in thyroid volume occurred in association with pregnancy. In European regions with an apparently sufficient iodine intake, results showed thyroid volume increments of 10 to 15 percent on average, consistent mainly with vascular swelling of the gland during pregnancy. In other iodine-deficient regions, results showed 20-35 percent on average increments in thyroid volume, with many women exhibiting a doubling in thyroid size between the first trimester and term (Glinoer 1997).

Grade 1 goiter was present in 23 percent of the normal pregnant subjects and this may be due to pregnancy induced changes. Rest of the subjects did not show goitrogenesis. Whereas, the subjects whose medicine had to be started or were prior on medication showed higher prevalence of goiter (50% -60%).

The human fetus develops along a narrow growth trajectory that must balance the demands of the fetus with the capabilities of the mother. The mother is the supplier of oxygen and essential nutrients to the fetus via the placenta. Maternal diet, caloric intake, and metabolic function each have an important role to play in supplying nutrients to the fetus. In addition, alterations in maternal metabolism in response to hormonal signals ensure a redirection of required nutrients to the placenta and mammary gland. Increased caloric intake is necessary during the second and third trimesters to cope with most fetal and placental growth (Murphy, *et al.* 2006).

Both maternal and fetal thyroid status *in utero* may be critical in brain development. Observational studies performed in iodine deficient parts of the worlds have shown that iodine supplementation before pregnancy and in the first and second trimesters reduces the incidence of cretinism but supplementation beginning later in pregnancy does not improve the neurodevelopmental status of the offspring (Cao, *et al.* 1994). Even children of marginally iodine- deficient mothers show psychomotor and cognitive impairment. Such data indicates the sensitivity of the developing central nervous system (CNS) to maternal thyroid metabolism *in utero* (Chan and Kilby 2000).

Maternal hypothyroidism, not necessarily due to iodine deficiency, has been associated with poorer neuropsychological outcome in offspring. In humans, both T_3 and T_4 can be detected in the first trimester brain before the fetal thyroid gland becomes active, possibly indicating that thyroid hormones play an important role. T_3 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 (Chan and Kilby 2000, Morreale de Escobar, Obregon and Escobar del Ray 2004). Even after the fetus begins to produce its own thyroid hormones in the second trimester, maternal thyroid hormones makes a significant contribution towards the supply to the fetal brain. This is

indicated by positive correlations between maternal serum T_4 concentrations, fetal cerbro-cortical T_4 and maternal urinary iodine excretion at this stage (Sinha, *et al.* 1997).

Studies using rats have shown that maternal T_4 transported across the placenta can provide normal concentrations of T_3 in hypothyroid fetal brains (Calvo, *et al.* 1990). Mildly iodine-deficient and hypothyroid mothers do produce neuro-developmentally compromised offspring, fetuses in such circumstances are known to have normal T_4 concentrations but reduced T_3 concentrations in the brain.

Fetal development and growth depend on several endocrine, paracrine and autocrine events within the feto-placental unit. Malfunction of this unit can result in intrauterine growth restricted (IUGR) fetuses, with brain weight usually maintained relative to body weight while other organs like liver are significantly smaller. IUGR babies contribute significantly to perinatal and neonatal mortality. *In utero* fetal blood sampling has shown that fetuses with severe IUGR have significantly lower levels of circulating FT_4, FT_3 and a slight elevation in TSH (Chan and Kilby 2000).

The pregnancy outcome observed in our subjects was good. There was very low incidence of still birth, preterm deliveries, deaths etc. None of the babies were affected due to intrauterine growth retardation. This might have been due to sufficient iodine status of the population and also the thyroid hormones of mother were maintained within the reference ranges. They were serially followed throughout pregnancy and those found to be thyroid-insufficient were placed on levothyroxine dosage as soon as diagnosed. It was observed that the absolute percentage increase in dose during pregnancy in newly diagnosed hypothyroid subjects were 45.58 µg/day and 25.09 µg/kg day. The dosage of 64.1 percent of subjects already on levothyroxine before pregnancy was increased as a result of increased demand of the hormone due to pregnancy. Other studies have also shown that 35-75 percent of women with pre existing hypothyroidism will require more thyroxine during pregnancy (Bach-Huynh and Jonklaas 2006).

We found positive correlation between cord blood FT_4 and maternal first and third trimester FT_4 which supports the belief that maternal T_4 crossed the placenta even in late gestation. The lack of association between maternal TSH and cord TSH in our study is

consistent with the suggestion that TSH is poorly transferred across the placenta (Shields, *et al.* 2011).

A positive association was found between FT_4 in the cord blood at birth and birth weight, birth length and head circumference. This suggests that fetal thyroid hormone may influence generalized growth, including skeletal size. The association between thyroid hormone and measurements of fetal growth is consistent with the previous studies showing an association between overt maternal hypothyroidism and low birth weight (Blazer, *et al.* 2003, Idris, *et al.* 2005). In normal pregnancy, the role of thyroid hormone in fetal growth is indirectly supported by previous work showing that, babies born to women with inadequate dietary iodine intake in the third trimester had lower birth weights than those born to women with adequate dietary iodine intake (Alvarez-Pedrerol, *et al.* 2009).

Although the optimal TSH value for pregnant women with hypothyroidism has not been rigorously established, it has been suggested that the goal of therapy should be to maintain TSH levels between 0.5 and 2.5 mIU/L. Free thyroxine should be brought into the upper normal range. As pregnancy is a time of complex hormonal changes affecting T_4 availability to the mother and fetus, therefore it is necessary to establish trimester-specific- reference intervals of TSH and FT_4 as use of non-pregnant reference intervals maybe misleading during this time. This can lead to misdiagnosis of many at-risk women. In this study, we tried to establish trimester- specific reference intervals for TSH and FT_4 using a rigorously selected euthyroid iodine sufficient cohort since there is no data available for these subjects. Thus the study concludes emphasizing the need of a Thyroid scan as a mandatory approach to be put into implementation as a health parameter for all pregnancies across the country. The approach would help essentially in preventing fetal brain damages for all populations. Incorporation of Iodine rich sea weeds in regular diets of pregnant mothers needs to be popularized. Further it is of importance to emphasize good nutrition, adequate antenatal and post natal care to ensure a healthy baby's arrival in this world.

Summary and Conclusions

5. SUMMARY AND CONCLUSIONS

Iodine is a trace element found in human body in very little amounts (15-25 mg) almost exclusively in the thyroid gland (Hays 2001). It is a fundamental component of thyroid hormones, conferring approximately 65 percent of thyroxine (T_4) and 59 percent of triiodothyronine (T_3) weight. Thyroid gland is the only organ that uptakes iodine actively, organifies it, and forms iodized compounds. These compounds are used for the synthesis of thyroid hormones that regulate a number of metabolic functions and promotes normal growth, development, and maturation of many organs, especially of brain.

The relationship between the level of iodine intake and the risk of occurrence of thyroid diseases is U-shaped, which means that there is an increasing risk with both low and high iodine intake (Laurberg, *et al.* 2001). An adequate iodine intake is essential for normal thyroid hormone synthesis and normal thyroid function. Insufficient dietary iodine intake leads to adaptation mechanisms, which include decrease of intrathyroidal iodine, preferential T_3 synthesis and secretion as opposed to decreased T_4 secretion. These responses are maintained by increased secretion of thyroid- stimulating hormone (TSH) which consequently leads to development of goiter, hypothyroidism, impaired growth and intellectual development, and other iodine deficiency disorders.

Thyroid disease is the second most common endocrine disorder (after diabetes mellitus) affecting women of reproductive age. The incidence of hypothyroidism during pregnancy is estimated to be 0.3% to 0.5%for overt hypothyroidism and 3%to 5% for subclinical hypothyroidism (Casey, Dashe and Wells, *et al.* 2006, Casey, Dashe and Spong, *et al.* 2007). Overt hypothyroidism is defined as symptomatic thyroid hormone deficiency (low free thyroxine hormone, elevated thyroid stimulating hormone), whilst subclinical hypothyroidism refers to biochemical evidence of thyroid hormone deficiency (normal free thyroxine but elevated TSH), in women with few or no clinical features. Thyroid hormone helps the body to make energy, keeps body temperature regulated and assist other organs in their functions.

Over the last decade there has been enhanced awareness of the appreciable morbidity of thyroid dysfunction, particularly thyroid deficiency (Lazarus 2005, Casey, Dashe and

Spong, *et al.* 2007, Abalovich, *et al.* 2007). The increasing evidence of impaired neuropsychological development in the children of women with variously defined hypothyroidism has fuelled a resurgence of interest.

Worldwide, particularly in mountainous regions and in Central Africa, South America and northern Asia, the most common cause of hypothyroidism is iodine deficiency (Jameson and Weetman 2008). In areas of iodine sufficiency, the most common cause of hypothyroidism in pregnant women is Hashimoto's (chronic thyroiditis), an autoimmune disease where the bodies own antibodies attack the thyroid.

Both clinical and subclinical hypothyroidism have significant adverse effects on pregnancy and fetal development, more frequently seen in symptomatic women. The obstetric complications of hypothyroidism contribute to the overall increase in frequency of adverse neonatal outcomes, which include preterm birth, low birth weight, increased perinatal morbidity and mortality. Congenital cretinism is a well documented syndrome of growth restriction, deafness and neuropsychological impairment, resulting from severe iodine deficiency or untreated congenital hypothyroidism (Cao, *et al.* 1994).

The literature suggests an increased thyroxine requirement for hypothyroid women in pregnancy, on average 30 percent to 50 percent above preconception dosage (Reid, *et al.* 2010). The timing of the increase in thyroxine requirement during pregnancy is still controversial.

Clinical hyperthyroidism has a reported prevalence of 0.1 to 0.4 percent. It is associated with ovulatory dysfunction, miscarriage and difficulties conceiving. Prompt diagnosis and treatment of hyperthyroidism are of paramount importance in preventing maternal and fetal morbidity and mortality.

The establishment of trimester- specific- reference ranges for thyroid hormones in iodine sufficient and deficient pregnant women could be useful in identifying at- risk women as maternal thyroid hormone concentrations are important for normal fetal development.

Thus, keeping all these aspects in mind, a study was designed with the broad objective of screening pregnant women for thyroid disorders so as to prevent brain damage of the fetus. The specific objectives of the study were to establish trimester specific reference

ranges for thyroid hormones, neonatal screening, compare normal pregnancies with thyroid insufficient pregnancies and to provide nutrition health education to all the women and assess their knowledge, attitude and practices regarding iodized salt and consumption.

Along with the hormonal induction dietary supplements such as seaweeds can also help in sustaining the circulatory levels of iodine as they have rich iodine content. So, a trial study was carried out to supplement iodine rich seaweed to iodine deficient or thyroid insufficient pregnancies.

Keeping the above objectives in mind the study was carried out and results are summarized under following heads:

5.1 Enrollment of the subjects

Total of 341 pregnant women in the first trimester of pregnancy [189 women with non specific thyroid status comprised group I and 145 women who were already on hormone replacement therapy comprised group II] were enrolled from various hospitals of Delhi. The subjects in both the groups were serially followed throughout pregnancy and upto six months postpartum. Only ninety two women in Group I and one hundred and two women in Group II regularly visited in all the three trimesters of the pregnancy.

Thirty pregnant women in any trimester were enrolled from five *anganwadis* of Vadodara (Group III). These women were followed for a period of one month.

One hundred and fifty non-pregnant women (Group IV) in the same age group were also recruited for the study to compare the difference in thyroid function between pregnant and non-pregnant women.

History of repeated abortions, known complications like diabetes and hypertension, autoimmune disorder and family history of thyroid disorder was present more in group II subjects.

5.2 General with non-specific thyroid status group (Group I)

The subjects in this group were subdivided into normal (Group I A) and medicine started during the course of pregnancy (Group I B). Complete three trimester data of 68 subjects in group I A and 24 subjects in group I B was available; hence full analysis of these subjects was carried out.

Group I A (n=68):

- Thirty percent of the subjects were underweight at the time of registration and those with above normal BMI had weight gain throughout pregnancy within the recommended levels.
- Majority (60 percent) of the subjects had normal hemoglobin concentration throughout pregnancy.
- The median urinary iodine concentration showed that population is iodine sufficient in all the trimesters (UIC being 189.2, 200 and 196.9 µg/L in first, second and third trimester, respectively) of the pregnancy. In pregnant women, UIC was significantly ($p<0.001$) elevated, compared with postpartum (UIC was 139.1, 142 and 141 µg/L after 6 weeks, 12 weeks and 24 weeks postpartum, respectively) and non-pregnant control group IV (UIC was 141.5 µg/L).
- TPO-Ab positivity was present in 7.4 percent of the subjects and 23.5 percent (16 out of 68) had goiter. No association was observed between TPO-Ab positivity and goiter.
- The mean FT_3 and FT_4 decreased significantly ($p<0.05$) in second (by 9%) and third (by 10%) trimester when compared to first trimester. There was no difference between first trimester and non-pregnant values. Postpartum the FT_3 and FT_4 concentration increased significantly ($p<0.05$) when compared to second and third trimester and reached almost the non-pregnant values.
- Serum TSH decreased significantly during the first (by 20.7 percent, $p<0.05$) trimester when compared to non-pregnant state and increased significantly by 25.1 percent in second trimester when compared to first trimester. Postpartum the TSH concentration showed non-significant decline but the mean remained higher than the first trimester value.
- In the first trimester, a significant negative correlation ($r=-0.251$, $p<0.05$) was found between urinary iodine and TSH ($p<0.05$) whereas in all the other trimesters the correlation was non-significant. Serum TSH was significantly ($p<0.05$) lower in iodine sufficient (UIC >150 µg/L) group *vs.* iodine deficient group (UIC <150 µg/L) in first trimester whereas non-significantly lower in all the other trimesters and postpartum. No significant difference in mean FT_3 and FT_4 during trimesters and postpartum between the two groups was observed.

Group I B (n=24):

- One- third of the subjects in this group were anemic (Hb <11 g/dl) but none of them had severe anemia.
- The median urinary iodine concentration of the group showed iodine sufficiency throughout pregnancy (180.2, 179.9 and 199.05 µg/L in first, second and third trimester, respectively) and postpartum (134.41, 123.8 and 140.75 µg/L in 6 weeks, 12 weeks and 24 weeks postpartum, respectively).
- Sixty seven percent of the subjects had TPO-Ab positivity and almost 40 percent of the total subjects had goiter.
- The mean FT_3 and FT_4 decreased significantly ($p<0.05$) throughout pregnancy and increased significantly ($p<0.05$) postpartum.
- The subjects had abnormal TSH in the first trimester ranging from 0.03 to 100 µIU/ml and it decreased significantly ($p<0.05$) in second and third trimester when compared to first after the initiation of levothyroxine treatment. The median TSH decreased significantly ($p=0.026$) by 29 percent from first to third trimester.
- A significant positive correlation ($r=0.452$, $p<0.05$) was observed between first trimester TSH and TPO-Ab. A significant negative correlation ($p<0.01$) was observed between urinary iodine and FT_4 in first ($r=-0.633$) and second trimester ($r=-0.509$).
- The comparison between TPO-Ab positive (n=8) and TPO-Ab negative (n=16) subjects revealed that median FT_3 and FT_4 of TPO-Ab positive subjects was significantly ($p<0.05$) lower when compared to TPO-Ab negative subjects. The median TSH of TPO-Ab positive group was non-significantly higher than the other group.
- The levothyroxine dose was started in first trimester in 80 percent of the subjects while in others it was started in second or third trimester.
- The L-T4 minimum dosage in first trimester was 0 µg/day whereas at the end of pregnancy it was 25 µg/day. The absolute percentage increase in dose during pregnancy was 45.58 µg/day and 25.09 µg/kg day.

5.3 Subjects already on hormone replacement therapy (Group II)

The subjects in this group were subdivided on the basis of thyroid dysfunction into hypothyroidic (Group II A) and hyperthyroidic (Group II B). Complete three trimester data of 92 subjects in group II A and 10 subjects in group II B is available; hence full analysis of these subjects was carried out.

Group II A (n=92):

- The pre pregnancy mean FT_4 and TSH of the subjects was 16.88 ± 2.2 pM/L and 2.3 ± 0.99 µIU/ml, respectively which was within the reference range (FT_4: 12-22 pM/L and TSH: 0.27-4.2 µIU/ml). The baseline L-T4 dose was 88.3 ± 35.4 µg/day.
- Majority of the women (n=59) had to increase their dose one or more times during the course of pregnancy and formed group 'b'. No change in dose was observed in thirty subjects and formed group 'a'.
- There was no significant difference in age, parity, BMI and duration of hypothyroidism between the pregnancies where the doses had to be changed and those where it did not.
- The mean TSH was significantly ($p<0.05$) higher during all the trimesters in the group 'b' when compared to group 'a'. There was no significant difference found between FT_4 values. The median L-T4 dose was significantly higher by 25 percent in group 'b' when compared with group 'a'.
- Nearly half of the patients in both the subgroups 'a' and 'b' were TPO-Ab positive.
- Seven subjects (23.3 percent) in group 'a' and 14 (23.7 percent) in group 'b' were subclinical hypothyroidic (SCH). Rest of them were overt hypothyroidic (OH).
- Out of 59 subjects, 40 (67.8 percent) required dose increment in first trimester itself. In these subjects, mean TSH and L-T4 dose significantly ($p<0.001$) increased during gestation as compared to before pregnancy while FT_4 significantly ($p<0.05$) decreased.
- The mean L-T4 dose significantly ($p<0.05$) increased by 17.7 percent from the start of pregnancy till the end of pregnancy.
- On an average, the entire group 'b' required a cumulative increase in thyroid hormone dosage from baseline of 14.3 percent in the first trimester, 33 percent in the second

trimester and 37.5 percent in the third trimester (Mann-Whitney U test, p=0.02, p=0.00, p=0.00, respectively).

- The median cumulative percentage L-T4 dose increase in subclinical hypothyroidic patients (n=14) was high in comparison to overt hypothyroidic subjects (n=45). It was found that the median cumulative increase in L-T4 dose from baseline in OH subjects was statistically significant ($p<0.005$ in all the three trimesters) whereas in SCH subjects it was non-significant (p=0.194) in the first trimester and significant (p=0.035, p=0.027) in second and third trimester, respectively.
- The OH group before pregnancy and final L-T4 dose (µg/day) was significantly higher by 33.9 percent and 20.7 percent, respectively when compared to SCH group.
- The increment in Δ% of absolute doses was higher in SCH than OH group.
- The subjects when divided according to the time they reached definitive therapeutic dose, it was found that 23.7 percent reached definitive dose in first trimester itself, 28.8 percent by second trimester and 47.5 percent by third trimester.
- No significant difference was found between the three groups when comparing initial and final doses of L-T4 expressed either as µg/day or µg/kg day.
- The median UIC showed iodine sufficiency in the population (175.43, 189.4 and 179.49 µg/L in first, second and third trimester, respectively).

Group II B (n=10):

- The median duration of hyperthyroidism before conception was 7.5 years (2 year to 12 year).
- All the subjects were on anti-thyroid drug propyl thio uracil (PTU) and had to decrease their dosage during pregnancy.
- The median urinary iodine concentration showed iodine sufficiency in the group.

5.4 Seaweed group (Group III)

- Total of thirty pregnant women irrespective of either trimester of pregnancy were enrolled for this part of the study.
- The women who had UIC below recommended levels (<150µg/L) formed group III B (n=10) and were supplemented with iodine rich seaweed.

- For preparation of *ladoos* rich in iodine, four species of seaweeds namely *Caulerpa scalpelliformis, Caulerpa racemosa, Caulerpa veravelense* and *Padina tretastromatica* were collected from Gujarat coast and were analyzed at National Institute of Nutrition for proximate and mineral composition.
- After analysis it was found that *Caulerpa racemosa* has the highest content of iodine (28.31 mg/100 g) as well as iron (201.89 mg/100 g) when compared to other seaweeds.
- Daily 0.17g of the seaweed in 20 g wheat flour *ladoo* was supplemented to these subjects so as to provide 50µg/day of iodine and 0.343 mg/day of iron.
- A slight non-significant increase (104.75 to 121.05 µg/L) in median UIC of the supplemented group III B was observed after one month of supplementation but it was still below the normal levels suggesting prolonged period of supplementation may bring a positive change in iodine status.
- The TSH of supplemented group III B before supplementation was significantly ($p<0.05$) higher than that of non-supplemented group III A. No significant effect of supplementation was observed on thyroid function parameters of the subjects.
- The hemoglobin profile of supplemented group was better than that of non-supplemented group both before and after supplementation.

5.5 Establishment of thyroid hormones trimester- specific reference intervals

- Out of sixty eight women from Group I A with complete three trimesters data, women were excluded on the basis of laboratory results that revealed positive serum thyroid peroxidase antibody (TPO-Ab > 35 IU/mL). All the women having goiter, overt hypothyroidism or overt hyperthyroidism were excluded.
- The values of serum FT_3, FT_4 and TSH from this pregnant women group (n= 46) and nonpregnant women (n=125) who were considered as 'normal subjects' were used to derive thyroid function test reference intervals. These subjects constituted the normal reference study population.
- The 95^{th} percentile was selected to represent the upper reference limit so as not to omit any cases of hypothyroidism. The 2.5^{th} percentile was used to represent the lower reference limits.

- The results of this study have provided information on the reference intervals of thyroid hormones during pregnancy from thyroid antibody- negative, iodine-sufficient population. These reference intervals for FT_3, FT_4 and TSH determined for each trimester of pregnancy [FT_3: 1.7-5.39, 2.38-5.12 and 2.21-5.18 pM/L; FT_4: 10.12-18.56, 8.29-19.02 and 9.27-17.69 pM/L; TSH: 0.03-4.31, 0.54-4.02 and 0.78-4.29 µIU/ml] are recommended for evaluation of pregnant Indian women using IA, Roche Elecsys 1010 analyzer method or similar methods.

5.6 Prevalence of thyroid dysfunction in Group I subjects

The prevalence of thyroid dysfunction was assessed and compared using four different classification criteria:

i. Trimester- specific reference intervals established by our study (Method I)
ii. Manufacturer's reference interval (Method II),
iii. Trimester- specific reference interval established by previous Indian study (Marwaha, *et al.* 2008) (Method III),
iv. TSH reference limit given by Endocrine Society Clinical Practice Guideline (Abalovich, *et al.* 2007) (Method IV).

- Our reference intervals (Method I) showed that the prevalence of OH (5.8 percent) in first trimester was lower than found by other methods.
- The prevalence of SCH in all the three trimesters by method I increased in comparison to method II & III whereas it decreased in comparison to method IV due to higher upper TSH reference limit.
- The prevalence of isolated hypothyroxinemia according to Method I was lower when compared to all the other methods.
- According to all the methods the prevalence of overt hyperthyroidism was approximately 1.5 percent in first trimester.
- No case of overt hyperthyroidism was observed in second and third trimester by any of the methods.

5.6 Nutritional status of the study subjects

- The average energy consumption of group I, II and III pregnant women at the time of registration was considerably low by 34 percent, 27 percent and 39 percent, respectively when compared to RDA (2525 kcal/day).
- In third trimester, an energy deficit of 17 percent and 16 percent was found in group I and II women, respectively whereas a deficit of 33 percent was found in group III subjects after one month.
- In the study, a protein deficit of 25 percent, 12 percent and 35 percent was found in group I, II and III subjects, respectively at the time of initial presentation when compared to RDA (65 g/day).
- In third trimester, an excess intake of 10 and 20 percent of protein was found in group I and II subjects, respectively.
- Though NHE was provided no much difference in goitrogen and iron rich food consumption pattern by group I and II subjects was observed.
- After NHE an improvement in consumption of vitamin-C rich foods was observed.

5.7 Knowledge, attitude and practices (KAP) regarding iodized salt

- An improvement in knowledge of respondents about iodine, attitude regarding iodized salt and practices used for its consumption and storage was observed after NHE.
- After NHE there was a 15- 20 percent increase in knowledge about importance of iodine in group I and II subjects.
- About 30 percent increase in knowledge about effects of iodine deficiency was found in both group I and II subjects after NHE.
- The percentage of subjects who were able to recognize iodized salt increased from 25 to 63 percent in group I and 47 to 66 percent in group II subjects after NHE was given.
- Majority (80-90 percent) of the subjects in both group I & II used to add salt in beginning while cooking whereas after NHE the percentage reduced to 20-30 percent.
- Tata salt was consumed by majority of the subjects in all the groups as it is readily available.

- In group III NHE was not provided and only 20 percent of the subjects were able to recognize iodized salt. Only 7 percent of the subjects knew about the consequences of iodine deficiency.

5.8 Pregnancy outcome of the study subjects

- Pregnancy outcome of total 184 subjects (69 in group I A, 16 in Group I B, 90 in Group II A and 9 in Group II B) was available as some subjects had miscarriage or dropped out from the study or had home delivery.
- Majority (60 percent) of the subjects had normal delivery.
- The parameters of the neonates showed that they had normal mean birth length, birth weight and head circumference.
- The data when classified according to Z-scores revealed that 15 percent, 13 percent and 11.2 percent of the subjects were stunted, wasted and underweight, respectively.
- According to our data 28.5 percent neonates had TSH level above 10 µIU/ml which indicates moderate iodine deficiency.
- Various neonatal parameters like head circumference, length and weight were found to have no correlation with cord blood TSH. A significant ($p<0.05$) positive correlation of cord FT_4 was found with birth weight, birth length and head circumference.
- There was no significant relationship between maternal thyroid function tests and fetal cord blood TSH.
- Cord blood FT_4 was found to be positively correlated with maternal first trimester FT_4 and third trimester FT_4.

CONCLUSIONS

Following conclusions can be drawn from the results obtained in the present study:

- The study population was iodine sufficient but neonatal data showed moderate iodine deficiency, so efforts should be targeted in maintaining iodine sufficiency.
- The hemoglobin status of the subjects was normal as majority of the subjects were taking iron and folic acid supplementation.
- Almost 20 percent of the subjects during pregnancy had to start levothyroxine due to thyroid insufficiency.

- A decrease in free triiodothyronine and free thyroxine was observed during the course of pregnancy. The thyroid stimulating hormone concentration decreased in the first trimester and then increased in the second and third trimester.
- All pregnant women should be screened for iodine deficiency and/ or thyroid dysfunction as soon as the pregnancy is confirmed.
- Large number of subjects were found to be subclinically hypothyroid during pregnancy and they should be carefully monitored and if necessary should be put on levothyroxine.
- A global increase in thyroxine in early pregnancy is not appropriate. The hypothyroid women should get FT_4 and TSH tests done every month until the end of pregnancy to prevent risk of adverse maternal and neonatal outcomes.
- The trimester- specific- reference intervals for the Indian population using IA, Roche Elecsys 1010 analyzer method have been established.
- Seaweed such as *Caulerpa racemosa* can be used as an iodine supplement as slight improvement in UIC was observed but prolonged period of supplementation needs to be carried out.
- Pregnant women should be made aware about the importance of iodine during pregnancy and lactation and adverse effects due to iodine and/ or thyroid hormone deficiency.

RECOMMENDATIONS

- All women with the possible exception of those known to have Graves' disease, should take an iodine supplement from before conception until completion of pregnancy.
- All the women who already have known thyroid dysfunction should immediately go for thyroid function tests as soon as the pregnancy is confirmed.
- Careful monitoring of the medicine should be done during the course of pregnancy.
- All women should be screened for iodine deficiency and / or thyroid dysfunction as soon as pregnancy is confirmed.
- The trimester- specific reference intervals for thyroid hormones established for pregnant Indian population after serially following the pregnant women should be used to identify at- risk women.
- Seaweeds rich in iodine can be used as dietary supplements to increase the iodine levels of the pregnant women.

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Appendix

ANNEXURE I

CONSENT FORM

Study Title: **"Screening of thyroid disorders for early prevention"**

Name of the participant :
…….....

Address :
…….....……
……
……

- I feel free to accept or refuse to participate in the study.
- I have had a chance to ask questions and all my questions have been answered to my satisfaction.
- I have been given and I understand the information on the above study concerning its nature, purpose and duration as well as the procedures involved in the study, including any known or expected inconveniences, risks, discomforts or potential side effects and of their implications as far as they are currently known by the researchers.
- My medical data is strictly confidential and I authorize its consultation and publication only by persons involved in the research.
- By signing this form, I give my free and informed consent to take part in the study.

Signature of the participant : …… Date: ….....

Name of the investigator : Ms. Juhi Agarwal

Signature of the investigator : …… Date: ……..

ANNEXURE II

QUESTIONNAIRE CUM INTERVIEW SCHEDULE

Case No. :
Date :

Name:
Husband's name:
Address:
Contact No.:

A. FAMILY BACKGROUND

No.	Question	Category	Code
1.	Age	_____ Years	☐☐
2.	Area of residence	1. Urban 2. Rural	☐
3.	Religion	1. Hindu 2. Muslim 3. Sikh 4. Christian	☐
4.	Education in completed years		☐☐
5.	Type of Family	1. Nuclear 2. Joint	☐
6.	Monthly family income	Rs.	
7.	Number of family members	1. Elders 2. Children	☐ ☐

B. MEDICAL HISTORY OF THE RESPONDENT

8.	Gravida (G)		☐
9.	Parity (P)		☐
10.	No. of abortions (A)		☐
11.	No.of living children (L)		☐
12.	No. of dead children (D)		☐

13.	Week of pregnancy		☐☐
14.	Last menstrual period (LMP)		☐☐ ☐☐ ☐☐
15.	Expected date of delivery (EDD)		☐☐ ☐☐ ☐☐
16.	Any known complication or medical disease in pregnancy	0. No 1. Yes (Specify)	☐
17.	History of repeated abortions	0. No 1. Yes Reason, if known	☐
18.	Any known or diagnosed autoimmune disorder	0. No 1. Yes	☐
19.	a. Known thyroid disease b. If yes, for how much time	0. No 1. Yes _____months	☐ ☐☐
20.	a. Family history of thyroid disease b. If yes, then who	0. No 1. Yes 1. Mother 2. Father 3. Sister 4. Brother 5. Other blood relative	☐ ☐☐☐
21.	a. Drug history b. Dosage (For Hypothyroid patients)	1. L-thyroxine 2. PTU 3. Iodine 4. Carbimazole 5. NMZ 6. Radioactive I µg/day µg/day µg/day µg/day µg/day	☐

		µg/day µg/day	
	b. Dosage (For Hyperthyroid patients)	mg/day mg/day mg/day mg/day mg/day mg/day mg/day	
22.	Height	_____cms.	☐☐☐
23.	Weight	_____ Kgs. _____ Kgs. _____ Kgs. _____ Kgs. _____ Kgs. _____ Kgs.	
24.	Body Mass Index (BMI)	_____ Kg/m^2 _____ Kg/m^2 _____ Kg/m^2 _____ Kg/m^2 _____ Kg/m^2 _____ Kg/m^2	
25.	Pulse	_____ min _____ min _____ min _____ min _____ min _____ min	☐☐ ☐☐ ☐☐ ☐☐ ☐☐ ☐☐
26.	Blood Pressure a. Systolic	_____ mm Hg _____ mm Hg _____ mm Hg	

	b. Diastolic	_____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg _____ mm Hg	
27.	Pedal Edema	0. No 1. Yes	☐
28.	Pallor	0. No 1. Yes	☐
29.	Goiter Grade	0. 0 1. I 2. II 3. III	☐
30.	a. Folic Acid supplementation b. If yes, from which month of pregnancy	0. No 1. Yes	☐
31.	a. Iron supplementation b. If yes, from which month of pregnancy	0. No 1. Yes	☐
32.	a. Calcium supplementation b. If yes, from which month of pregnancy	0. No 1. Yes	☐

33. Investigations

Investigations	Pregnancy (Trimesters)			Postpartum (PP)		
	Ist	IInd	IIIrd	Ist	IInd	IIIrd
FT_3 (pM/L)						
FT_4 (pM/L)						
TSH (µIU/ml)						
TPOAb (U/ml)						
Hb						
UIC (µg/L)						

C. BABY'S DETAILS

34.	Gestational age	_____ Weeks	☐☐
35.	Date of Birth		☐☐ ☐☐ ☐☐
36.	Sex	1. Male 2. Female	☐
37.	Birth place	1. Hospital 2. Home	☐
38.	Birth weight	_____ gms.	☐☐☐☐
39.	Birth length	_____ cms.	☐☐☐☐
40.	Type of delivery	1. Normal 2. Caesarian 3. Other	☐
41.	IUGR	0. No 1. Yes	☐
42.	APGAR Score		☐☐☐
43.	Cord blood TSH	_____ µIU/ml	☐☐☐☐
44.	Cord blood FT_4	_____ pM/L	☐☐☐☐
45.	4th to 7th day TSH	_____ µIU/ml	☐☐☐☐
46.	Head Circumference	_____ cms.	☐☐☐☐

D. DIETARY PROFILE

47. Eating habit : 1. Vegetarian
2. Non-vegetarian
3. Eggetarian

48. 24-Hr. Dietary Recall

Meal	Description	Household measure	Amount

49. What type of oil do you use : 1. Refined oil
2. Desi Ghee
3. Butter
4. Mustard oil
5. Any other

50. Type of milk consumed : 1. Full cream
2. Toned
3. Double toned
4. Cow's milk

51. Food Frequency for consumption of Goitrogens, Iron rich foods and Vitamin C rich foods

Food Item	Daily	5-6 times a week	2-4 times a week	Once a week	Fortnight	Once a month	Occasionally	Seasonally	Never
Goitrogens									
Cabbage									
Cauliflower									
Turnip									
Mustard leaves									
Knol-Khol									
Radishes									
Spinach									
Peaches									
Bajra									
Jowar									
Soya bean									
Iron rich foods									
Bajra									
Jowar									
Wheat flour									
Rice flakes									
Onion Stalks									
Shepu									
Colocasia leaves									
Amaranth									
Fenugreek leaves									
Spinach									
Vitamin C rich foods									
Amaranth									
Cabbage									
Drumstick									
Tomato									
Capsicum									
Amla									
Guava									
Oranges									
Sweet lime									

E. KNOWLEDGE, ATTITUDE AND PRACTICES

52.	Is adequate iodine intake important for pregnant women and children	0. No 1. Yes	☐
53.	Do you know what happens due to iodine deficiency	0. No 1. Goitre 2. Abortion 3. Still birth 4. Any other	☐
54.	Which type of salt do you use	1. Loose 2. Packed	☐
55.	Do you consume iodized salt	0. No 1. Yes	☐
56.	From where have you heard about iodized salt	0. No 1. TV 2. Radio 3. Newspaper 4. Other	☐
57.	Can you recognize iodized salt	0. No 1. Smiling sunlogo 2. By reading logo 3. By color & texture 4. Other	☐
58.	Which brand of salt are you using		
59.	Reasons for using that particular brand of salt	1. Iodized 2. Free flowing 3. Less price 4. Readily available 5. Others (specify)	☐ ☐ ☐
60.	How frequently do you purchase salt	1. Weekly 2. Fortnightly 3. Monthly	☐
61.	What is the approximate quantity of salt purchased per month	_____ Kg	

62.	When do you add salt while cooking	1. Beginning 2. In- between 3. End	☐
63.	Does loss of iodine occur during cooking & storing	0. No 1. Yes	☐
64.	How do you store salt	1. In jar with cap 2. In packet 3. In open container	☐
65.	Do you feel iodized salt is costlier than non-iodized salt	0. No 1. Yes	☐

Iodine importance during pregnancy and its effect on child's brain development

Eat a variety of foods within your recommended intake