RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSION

4.2.9 KAP

4.2.10 Food Frequency

4.2.11 Maternal health and child care

4.3 Phase III: Screening of neonates

4.3.1 Characteristics of neonates

4.3.2 Thyroid profile of neonates

4.3.3 Newborn screening

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

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

4.4.1 Characteristics of women during postpartum period

4.4.2 Comparison of dietary intake during pregnancy and postpartum

4.4.3 Micronutrient deficiency during pregnancy and postpartum

4.4.4 Prevalence of thyroid dysfunction during postpartum period

4.4.5 Comparison of diagnostic test

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

4.4.7 Characteristics of infants

4.4.8 Nutritional status of infants

4.4.9 DFS supplementation to combat anemia

4.4.10 Effect of early gestation thyroid dysfunction on infant development

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

Phase 1-Screening of pregnant women

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

Phase 2-Follow up till delivery

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

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

Phase 3-Screening of neonates

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

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

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

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

RESULTS

Phase I

Screening of pregnant women during early gestation

4.1.1 General Characteristics

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

Table 4.1.1: General characteristics of pregnant women

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

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

Nutritional status of pregnant women in India

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

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

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

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

Time trends in dietary intake in pregnant women

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

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

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

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

Source: NNMB report, 1979-2002

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

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

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

Source: NNMB report, 1979-2002

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

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

Table 4.1.4: Change in weight during pregnancy

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

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

Table 4.1.5: Birth weight and socio economic status

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

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

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

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

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

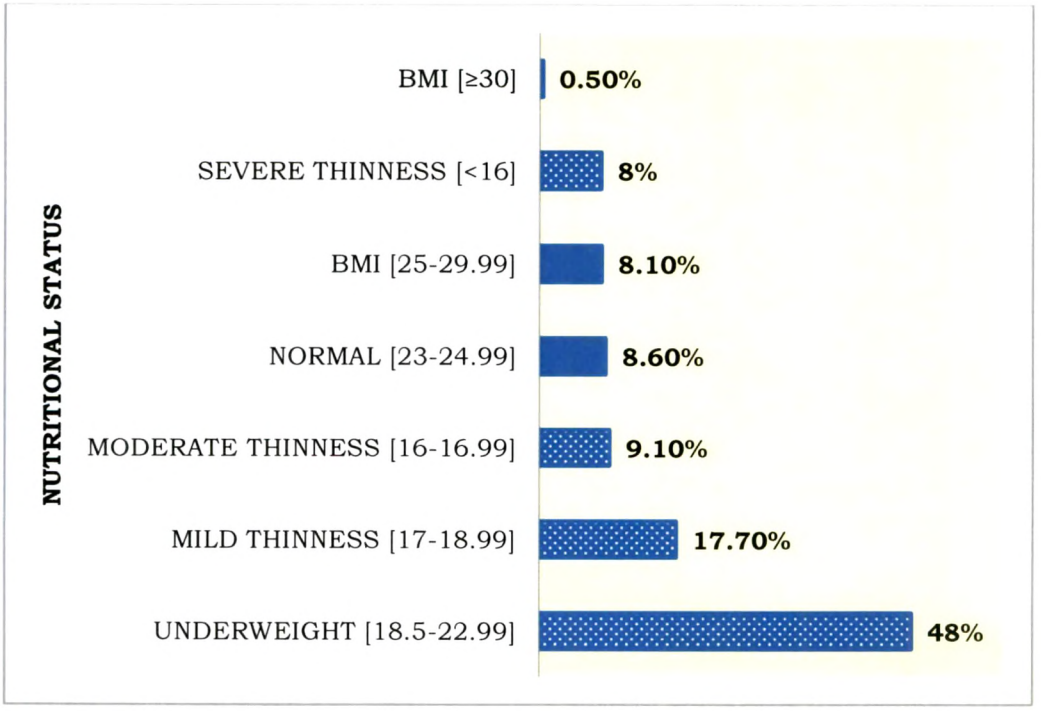
The National Rural Health Mission (NRHM) envisages that, there will be village health and nutrition days where in the ANM and AWW will

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

4.1.2 Anthropometric measurements and BMI of pregnant women

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

Figure 4.1.1: Nutritional status of pregnant women according to BMI



Nutritional Anemia during pregnancy

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

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

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

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

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

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

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

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

Source: Kalaivani, 2009

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

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

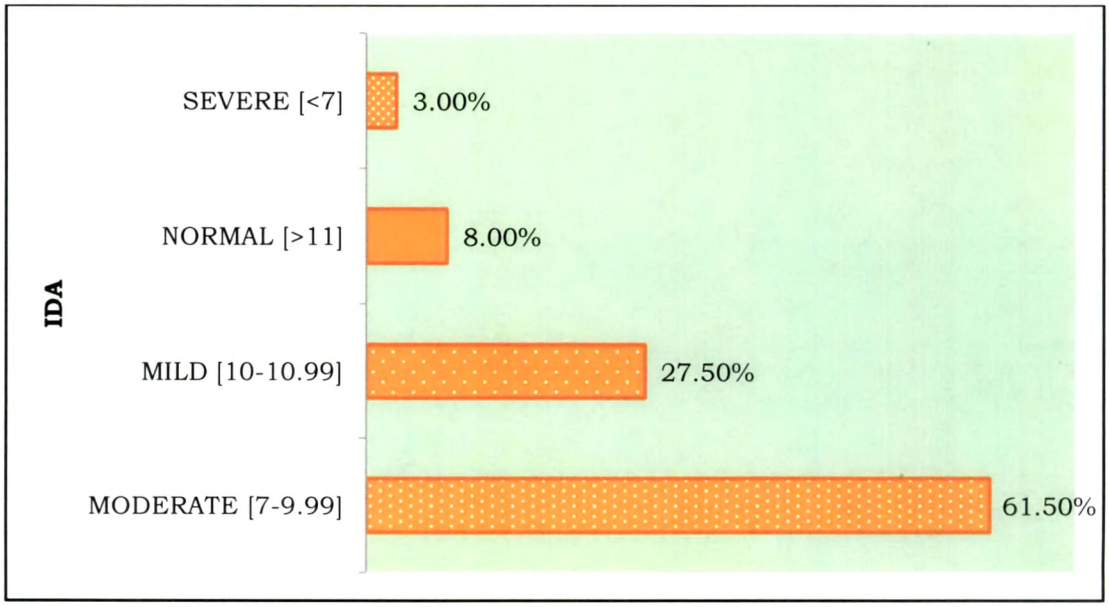
4.1.3 Iron Deficiency Anemia

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

Table 4.1.7: Hemoglobin level of pregnant women

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

Figure 4.1.2: Iron Deficiency Anemia using hemoglobin



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

Iodine Deficiency Disorders (IDD) during pregnancy

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

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

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

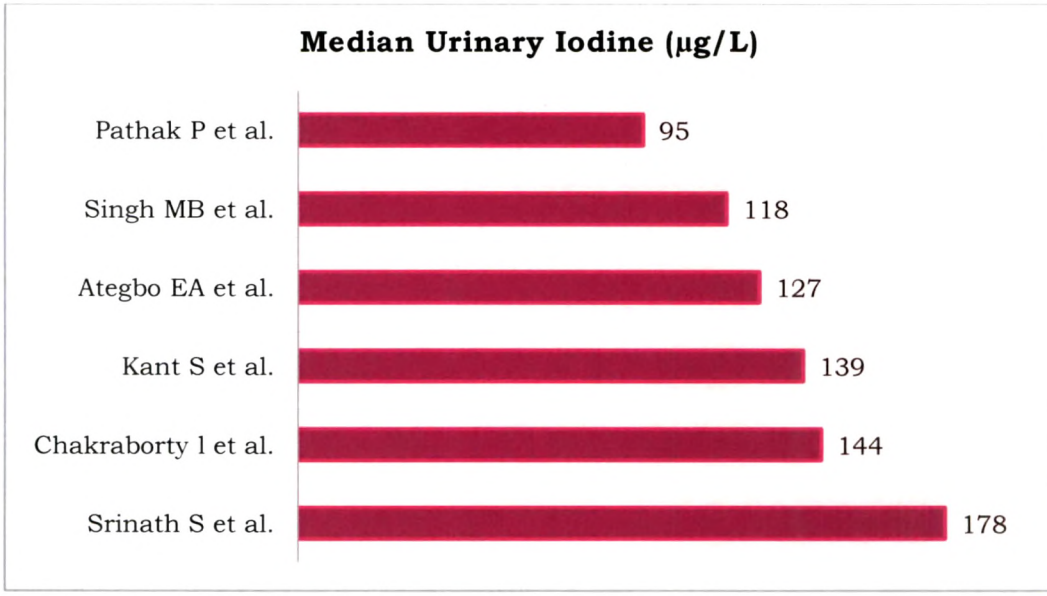
Table 4.1.8: Summary of studies on iodine nutrition status of pregnant women in India

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

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

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

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



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

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

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

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

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

4.1.4 Iodine Deficiency

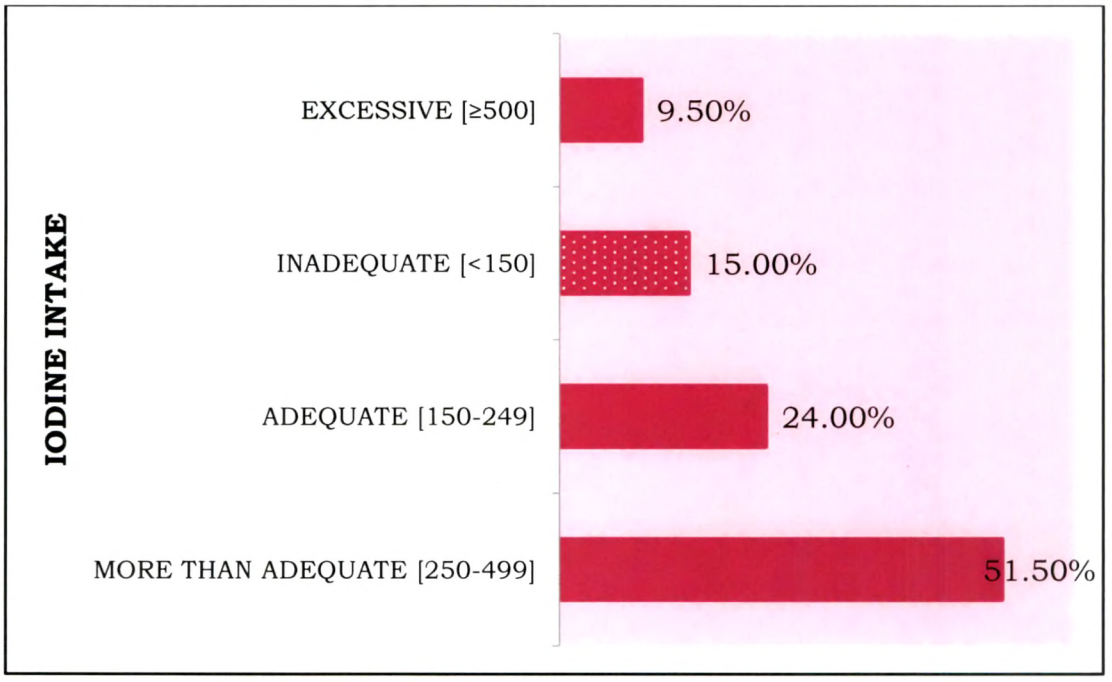
Median urinary iodine was 283.8 µg/L (Table 4.1.9) indicating adequate iodine intakes among these women. All these women were receiving iodized salt from their respective *Angawadi Centres.* Percentage of women having inadequate, adequate, more than adequate and excessive iodine intake was 15%, 24%,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

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

Table 4.1.9: Urinary iodine level of pregnant women

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

Figure 4.1.4: Iodine intake of pregnant women using UI



4.1.5 Thyroid dysfunction

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

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

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

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

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

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

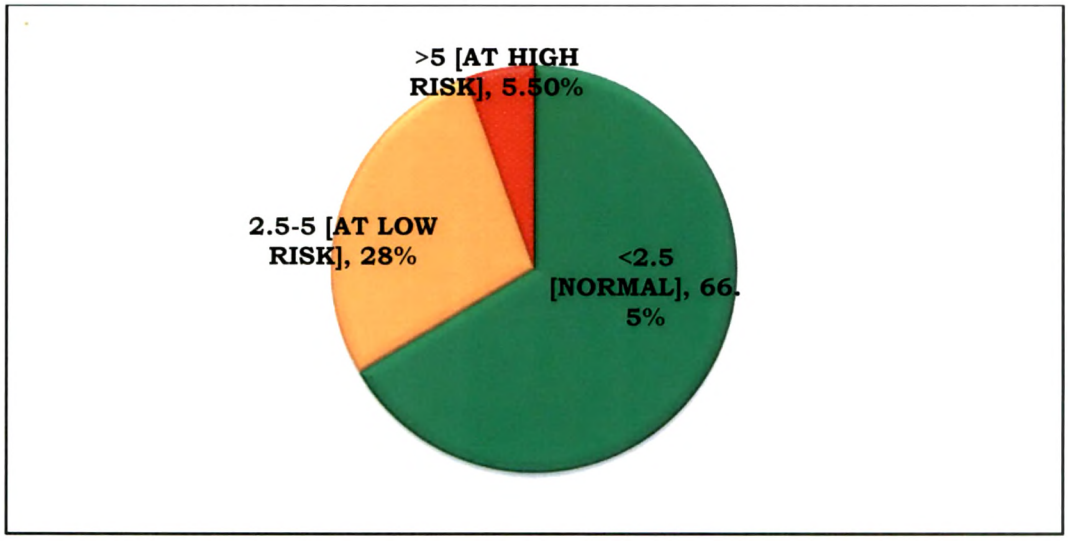
these women may be associated with increased risk of adverse pregnancy and perinatal outcomes.

Table 4.1.10: Thyroid Hormone level of pregnant women

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

*Normal range for non-pregnant adults

Figure 4.1.5: Percentage of at risk women for developing hypothyroidism



4.1.6 Screening of pregnant women for thyroid dysfunction during early gestation

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

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

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

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

Recommendation on universal screening for thyroid dysfunction-

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

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

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

Mitchell and Klein from USA (2004) suggested that-

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

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

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

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

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

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

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

RESULTS

Phase II

Follow-up of pregnant women
&
Intervention

4.2.1 General characteristics

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

4.2.2 Dietary information

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

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

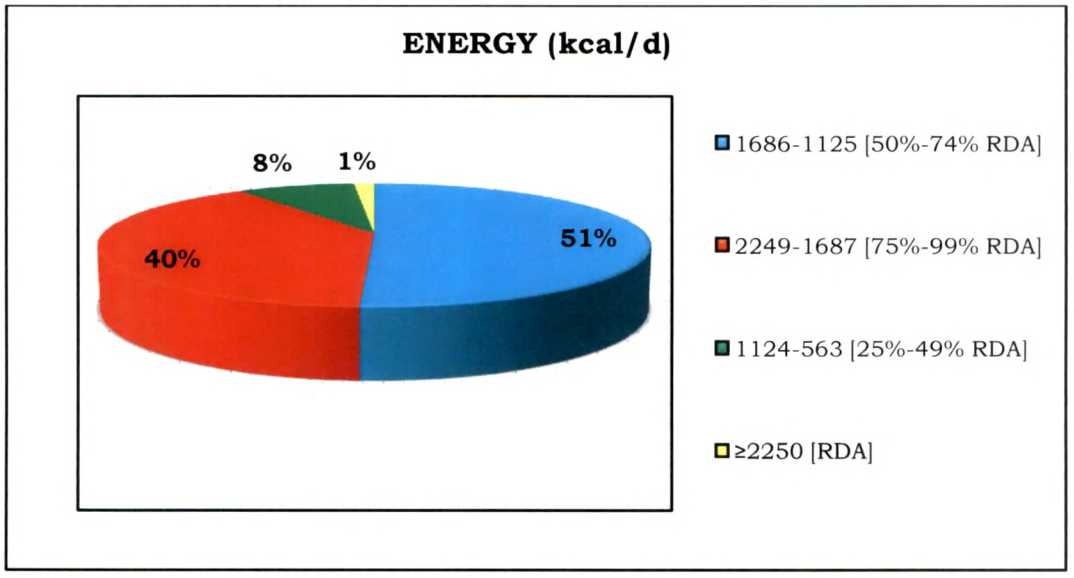
Parikh and Nair (2012) carried out dietary survey for urban pregnant women (Vadodara) and found that their mean calorie, protein and fat intake was 1022 kcal, 31 g and 45 g respectively.

Energy intake

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

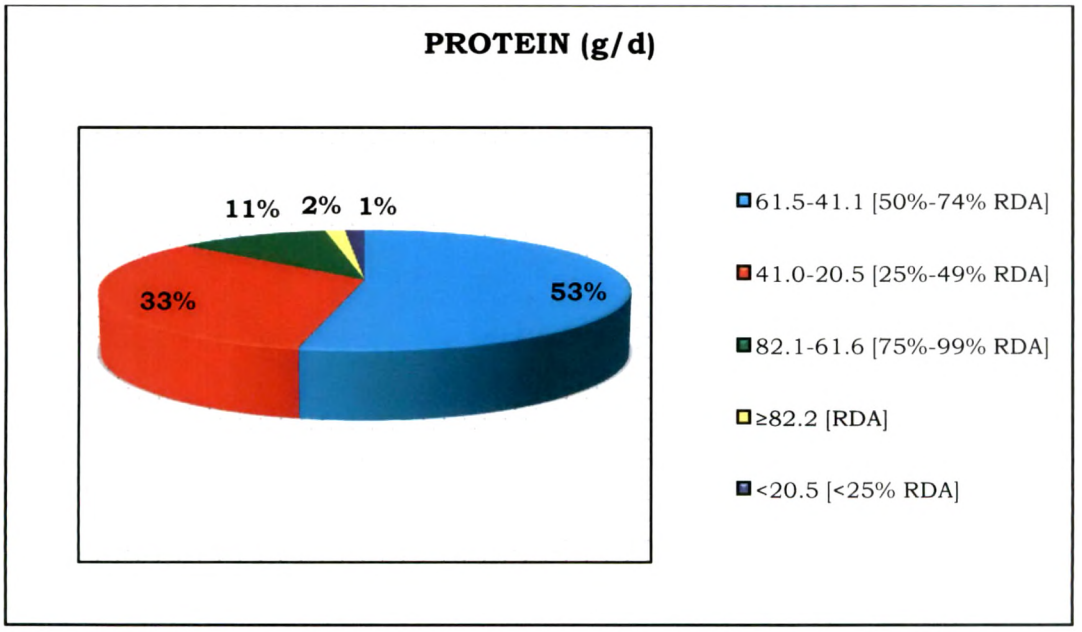
1.4% pregnant women met the requirements for daily calorie needs (Figure 4.2.1). Half (50.74%) of the population had calorie intake between 50-74% RDA.

Figure 4.2.1: Energy intake of pregnant women according to RDA



[RDA for energy-2250 kcal]

Figure 4.2.2: Protein intake of pregnant women according to RDA



[RDA for protein-82.2 g]

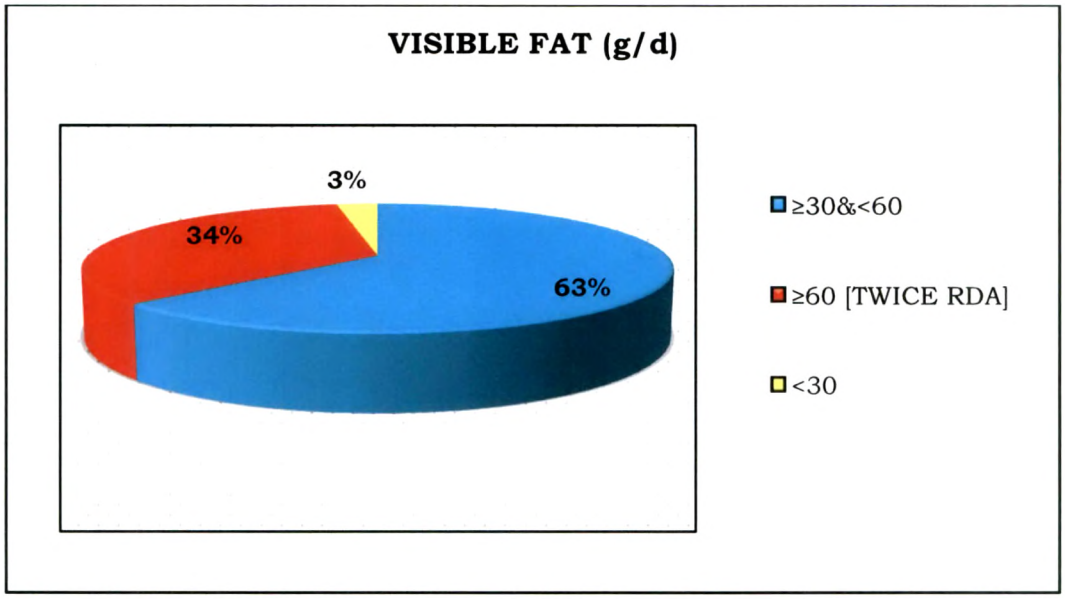
Protein Intake

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

Visible Fat

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

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



[RDA for fat inatke-30 g]

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

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

4.2.3 Anthropometric measurements

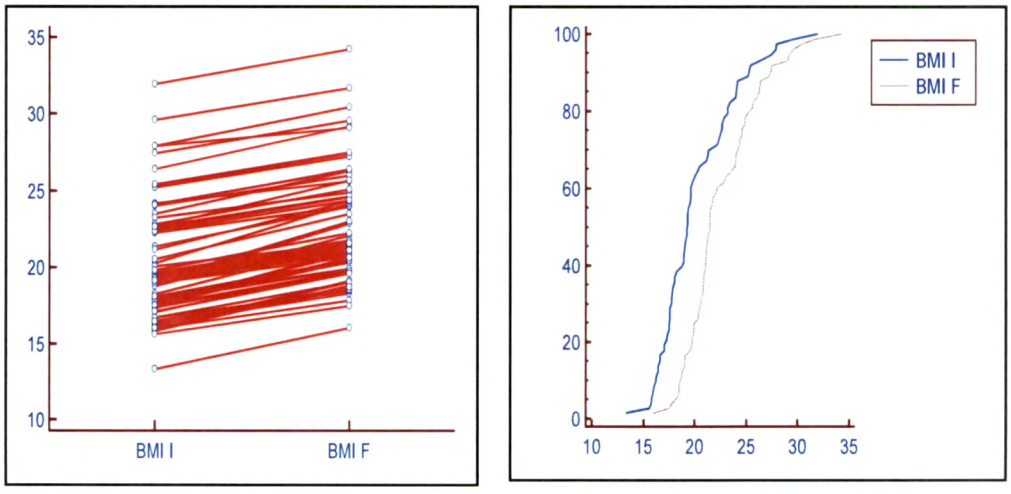
Low pre-pregnancy BMI is a risk factor for poor birth outcome and delivery complaints. Mean height and mean weight gain of pregnant women was found to be 151.4(5.3) cm and 5.4(1.3) kg respectively (Table 4.2.1).

Table 4.2.1: Anthropometric measurements of pregnant women

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

M-month

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



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

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

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

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

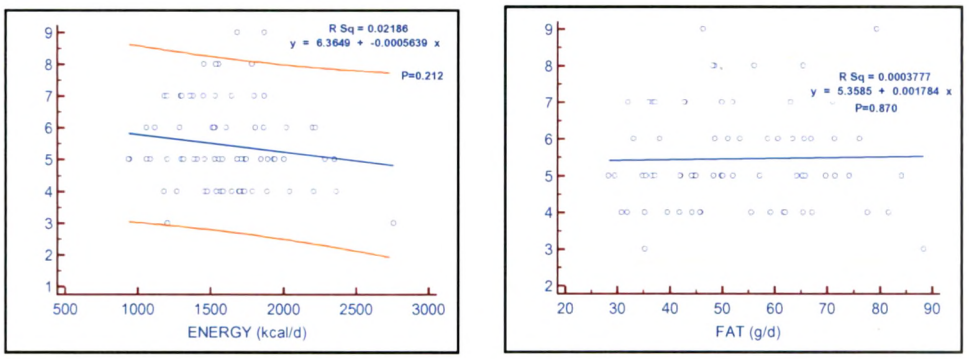
M-month

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

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

Figure 4.2.5: Correlation and regression

[Orange line denotes 95% prediction interval]



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

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

DFS supplementation during pregnancy

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

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

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

4.2.4 Iron Deficiency Anemia

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

4.2.4.1 Iron status

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

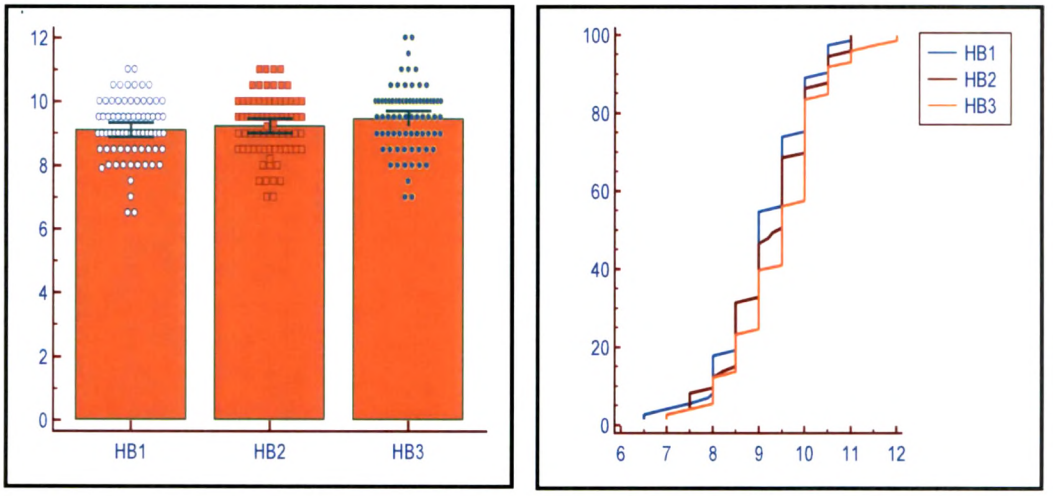
and 90th percentile value did not change with advancing gestation, suggesting an overall improvement in iron status of these pregnant women.

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

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

P-Percentile

Figure 4.2.6: Box plot of hemoglobin and cumulative frequency



HB1, HB2 and HB3 denote HB during trimester

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

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

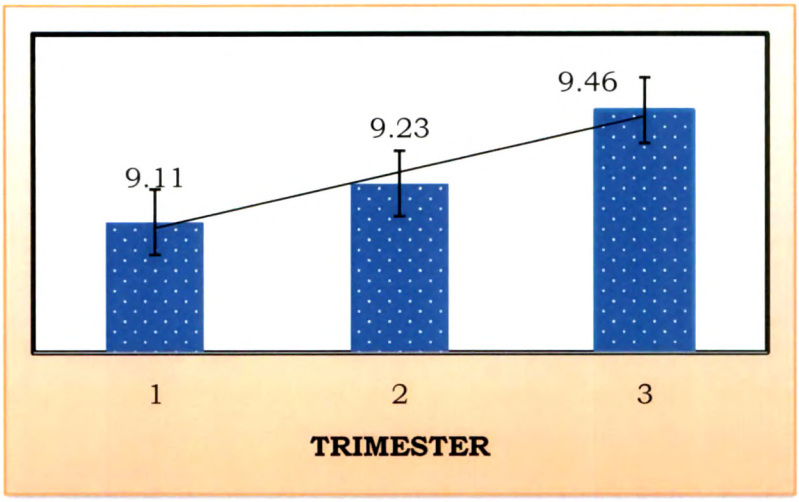


Table 4.2.5: Repeated measures of ANOVA, trend analysis and post hoc test for hemoglobin

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

4.2.4.2 IDA prevalence during each trimester

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

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

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

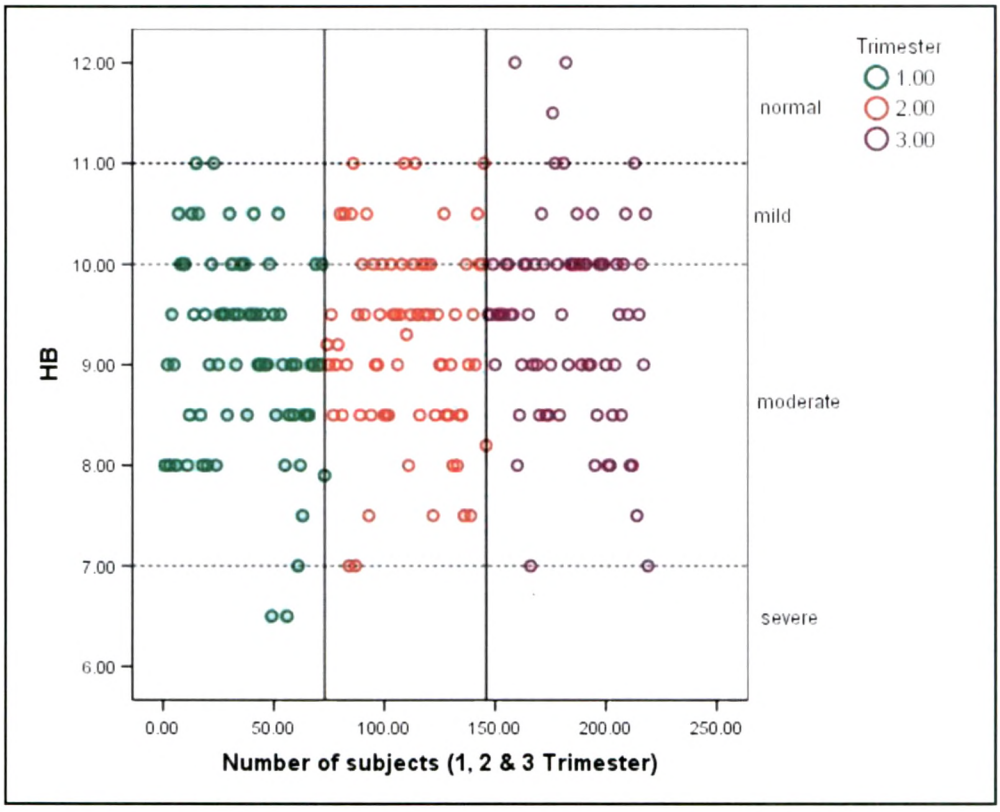
Figure in parenthesis denote number of subjects

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

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

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

Figure 4.2.8: Subject-wise prevalence of IDA



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

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

4.2.5 Iodine Deficiency during pregnancy

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

4.2.5.1 Iodine status

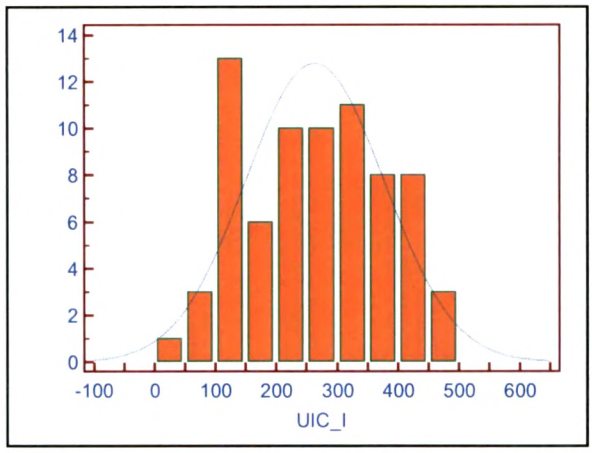
Urinary iodine excretion is an appropriate indicator of dietary iodine intake since 90 per cent of ingested iodine is excreted in the urine. Median urinary iodine (µg/L) during first, second and third trimester was found to be 270.1, 292.7 and 284.7 respectively (Table 4.2.7 and Figure 4.2.9). These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

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

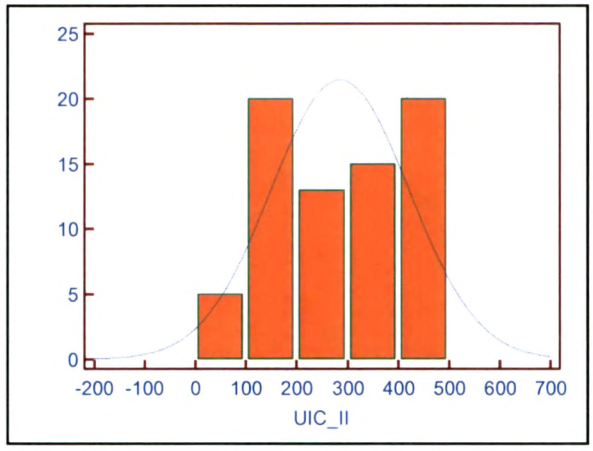
Parameter	Trimester	Median	95% CI of Median	20th Percentile	80th Percentile
UI	First	270.1	224.2-306.5	140.1	375.6
	Second	292.7	243.6-360.0	123.5	423.0
	Third	284.7	232.0-307.4	155.4	393.6

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

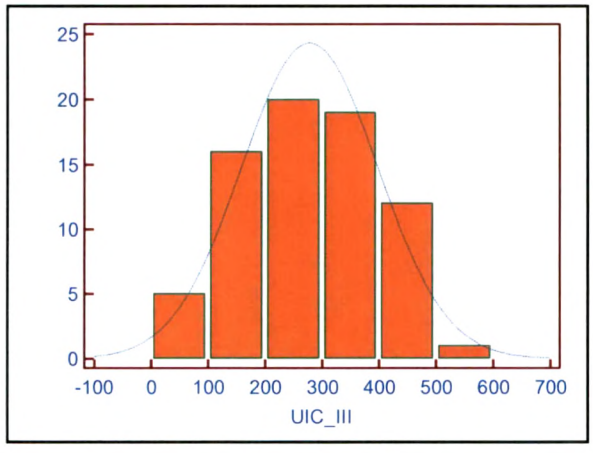
Figure 4.2.9: Frequency distribution of median urinary iodine during pregnancy



First trimester



Second trimester



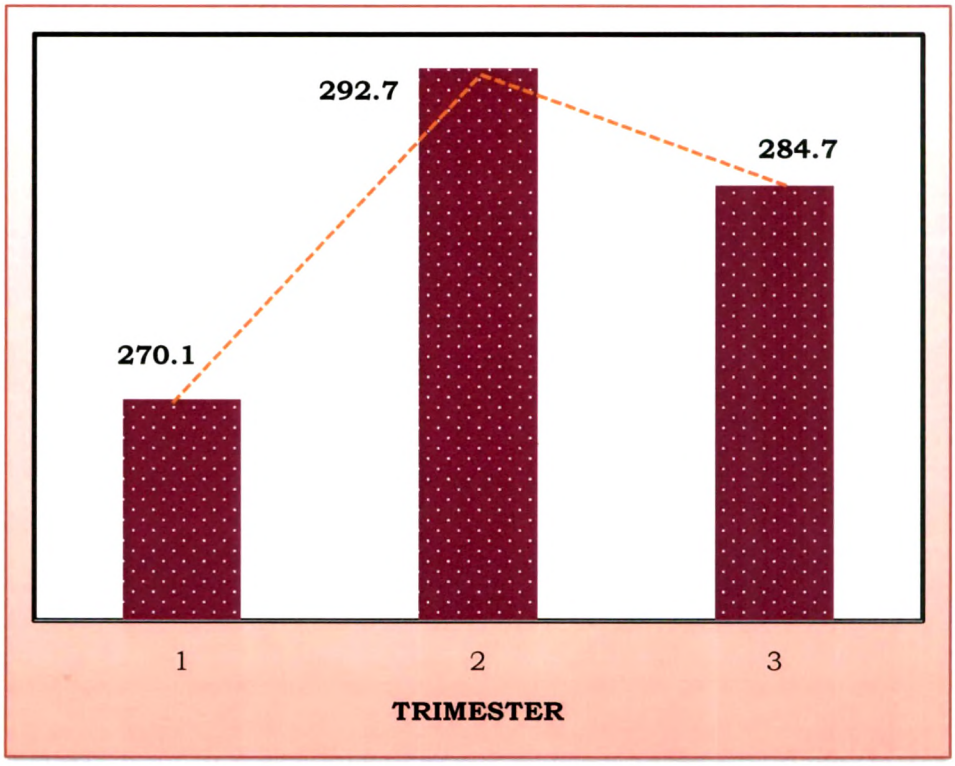
Third trimester

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

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

F value	DF1	P
2.1856	2	0.116^{ns}

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



4.2.5.2 ID prevalence during each trimester

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

Table 4.2.9: Iodine intake of pregnant women

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

Figure in parenthesis denotes actual number

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

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

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

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

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

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

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

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

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

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

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

4.2.6 Thyroid function during pregnancy

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

4.2.6.1 Thyroid status

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

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

Table 4.2.10: Mean and median thyroid hormone of pregnant women

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

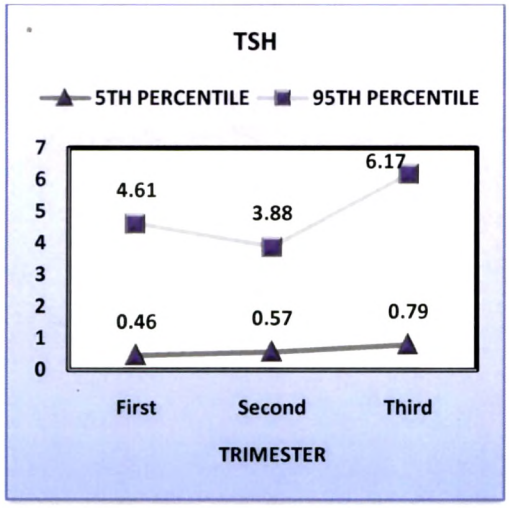
*geometric mean

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

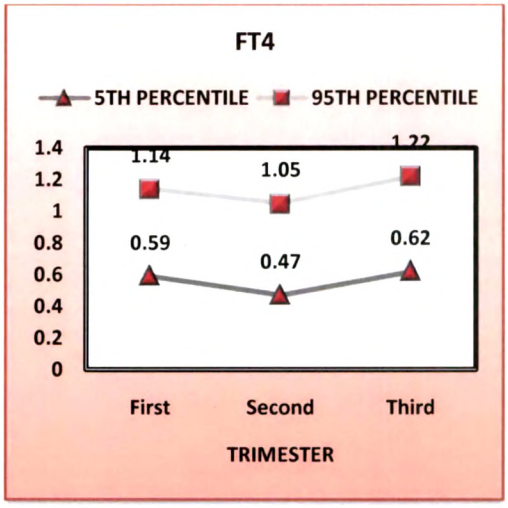
When compared, 5th percentile values of TSH, TT4 and TG, a similar trend was observed. We have observed a linear relationship between

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

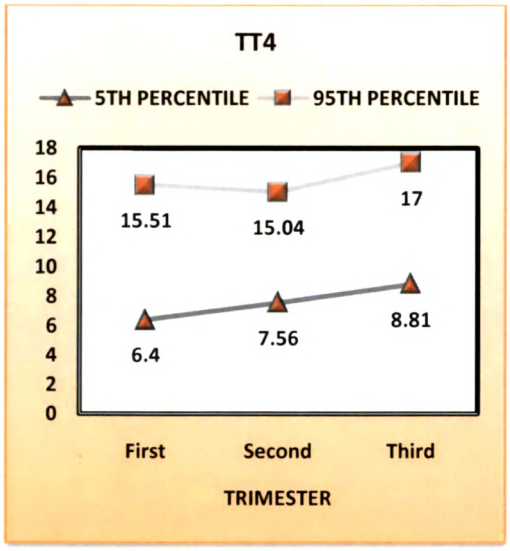
Figure 4.2.11: 5th and 95th percentile values of thyroid hormones



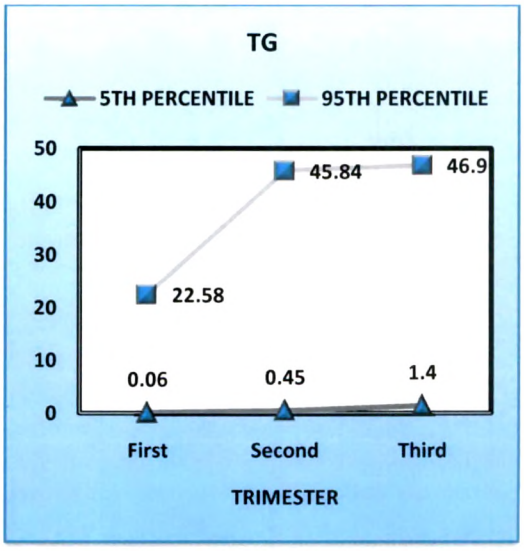
TSH



FT4



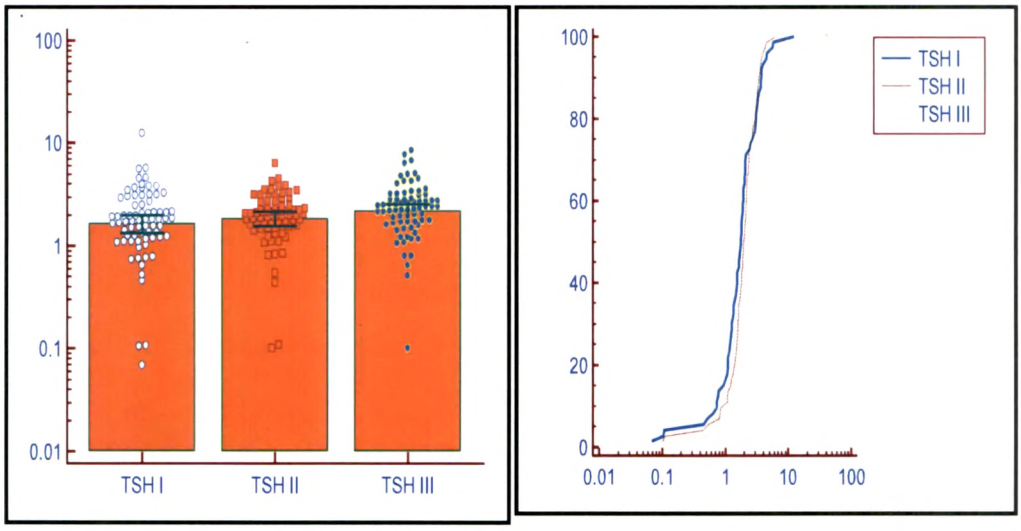
TT4



TG

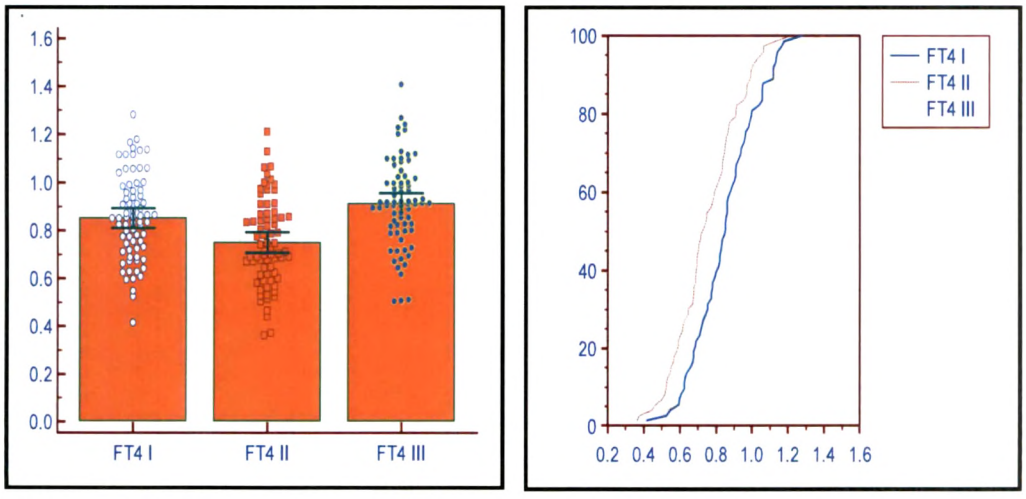
TSH and FT4 values of all pregnant women during first, second and third trimester can be seen in Figure 4.2.12 and 4.2.13 respectively. These figures are also helpful for outlier detection for trimester-specific reference interval generation. Cumulative frequency figure (right side) gives us the idea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

Figure 4.2.12: Box-plot and cumulative frequency of TSH



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

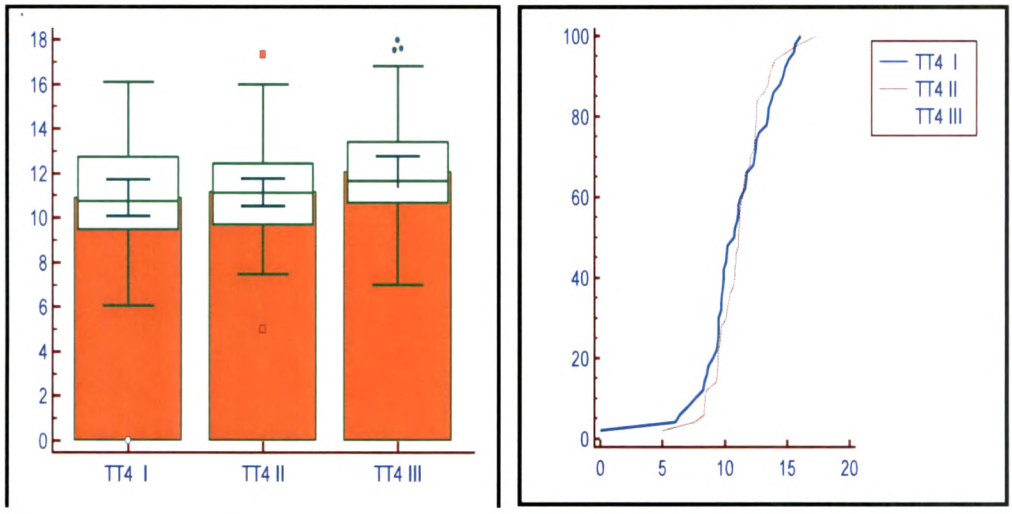
Figure 4.2.13: Box-plot and cumulative frequency of FT4



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

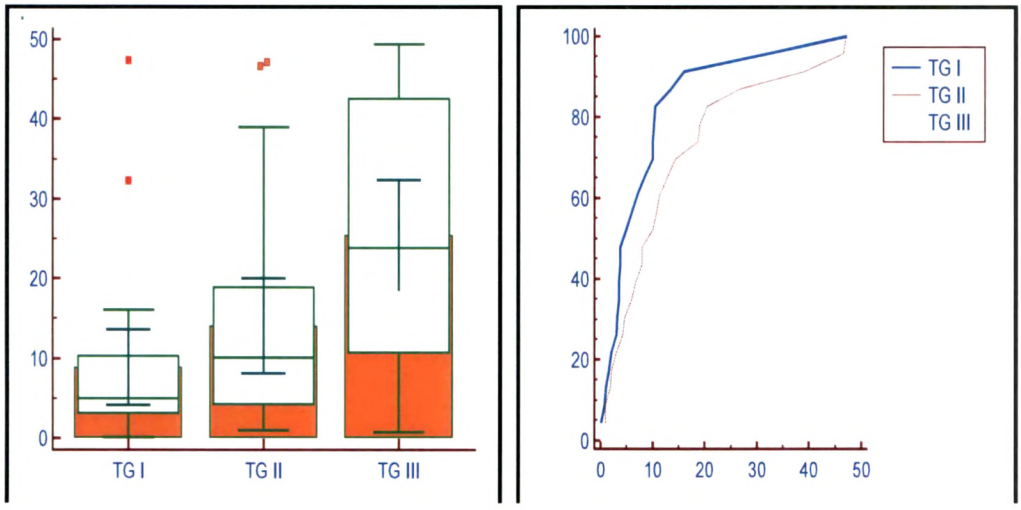
TT4 and TG values of all pregnant women during first, second and third trimester can be seen in Figure 4.2.14 and 4.2.15 respectively. Cumulative frequency figure (right side) gives us the idea of trend in TT4 and TG increase during each trimester. Variations were observed in TT4 and TG increase during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

Figure 4.2.14: Box-plot and cumulative frequency of TT4



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

Figure 4.2.15: Box-plot and cumulative frequency of TG



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

4.2.6.2 Thyroid dysfunction during each trimester

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

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

Table 4.2.11: Thyroid dysfunction among pregnant women

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

Figure in parenthesis actual number

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

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

Figure 4.2.16: Distribution of TSH during each trimester

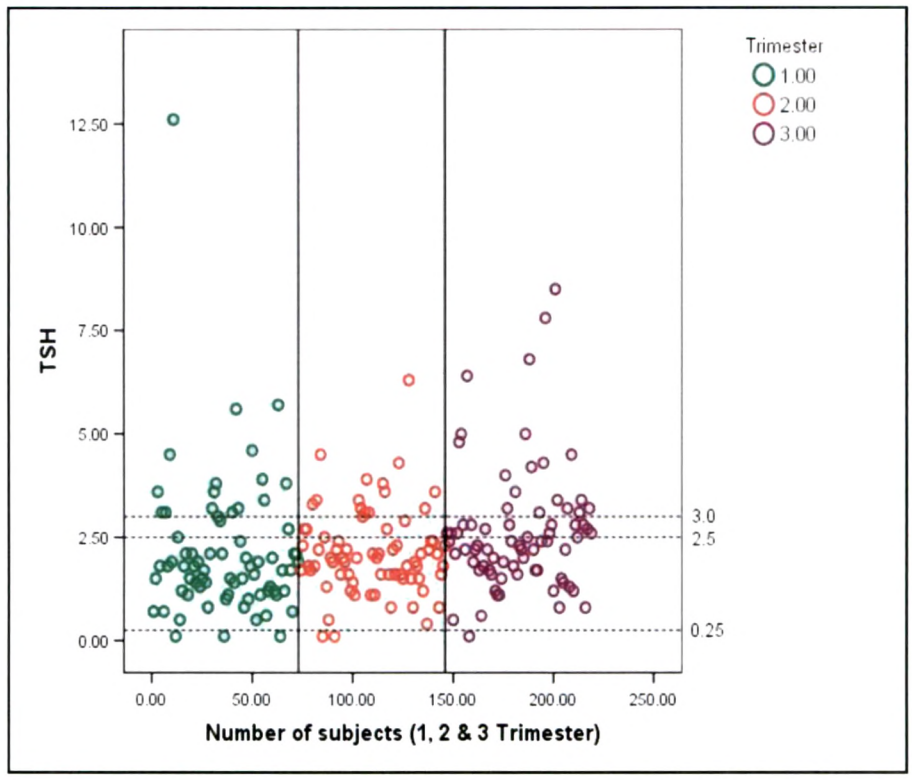
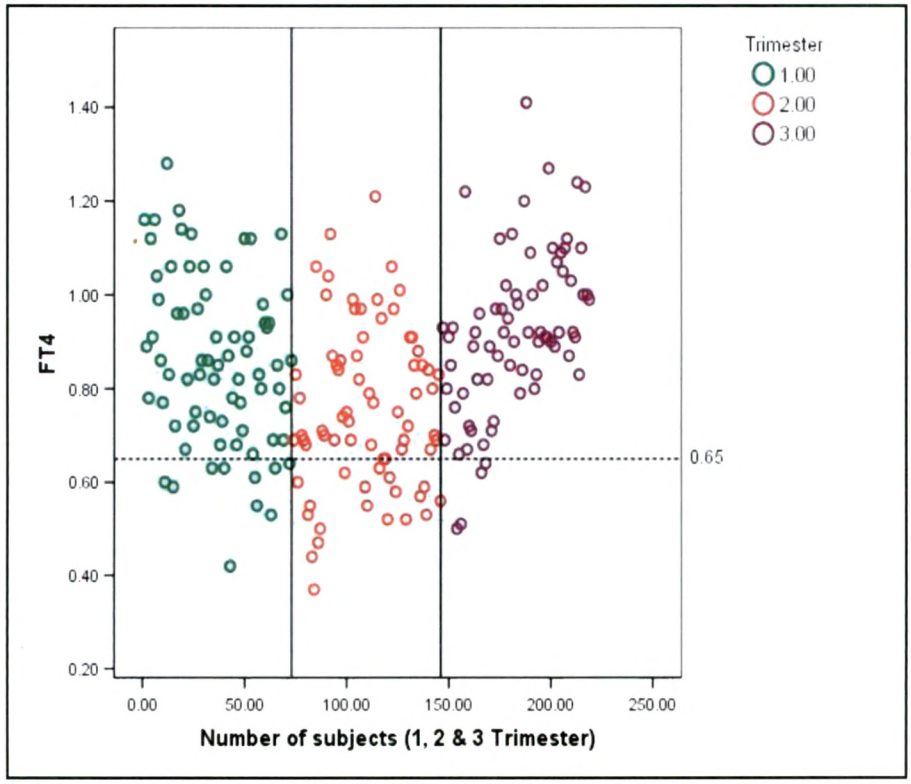


Figure 4.2.17: Distribution of FT4 during each trimester



4.2.6.3 TSH AND FT4 DURING PREGNANCY

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

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

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

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

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

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

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

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

Changes in FT4 concentrations during pregnancy have been controversial. Some authors had reported a decrease in FT4 (Boss and Kingstone 1979; Kurtz et al, 1979), whereas others had reported no change (Guillaume et al, 1985) or increase (Harada et al, 1979;Malkasian et al, 1970; Osathanondh et al, 1975) in FT4 concentration. Fantz et al (1999) mentioned that discrepancies in FT4 changes during pregnancy may have been attributable to the techniques used for free hormone measurement.

Another team (Roti et al, 1991) reported variability in serum free thyroid hormones in pregnant women at term among 10 commercially available methods. Albumin dependent methods gave 50% of subnormal values towards term, suggesting that such methods are unsuitable for use during pregnancy because of marked negative bias. Conversely, because of an increase in the pool of protein bound T4 during pregnancy, methods that require a high degree of sample dilution could be expected to show positive bias in relation to standard that contain a normal concentration of TBG.

Methods that are based on dialysis of free tracer to determine free fraction tend to overestimate free T4 in the presence of TBG excess, thus obscuring the normal decline in FT4 as pregnancy progresses. Regardless of the method, however, pregnant women, on an average had lower free thyroid hormone concentrations at term than non pregnant women.

Thus, thyroid function during pregnancy should be assessed using FT4 reference values that are both trimester-specific and method-specific. However, unlike TSH no recommendations on international basis have been given by endocrine societies on FT4 reference intervals that are both method and trimester specific.

Figure 4.2.18: Mean difference in TSH during first, second and third trimester

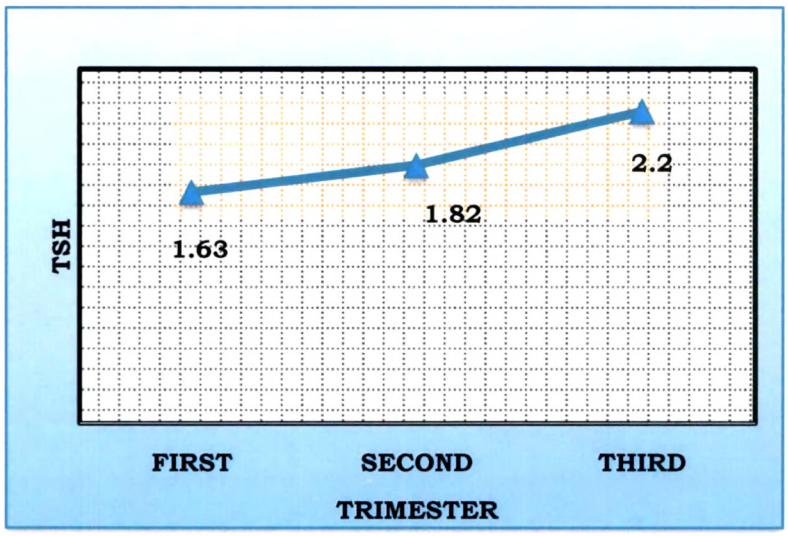


Table 4.2.12: Repeated measures of ANOVA for logTSH

Groups	**F value**	**DF**	**P**
Trimester (1,2 & 3)	10.41	2	<0.001***
	Trend analysis		
Trend	**t value**	**DF**	**P**
Linear	3.97	72	0.0002***
Factor	**Geometric Mean**	**95% CI**	
TSH_I	1.63	1.34-1.98	
TSH_II	1.82	1.56-2.13	
TSH_III	2.20	1.89-2.57	
Pair wise comparisons			
Factors	**Geometric Mean**	**95% CI**	**P**
TSH_I TSH_II	0.89	0.75-1.05	0.3242ns
TSH_II TSH_III	0.82	0.72-0.94	0.0024**
TSH_III TSH_I	1.35	1.12-1.63	0.0005***

Figure 4.2.19: Mean difference in FT4 during first, second and third trimester

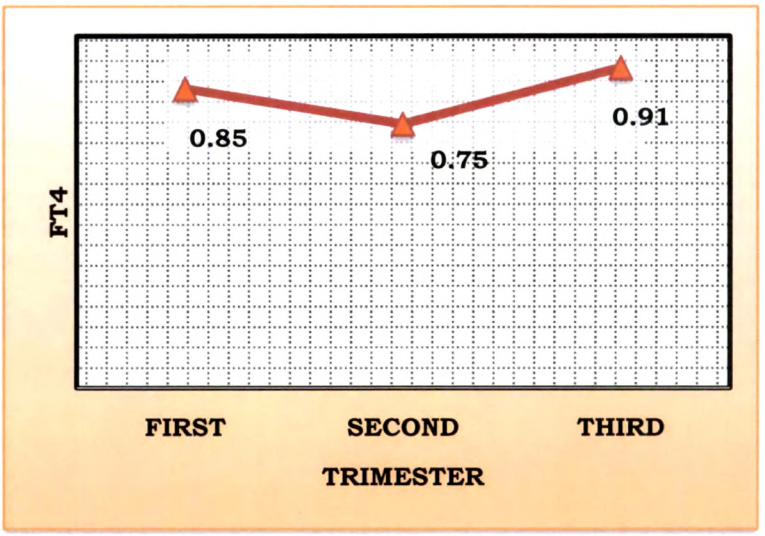


Table 4.2.13: Repeated measures of ANOVA for FT4

Groups		**F value**	**DF**	**P**
Trimester (1,2 & 3)		23.79	2	<0.001***
		Trend analysis		
Trend		**t value**	**DF**	**P**
Quadratic		7.64	72	< 0.0001****
Factor		**Mean**	**Std. Error**	**95% CI**
FT4_I		0.85	0.02	0.81-0.89
FT4_II		0.75	0.02	0.70- 0.79
FT4_III		0.91	0.021	0.87- 0.95
Pair wise comparisons				
Factors		**Mean Difference**	**Std. Error**	**P**
FT4_I	FT4_II	0.10	0.02	0.0001***
FT4_II	FT4_III	-0.16	0.02	<0.0001****
FT4_III	FT4_I	0.06	0.02	0.0820^{ns}

4.2.6.4 TT4 AND TG DURING PREGNANCY

Mean TT4 was found to be 10.89 µg/dl during first trimester. These were seen to rise through second trimester (3%) to a mean level of 11.15 µg/dl. The levels then further increased in the third trimester (8.3%) to 12.05 µg/dl. Levels of TT4 increase markedly during the first trimester of pregnancy, it reaches a peak at 20 weeks and then high levels of TT4 are maintained through the second and third trimesters.

Analysis of TT4 between each trimester showed significant differences (Table 4.2.14). Differences were primarily seen between second and third trimester and first and third trimester but not between first and second trimester (p=1.000). Trend analysis showed linear trend in TT4 increase between each trimester (Figure 4.2.20). A similar finding is echoed in study of Erem et al (2001), who investigated maternal thyroid function in 51 pregnant women without goiter in Turkey.

Mean TG was 8.8 ng/ml during first trimester. These were seen to rise through second trimester (74.3%) to a mean level of 14.0 ng/ml and then further increased in the third trimester (90.5%) to 25.3 ng/dl. Although thyroglobulin lacks specific hormonal activity, it can indicate the activity status of injury to the thyroid gland (Spencer and Wang, 1995). TG is frequently increased during pregnancy, reflecting the increased activity of the gland during pregnancy (Glinoer, 1997).

Analysis of TG between each trimester showed significant differences in mean values (Table 4.2.15). Differences were primarily seen between first and second trimester and first and third trimester but not between second and third trimester (p=0.0698). Trend analysis showed linear trend in TG increase between each trimester (Figure 4.2.21). Similar to our study Glinoer (1997) reported that increase in TG can be seen as early as the first trimester, but it is more pronounced during latter part of pregnancy.

Figure 4.2.20: Mean difference in TT4 during first, second and third trimester

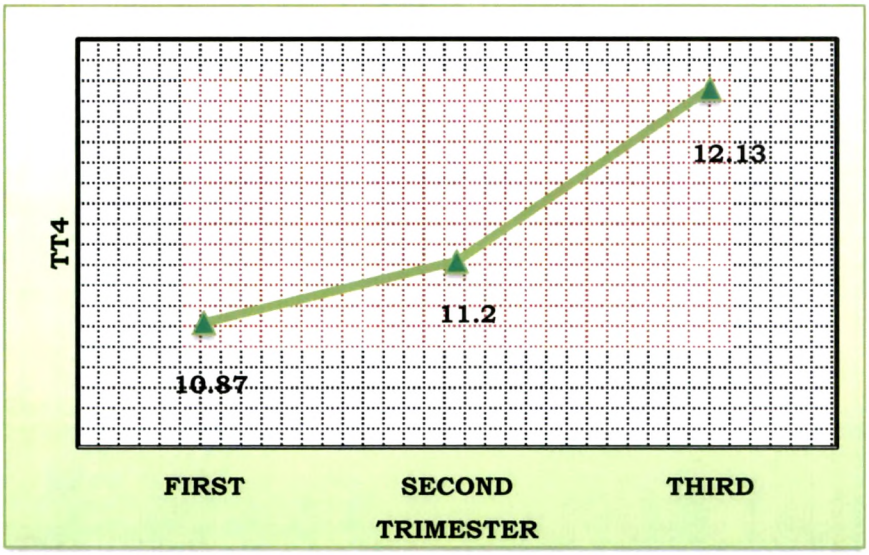


Table 4.2.14: Repeated measures of ANOVA for TT4

Groups	**F value**	**DF**	**P**
Trimester (1,2 & 3)	5.99	2	0.004**
	Trend analysis		
Trend	**t value**	**DF**	**P**
Linear	2.7775	72	0.0077**
Factor	**Mean**	**Std. Error**	**95% CI**
TT4__I	10.87	0.41	10.06-11.72
TT4_II	11.20	0.30	10.54-11.77
TT4_III	12.13	0.35	11.35-12.76
Pair wise comparisons			
Factors	**Mean Difference**	**Std. Error**	**P**
TT4__I TT4_II	-0.33	0.34	1.0000^{ns}
TT4_II TT4_III	-0.93	0.27	0.0059**
TT4_III TT4__I	1.26	0.41	0.0232*

Figure 4.2.21: Mean difference in TG during first, second and third trimester

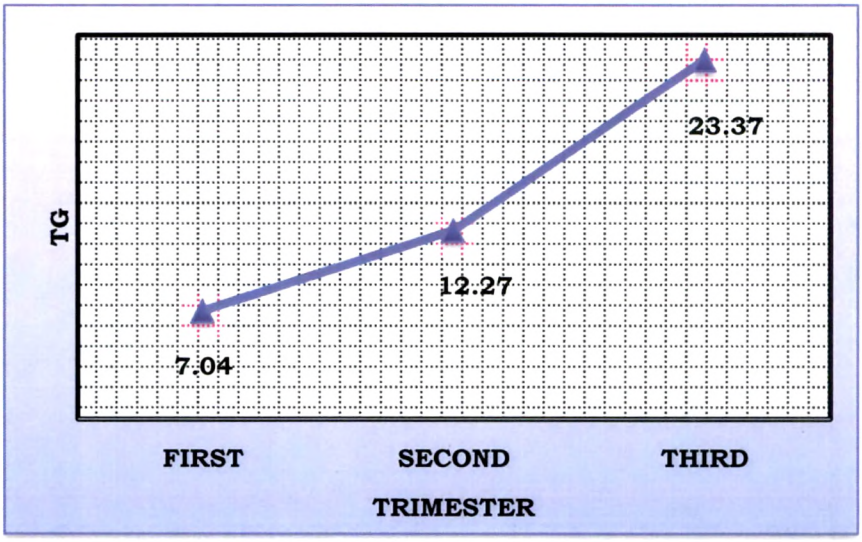


Table 4.2.15: Repeated measures of ANOVA for TG

Groups	**F value**	**DF**	**P**
Trimester (1,2 & 3)	10.11	2	0.002**
	Trend analysis		
Trend	**t value**	**DF**	**P**
Linear	2.10	72	0.0006***
Factor	**Mean**	**Std. Error**	**95% CI**
TG_I	7.04	2.27	4.13-13.55
TG_II	12.27	2.87	8.07-20.01
TG_III	23.37	3.33	18.46-32.32
Pair wise comparisons			
Factors	**Mean Difference**	**Std. Error**	**P**
TG_I TG_II	-5.23	1.95	0.0423*
TG_II TG_III	-11.10	4.65	0.0698^{ns}
TG_III TG_I	16.33	4.13	0.0018**

4.2.7 Reference interval for TSH and FT4

Trimester specific reference intervals for TSH and FT4 were developed using Robust method (CLSI C28-A3 Medcal software), 95 % CI of mean, upper limit and lower limit. Before developing reference intervals, outliers were tested and outside and far out values were excluded.

Table 4.2.16: Trimester specific reference intervals for TSH (μIU/ml) and FT4 (ng/dl)

Thyroid hormone	Trimester	N	Mean	Median	Lower limit	Upper limit
TSH	First	67	1.79	1.81	0.59	5.48
	Second	68	2.02	2.04	0.93	4.52
	Third	68	2.31	2.40	0.92	5.82
FT4	First	73	0.85	0.85	0.49	1.20
	Second	73	0.75	0.72	0.37	1.11
	Third	71	0.91	0.91	0.58	1.25

Table 4.2.16 shows mean and reference intervals for TSH (μIU/ml) during first, second and third trimester were 1.79 (0.59-5.48), 2.02 (0.93-4.52) and 2.31 (0.92-5.82) respectively. For FT4 (ng/dl) mean and reference interval were 0.85(0.49-1.20, first trimester), 0.75 (0.37-1.11, second trimester) and 0.91 (0.58-1.25, third trimester). Our trimester specific reference intervals were different from normal range for non-pregnant adults.

In 2007 International Guidelines for management of hypothyroidism during pregnancy and postpartum were published (Albanovich et al,

2007), according to these guidelines TSH (μIU/ml) upper limit of 2.5 during first trimester and 3.0 during second and third trimester should be considered for diagnosing hypothyroidism. However, till now no consensus has been reached for FT4 (ng/dl) lower limit.

Table 4.2.17: Comparison of trimester specific reference intervals

Thyroid hormone	Trimester	Reference interval (present study)		Reference interval (Marwah et al)		Manufacturer's range	
		Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
TSH	First	0.59	**5.48**	0.6	**5.0**		
(μIU/ml)	Second	0.93	**4.52**	044	**5.78**	0.25	**5.10**
	Third	0.92	**5.82**	0.74	**5.7**		
FT4	First	**0.49**	1.20	**0.93**	1.51		
(ng/dl)	Second	**0.37**	1.11	**0.73**	1.52	**0.65**	2.10
	Third	**0.58**	1.25	**0.87**	1.37		

Marwah et al were the first group who came up with trimester specific reference intervals for Indian pregnant women in 2008. They had studied 331 pregnant women (107 in first trimester, 137 in second trimester and 87 in third trimester). Their trimester specific upper limit for TSH was close to our range; however their lower limit for FT4 was far from our range (Table 4.2.17). The reason for these differences could be the study type, method used for thyroid hormone analysis and method used for developing reference ranges. Marwah et al study was a cross-sectional study and our study was a longitudinal study.

ECLIA method was used in Marwah et al study and we have used RIA method.

Trimester specific reference intervals can be developed using 3 methods, 1) normal distribution method 2) percentile method and 3) robust method for small sample size. In present study, since sample size was small we have used robust method. In contrast, Marwah et al have developed their reference ranges using percentile method [5th (lower limit) and 95th Percentile (upper limit)].

Looking into the variation caused by these 3 different factors, we recommend that each laboratory dealing with samples of pregnant women must develop their own trimester specific reference intervals using large sample size and with same subjects. Since more and more researchers are aware of the importance of evaluating maternal thyroid function during pregnancy by gestation-specific reference intervals manufacturer's reference range should not be used for pregnant women. If a non pregnant reference interval is used, a number of pregnant women with thyroid dysfunction could be potentially misclassified.

In 2007 Stricker et al have reported that 5.6-18.3% of misdiagnoses and missed diagnoses likely occur in clinical practice due to the use of non-pregnant reference values as basis for diagnosis. In China (Shan et al, 2009), Malaysia (Thevarajah et al, 2009) and Australia (Gilbert et al, 2008), the percentage of potentially misclassified cases of subclinical hypothyroidism and hypothyroxinemia in pregnant women was decreased by using trimester-specific reference ranges.

Many other authors from different parts of World have developed trimester specific reference intervals (refer ROL page 66 to 70). We

have observed vide variation in these reference intervals also, apart from 3 factors which we have mentioned other factors also contribute to variation in thyroid hormone reference intervals. These are storage of thyroid hormones, race and ethnic variation, dietary habits, presence and absence of iodine deficiency, data transformation (log or square root), sample size etc.

Distribution of pregnant women, according to 3 different reference intervals in shown in Figure 4.2.22 (present study reference interval), 4.2.23 (international guidelines for TSH during pregnancy) 4.2.24 (Marwah et al reference intervals for Indian pregnant women) and Table 4.2.17.

When we categorized subjects according to 3 different methods, we found that during first trimester, if we use method 2 then 27.4% subjects will have increased TSH and if we use method 1 and 3 only 4.1% subjects will have raised TSH. Similarly if we use method 3 then 68.5% subjects will have decreased FT4 and if we use method 2 then 13.7% and with method 1 only 1.4% subjects will have decreased FT4.

During second trimester, if we use method 2 then 19.2% subjects will reflect increased TSH and if we use method 1 and 3 only 4.1% subjects will have raised TSH. Similarly if we use method 3 then 50.1% subjects will have decreased FT4 and if we use method 2 then 30.1% and with method 1 only 1.4% subjects will have decreased FT4.

During third trimester, if we use method 2 then 26% subjects will have increased TSH and if we use method 1 and 3 only 5.5% subjects will have raised TSH. Similarly if we use method 3 then 33% subjects will have decreased FT4 and if we use method 2 then 6.8% and with method 1 only 4.1% subjects will have decreased FT4 (Table 4.2.18).

Table 4.2.18: Distribution of subjects according to 3 different reference intervals

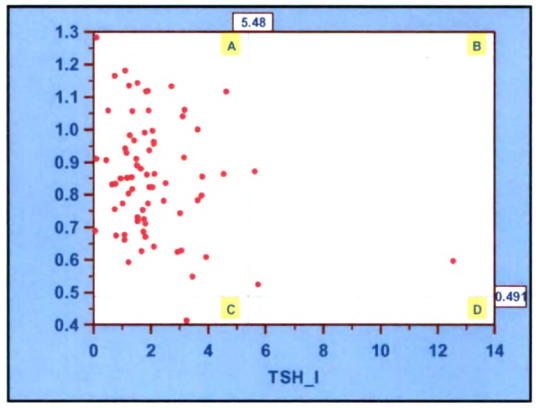
Trimester	Method 1*		Method 2**		Method 3***	
	TSH >upper limit	FT4 <lower limit	TSH >upper limit	FT4 <lower limit	TSH >upper limit	FT4 <lower Limit
First	4.1% (3)	1.4% (1)	27.4% (20)	13.7% (10)	4.1% (3)	68.5% (50)
Second	1.4% (1)	1.4% (1)	19.2% (14)	30.1% (22)	1.4% (1)	50.1% (37)
Third	5.5% (4)	4.1% (3)	26% (19)	6.8% (5)	5.5% (4)	33% (24)

*present study reference interval, **international guidelines (reduced TSH upper limit and FT4 kit value)***reference interval for Indian women

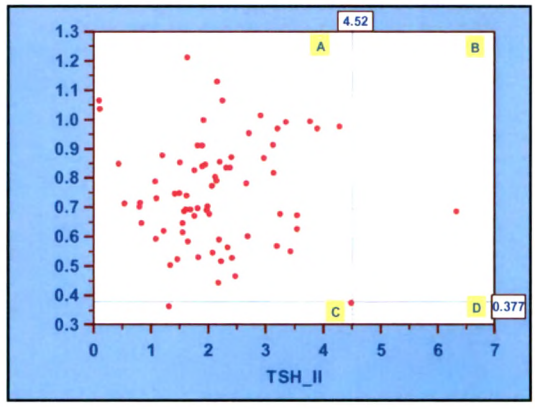
Figure in parenthesis denote actual number

From these figures it is concluded that, using different trimester-specific reference interval will result in different prevalence of thyroid dysfunction. Hence, choosing a right method is very important. Our reference intervals were self-sequential longitudinal reference intervals. In 2011 Wang et al have assessed thyroid function of pregnant Chinese women using self-sequential longitudinal reference intervals. They have screened 1,744 pregnant women with 3 different reference intervals: 1) self-sequential longitudinal reference intervals 2) Gestation-specific reference interval 3) non pregnant reference range. After comparing the results and pregnancy outcome, Authors have found that self-sequential longitudinal reference intervals had the best clinical specificity among the three reference intervals. Use of self-sequential longitudinal reference intervals can decrease the percentage of misclassification of thyroid dysfunction.

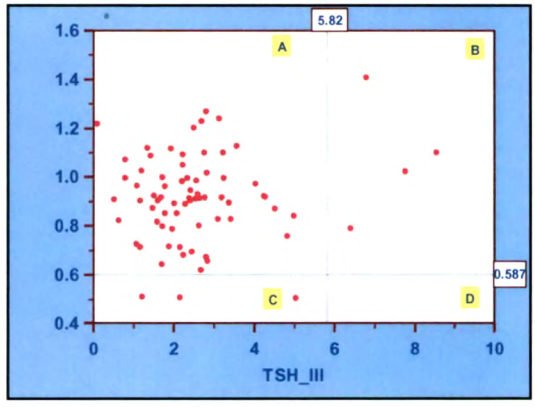
Figure 4.2.22: Distribution of pregnant women according to trimester-specific reference internal of present study (method 1)



First trimester



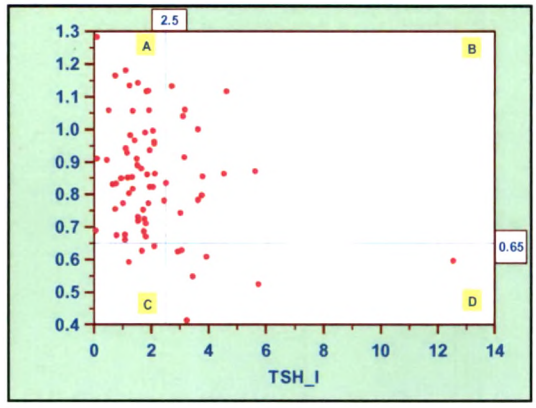
Second trimester



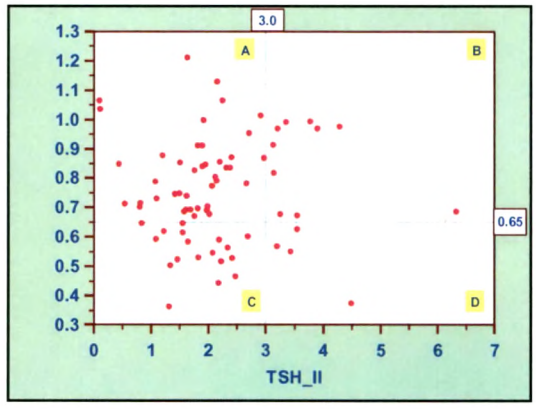
Third trimester

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (μIU/ml) upper limit

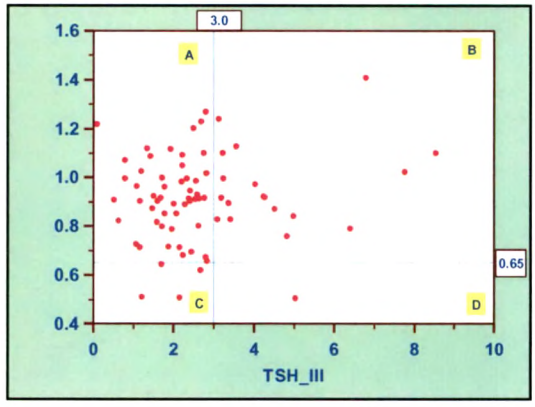
Figure 4.2.23: Distribution of pregnant women according to International guidelines (method 2) (Albanovich et al, 2007)



First trimester



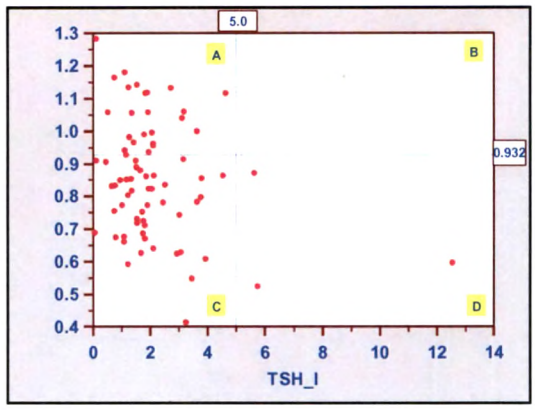
Second trimester



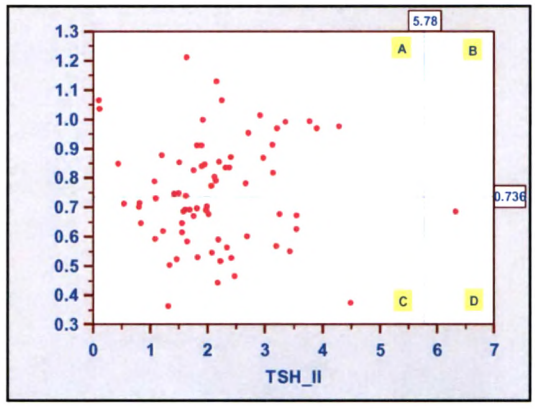
Third trimester

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (μIU/ml) upper limit

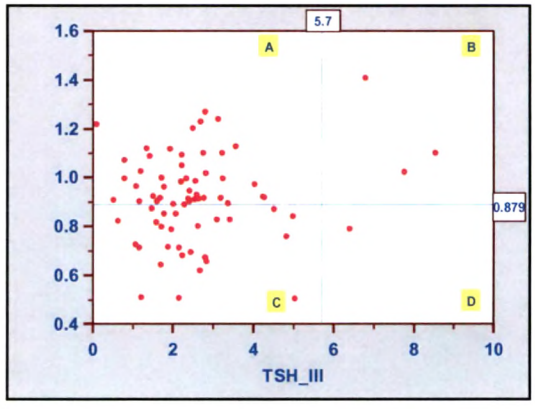
Figure 4.2.24: Distribution of pregnant women according to trimester-specific reference interval (method 3) [Marwah et al, 2007]



First trimester



Second trimester



Third trimester

Panel a) Normal subjects, b) Subclinical hypothyroidism, c) Hypothyroxinemia and d) overt hypothyroidism, horizontal reference line denotes FT4 (ng/dl) lower limit and vertical reference line denotes TSH (μIU/ml) upper limit

4.2.8 Correlation and regression analysis

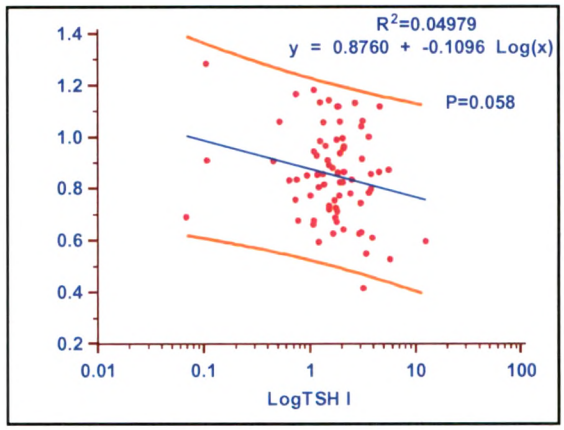
When correlation analysis was performed, we found positive correlation between per capita income and energy intake (r=0.2, p=0.012) and between gestation week and weight gain (r=0.3, p=0.008). A significant positive correlation was found between first trimester TSH and second trimester TSH (rho=0.77, p<0.0001), second trimester TSH and third trimester TSH (rho=0.677, p<0.0001) and between third trimester TSH and first trimester TSH (rho=0.665, p<0.0001). In case of FT4 a significant correlation was found between first and second trimester (r=0.388, p<0.0001) and between second and third trimester (r=0.520, p<0.0001) but not between third and first trimester (r=0.156, p=0.187).

We did not find any significant correlation between TSH, FT4, TT4 and UI, in contrast to other studies. One possibility is that our subjects were too homogenous regarding the presence and severity of iodine deficiency to show such a correlation. Correlation of thyroid hormones is shown in Figure 4.2.25 (TSH and FT4) and 4.2.26 (TSH and TT4). Log TSH had negative (non significant) correlation with FT4 and TT4 during each trimester. Correlation coefficient between log TSH and FT4 was found to be -0.22 during first trimester, -0.12 during second trimester and -0.008 during third trimester. Between log TSH and TT4, correlation coefficient during first, second and third trimester was -0.25, -0.012 and -0.091 respectively (Figure 4.2.26). A negative non significant association was found between TSH and urinary iodine during first (rho=-0.179, p=0.129), second (rho=-0.119, p=0.317) and third trimester (rho=-0.037, p=0.757).

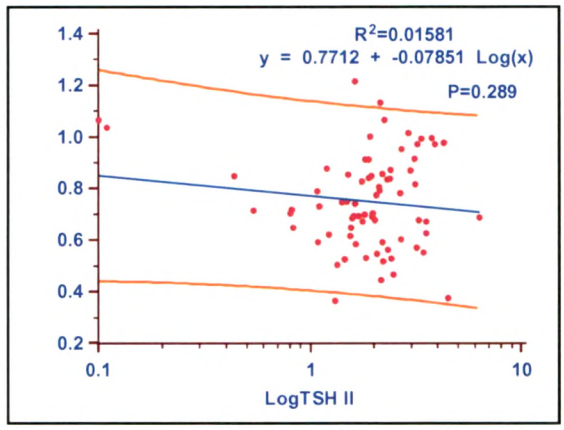
Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism (Hess et al, 2002). The mechanism by which iron status influences thyroid and iodine metabolism is unclear. IDA could impair thyroid metabolism through anemia and lowered oxygen transport (Surks et al, 1969; Galton 1972). When we correlated TSH with HB, we did not find any significant association (Figure 4.2.27).

Figure 4.2.25: Correlation (regression line) between TSH and FT4 during each trimester

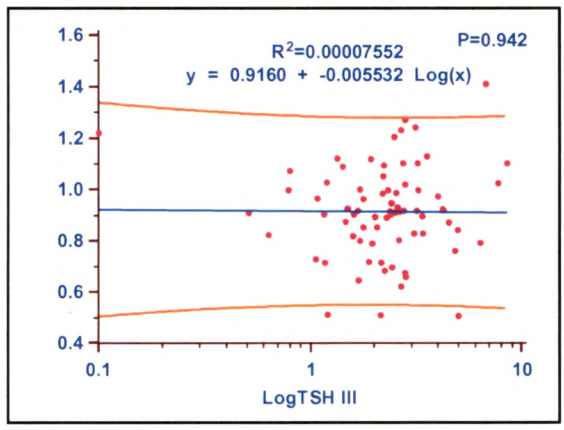
[Orange line denotes 95% prediction interval]



a) First Trimester



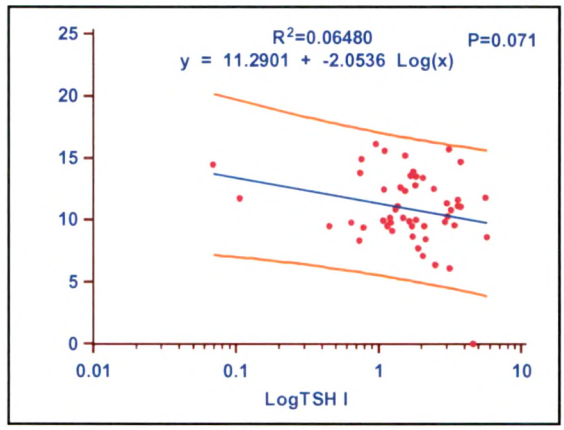
b) Second Trimester



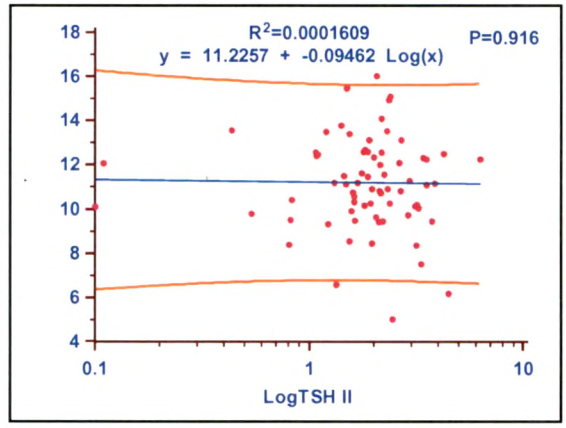
c) Third Trimester

Figure 4.2.26: Correlation (regression line) between TSH and TT4 during each trimester

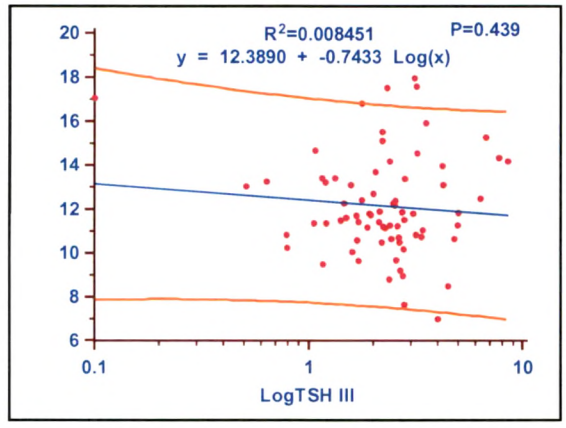
[Orange line denotes 95% prediction interval]



a) First Trimester



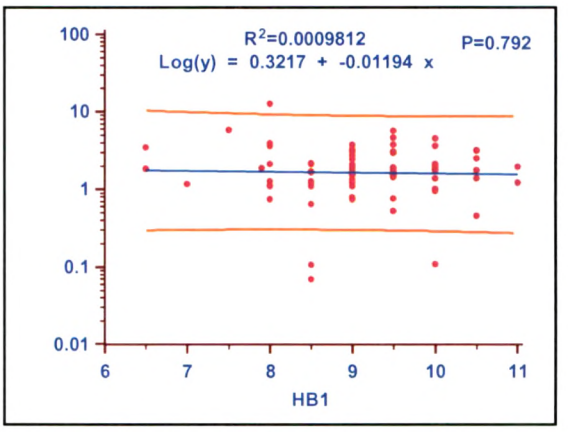
b) Second trimester



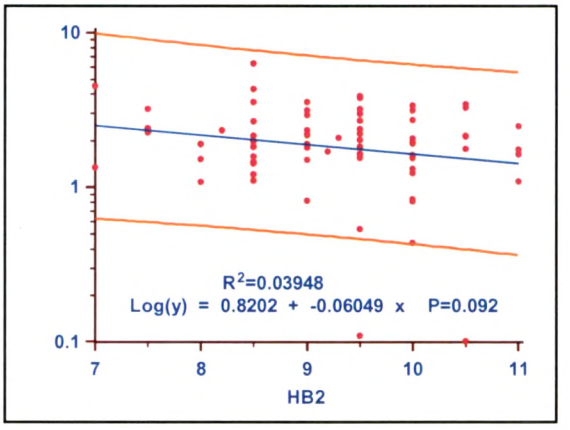
c) Third trimester

Figure 4.2.27: Correlation (regression line) between TSH and HB during each trimester

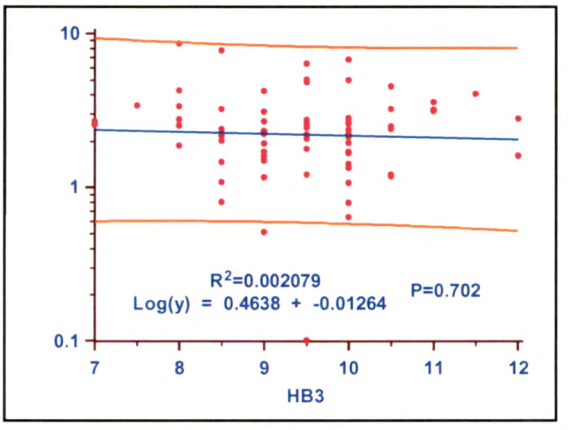
[Orange line denotes 95% prediction interval]



a) First trimester



b) Second trimester



c) Third trimester

4.2.9 Knowledge, Attitude and Practices

For improving Knowledge, Attitude and Practices (KAP) of pregnant women; Nutrition Health Education (NHE) was given to pregnant women during first trimester and pre data was collected. During second trimester reinforcement was carried out and post data was collected in third trimester.

Knowledge of pregnant women regarding critical role of iodine and iron during pregnancy was poor before intervention (Table 4.2.19). Percentage of women who have ever heard about iodized salt (IS) and IFA was 39.7% and 28.2% respectively. NHE has shown improvement in knowledge, attitude and practices of pregnant women. Percentage of women who could recognize IS increased by 62.8 % after providing NHE (Figure 4.2.28).

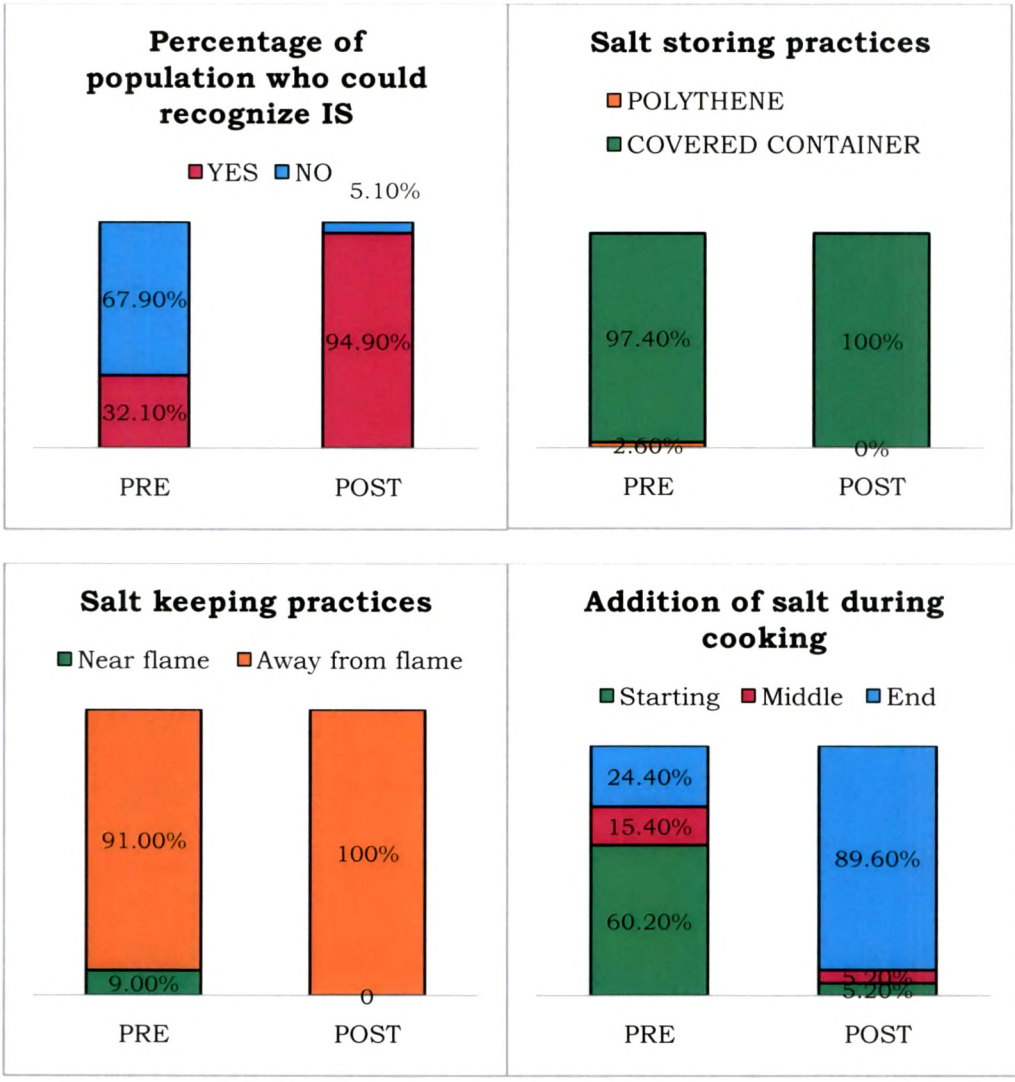
Table 4.2.19: Knowledge of pregnant women regarding iodine and iron

Knowledge about	Response
Iodine - Percentage of population who have heard about IS	39.7 %
Iron- Percentage of population who have heard about IFA	28.2%

According to Rana et al (2009) cooking losses of iodized salt ranges from 6.58- 51.08%. In order to get maximum iodine from our salt, it is necessary to take care of few small but important things like- proper storage of iodized salt, healthy cooking practices etc. A marked improvement was found in salt keeping practices of pregnant women. After intervention, everyone has started keeping iodized salt away from flame. Salt storing practices of the population were fair, and became good after NHE (Figure 4.2.28). A favourable change was observed in practices of pregnant women after NHE on addition of salt during

cooking. We observed that better cooking practices were followed by mothers like- adding salt after 75 % cooking is done, closing the lid while cooking etc. These small changes in their practices of storing, keeping and cooking iodized salt will definitely increase the iodine content of their diet.

Figure 4.2.28: Outcome of intervention (NHE) on Knowledge and Practices of population



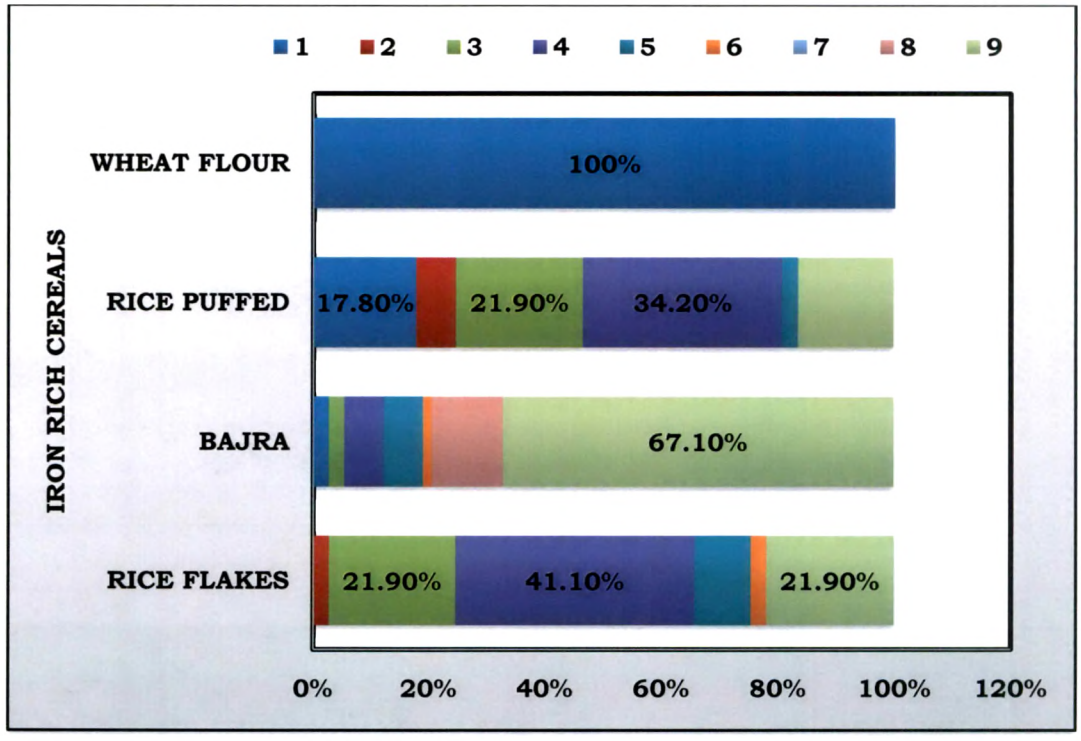
Since most of our subjects were educated till primary level (66%) only, our intervention in the form of NHE could help them improve their iodine and iron status. After receiving NHE, all pregnant women

became curious regarding brand name of their iodized salt. Earlier only few (19%) women were aware of the brand name of salt which they were consuming at home. Remembering brand name will definitely not increase the iodine content but it is an indicator of knowledge of pregnant women regarding importance of iodized salt.

4.2.10 Food Frequency

Data on consumption of iron rich foods, vitamin C rich foods and non vegetarian food items was obtained from pregnant women. In all frequency distribution figures legend were used, where 1 stands for daily, 2 specify three-four times a week, 3 stands for twice a week, 4 stands for weekly, 5 indicate bimonthly, 6 indicate monthly,7 means seasonally,8 means occasionally and 9 stands for never.

Figure 4.2.29: Frequency of consumption of iron rich cereals



Data on iron rich foods was obtained using a list of iron rich cereals, legumes and pulses, vegetables and iron rich fruits.

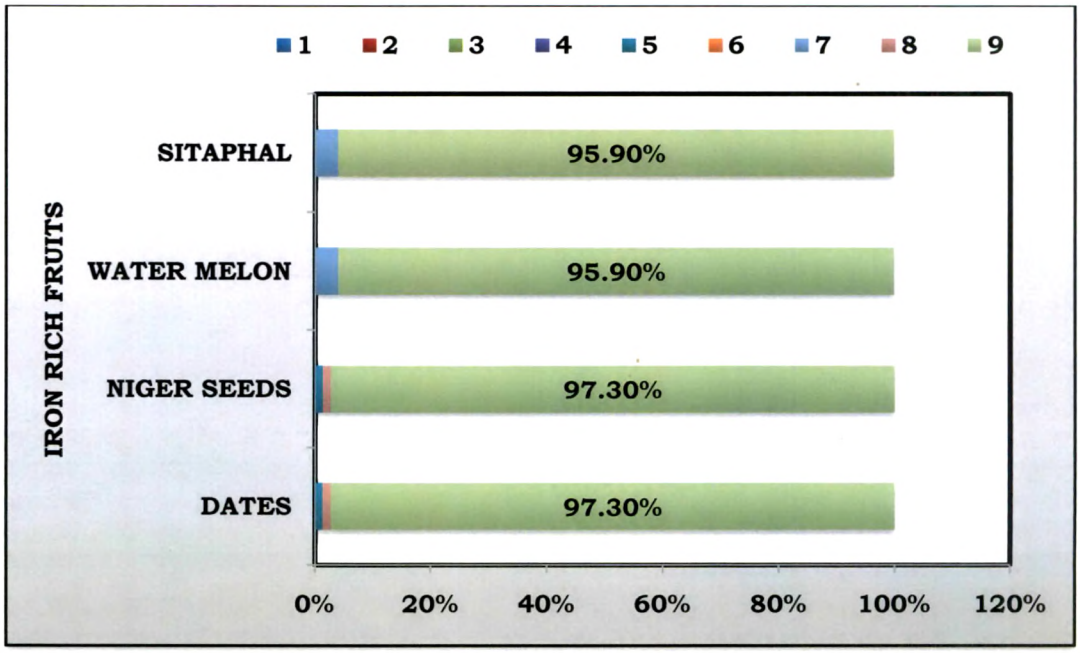
Iron rich cereals

Among the four cereals picked up, rice flakes contains maximum iron followed by bajra, puffed rich and wheat. Frequency distribution data reveals that 41.1% subjects consumed rice flakes once a week, 67.1% subjects never consumed bajra, 21.9% subjects consumed puffed rich twice a week while 34.2% subjects consumed it once in a week, and wheat flour was consumed daily by all the subjects (Figure 4.2.29).

Iron rich fruits

Among iron rich fruits that were selected, dates contain maximum iron followed by niger seeds, water melon and sitaphal. Though dates and niger seeds contain more iron than watermelon and sitaphal but amount of dates and niger seeds consumed daily by any person is generally less than the other two. Hence we can say that on an average these four food items will by and large provide same amount of daily iron. Frequency distribution reveals that only few subjects were consuming these iron rich fruits (Figure 4.2.30).

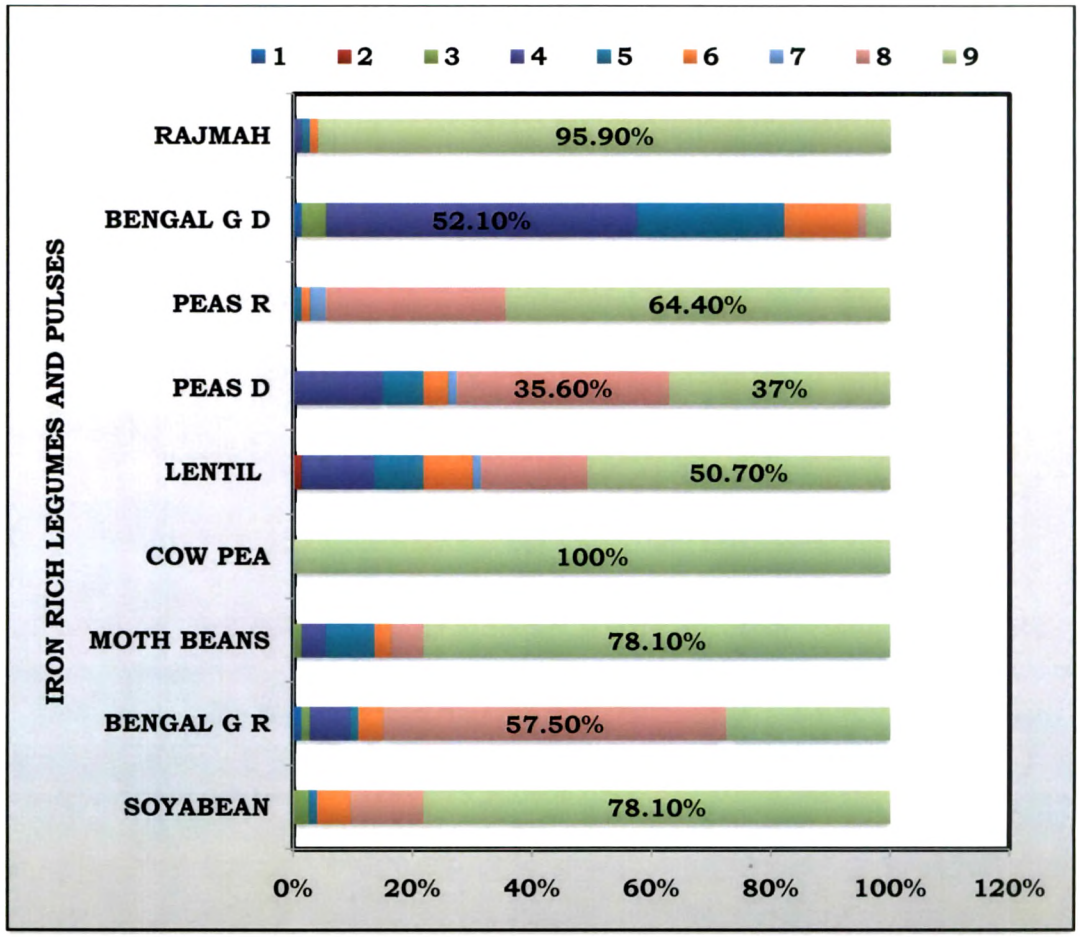
Figure 4.2.30: Frequency of consumption of iron rich fruits



Iron rich legumes and pulses

Among iron rich legumes and pulses, soyabean contained maximum iron followed by bengal gram roasted, moth beans, cow pea, lentil, peas dry, peas roasted, bengal gram dhal and rajmah. Soyabean and moth beans were never consumed by 78.1% subjects, bengal gram roasted and peas dry were occasionally consumed by 57.5% and 35.6% subjects respectively. Lentil, peas dry and peas roasted were never consumed by 50.7%, 37% and 64.4% subjects respectively. Bengal gram dhal was weekly consumed by almost half of the subjects. Rajmah was found to be the least consumed pulse, while none of the subjects consumed cow pea (Figure 4.2.31).

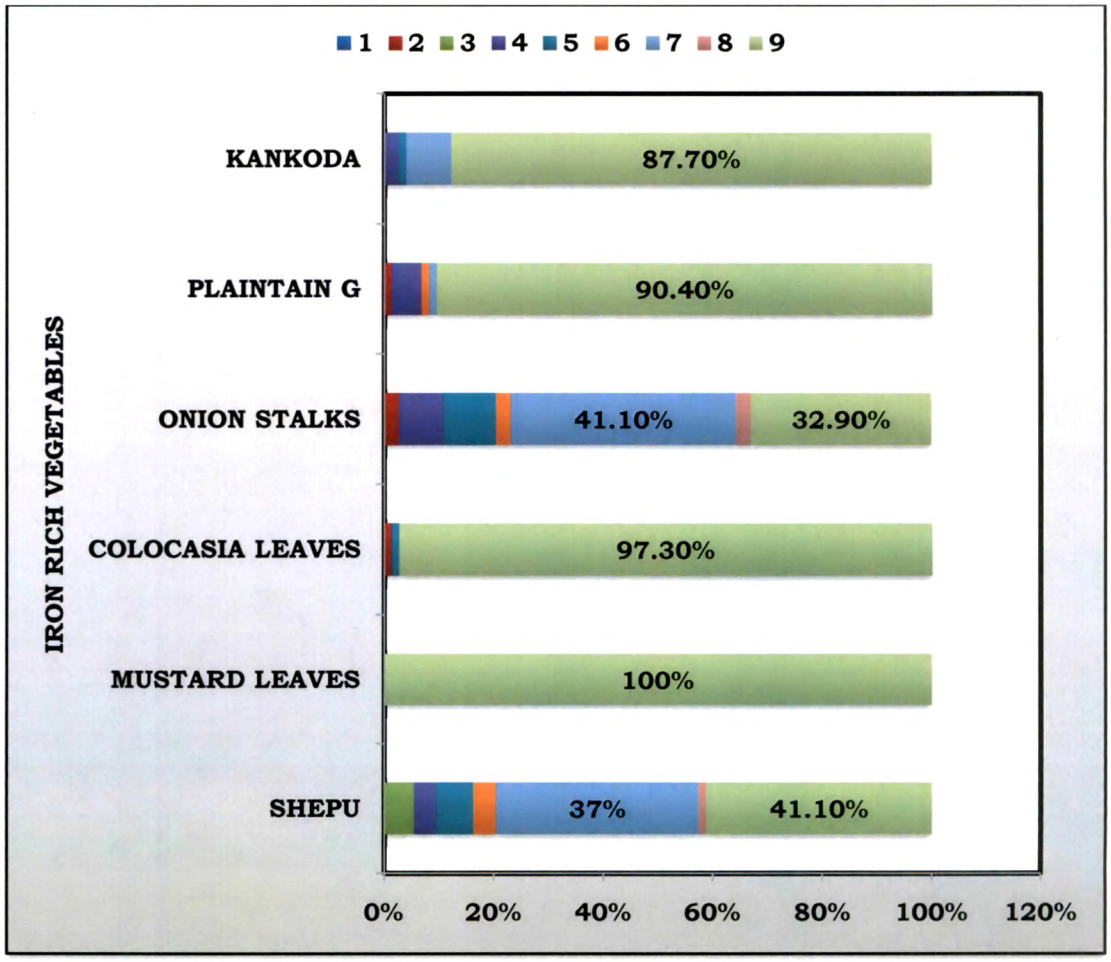
Figure 4.2.31: Frequency of consumption of iron rich legumes and pulses



Iron rich vegetables

Iron rich vegetables that were included in the list were, shepu with maximum iron content followed by mustard leaves, colocasia leaves, amaranth leaves, onion stalks, plantain green and kankoda. Shepu was seasonally consumed by 37% subjects, while 41.1% subjects never consumed it. Onion stalks were never consumed by 32.9% subjects, while 41.1% subjects consumed it seasonally. None of the subjects consumed mustard leaves. Vegetables like kankoda, plantain green and colocasia leaves were also never consumed by most of the subjects (Figure 4.2.32).

Figure 4.2.32: Frequency of consumption of iron rich vegetables



Vitamin C rich foods

It is a proven fact that vitamin C rich foods in the diet are enhancers of iron absorption, especially for those people who depend on vegetarian food items to fulfill their iron requirements.

Data on consumption of vitamin C rich fruits was obtained using a list of vitamin C rich fruits. Among the seven fruits which were included in the list, amla has maximum vitamin C followed by guava, sweet lime, pineapple, lemon, orange and tomato ripe. Ripe tomatoes were daily consumed by all the subjects. Like tomatoes, lemon was also consumed daily by most of the subjects, while fruits like amla and pineaaple were never consumed by 64.4% and 80.8% of the subjects respectively. Oranges, sweet lime and guavas were seasonally consumed by most of the subjects (Figure 4.2.33).

Figure 4.2.33: Frequency of consumption of Vitamin C rich foods

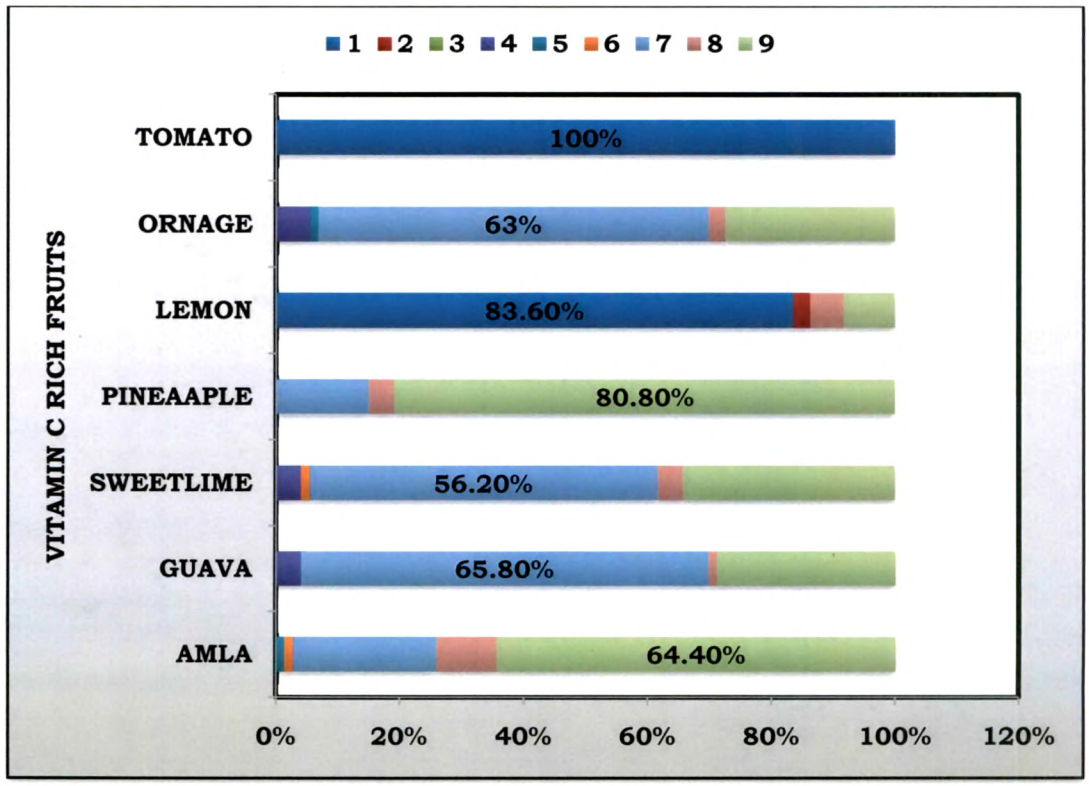
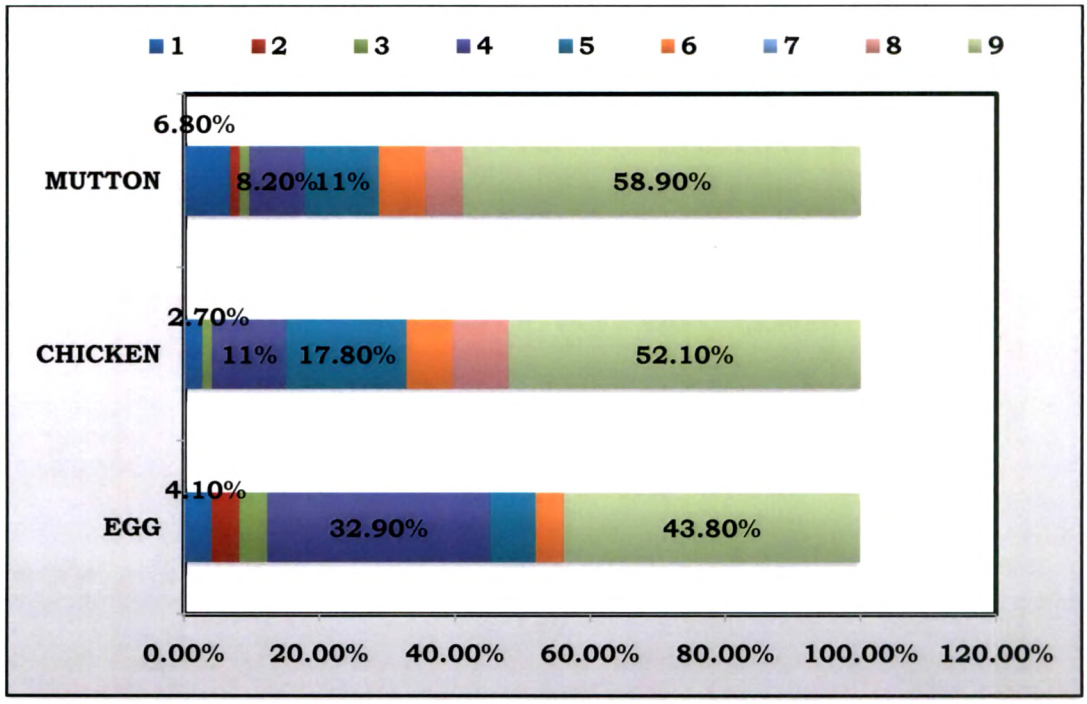


Figure 4.2.34: Frequency of consumption of non vegetarian food items



Non vegetarian food items

For obtaining data on frequency of consumption of non vegetarian food items- egg, chicken and mutton were used. Eggs, chicken and mutton were daily consumed by only few of the subjects. Percentage of subjects who consumed eggs, chicken and mutton weekly was 32.9%, 17.8% and 8.2% respectively (Figure 4.2.34). Many subjects (33%) reported that during pregnancy they have left non vegetarian food items. Reason given was they feel like vomiting due to smell of non vegetarian food. Percentage of subjects who were never consuming eggs, chicken and mutton was 43.8%, 52.15 and 58.9% respectively. Chicken was bimonthly consumed by 17.8% of subjects and mutton by 11%.

From the above discussed results it can be concluded that consumption of iron rich foods, vitamin C rich fruits and non vegetarian food items were not appreciable. Poor dietary intake of bio-available iron can result in anemia during pregnancy and lactation.

Dietary requirements of pregnant and lactating women are greater as compared to requirements of women during any other stage of life. However, In India it is observed that diet of pregnant and lactating women more or less remains the same as it was before pregnancy. Respite giving nutrition health education to pregnant women on importance of extra nutrients for delivering a healthy baby and keeping herself healthy, a marked improvement in their dietary habits were not observed. There is a need to further motivate pregnant women (LIG) to improve their dietary habits. This can be achieved by distributing food items rich in calories, proteins and minerals to pregnant and lactating women. In Vadodara, Gujarat Government is providing energy dense foods to pregnant and lactating women in the form of *sukhdi, upma* and *sheera* under NRHM (National Rural Health Mission) programme. There is a need to ensure that pregnant and lactating women are receiving these three food items. After they receive these food items again we must ensure that these items are being consumed by pregnant and lactating women. Often it happens that these foods are also consumed by other family members.

4.2.11 Maternal health and child care

Pregnancy and the first year of life are critical periods for human health. Maternal and child health are closely linked and the results are greatest when interventions are combined as packages that address the period before and during pregnancy, through birth and the neonatal stage, and then through early childhood (up to five years of age). Coverage of effective health interventions varies greatly within and between countries and across the continuum. It is highest for interventions that can be scheduled (e.g. antenatal care and immunization), but lower for interventions dependent on 24-hour service availability (such as skilled attendance at birth and care for sick newborns or children) and for behavioral and social change.

Maternal health indicators

Table 4.2.20 summaries the health status of pregnant women who participated in present study. Percentage of anemia (first trimester) in our subjects was found to be very high as compared to state data. However, prevalence of anemia was reduced with advancing gestation, indicating improvement in mean hemoglobin values due to IFA consumption.

Table 4.2.20: Maternal health indicators

Indicator	Present study Vadodara	NFHS3 Gujarat	NFHS2 Gujarat	NFHS1 Gujarat
Pregnant women with anemia	92%	61%	47%	NA
Three antenatal checkups	100%	65%	61%	61%
Institutional deliveries	94%	55%	46%	36%
Deliveries conducted by health personnel	94%	65%	53%	43%
Mothers received postnatal care within 2 days of delivery	97.3%	54%	NA	NA

For all four indicators except anemia better status was observed as compared to State data from NHFS 1, 2 and 3. In achieving these near to three digit figures for four maternal health indicators, NHE has played an important role. During their first visit to hospital (booking visit) we explained all components of MAMTA CARD to pregnant women.

Performance indicators for maternal health services

Coverage of antenatal services (Tetanus toxoid injection, completing three antenatal checkups, received IFA tablets) was more than 90 %.

Table 4.2.21: Performance indicators for maternal health services

Indicator	Present study	NFHS3	NFHS2	NFHS1
Coverage of antenatal services				
Tetanus toxoid injection (2 or more)	100%	80%	73%	63%
Completed 3 antenatal care visits(with abdominal examination and BP checkup)	97%	65%	60%	61%
Received IFA tablets	94%	82%	78%	69%
Place of delivery				
Institutional delivery	94%	55%	46%	36%
Domiciliary delivery	6%	45%	54%	64%
Institutional deliveries				
Government	45%	14%	11%	15%
NGO/trust	NA	2%	3%	NA
Private	55%	37%	32%	20%
Type of delivery				
Vaginal delivery	82.2%	91.1%	91.5%	97%
Caesarean section	17.8%	8.9%	8.5%	3%
Assistance during delivery				
Doctor	17.4%	52%	37%	29%
ANM/nurse/midwife/LHV	76.6%	11%	16%	14%
Dai	6%	37%	46%	47%

Percentage of institutional delivery was 94% and domiciliary delivery was 6%. Among institutional deliveries 45% were performed at government hospitals and 55% were at private hospitals. Table 4.2.21

gives us the comparison of performance indicators for maternal health services between present study and NFHS 1, 2 and 3. Of the above mentioned three performance indicators better results were observed in our study as compared to NFHS data.

Among type of deliveries, cases of caesarean section were more in our study compared to NFHS data (Table 4.2.21). When we compared our data with NFHS data on percentage of deliveries assisted by doctors we found that the percentage was less in our study. In our study 76.6% deliveries were assisted by nurses.

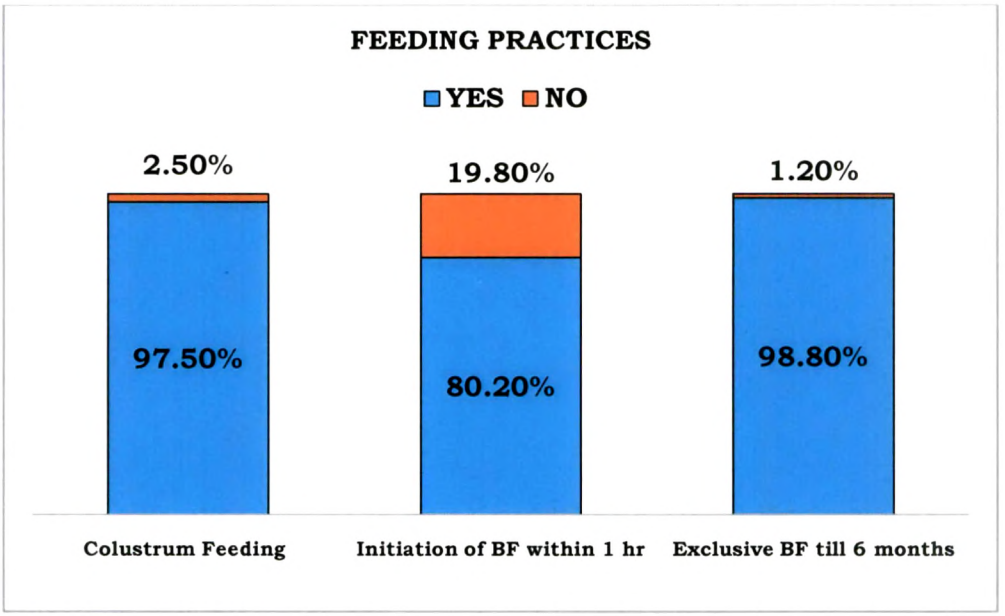
Child care indicators

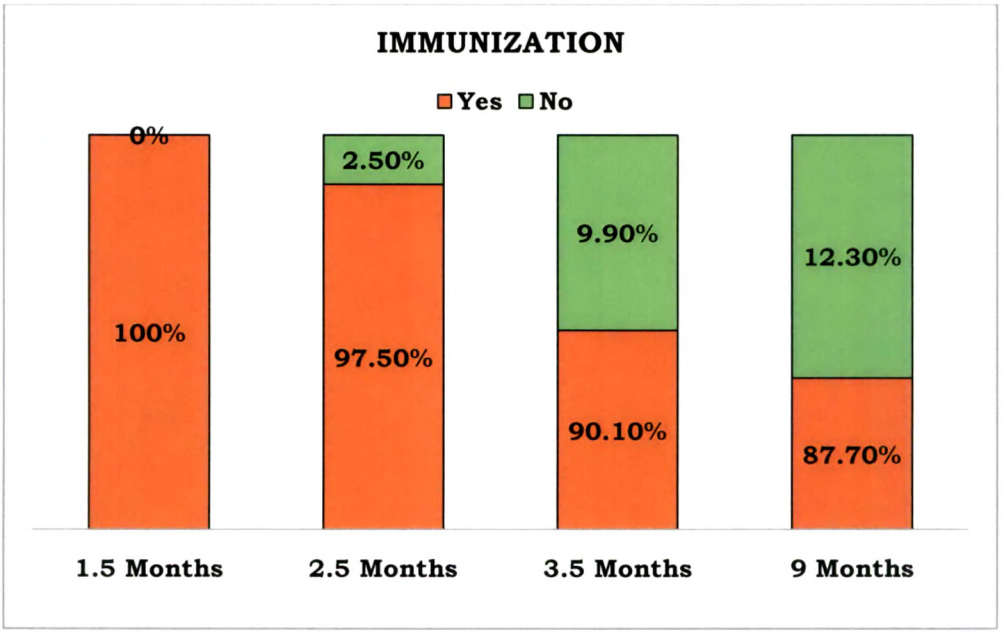
Colostrum feeding was done by 97.5% of mothers and the rest who did not feed the child responded that their relatives (elder females) had asked them not to give this yellow milk to child. Initiation of breast feeding within one hour was missed by 19.8% mothers (Figure 4.2.35). These mothers gave the reason that due to caesarean delivery they were not able to feed the child during first hour. Exclusive breast feeding till six months was done by 98.8% mothers. Respite giving counseling to these mothers, 1.2% mothers introduced water and biscuits to their child during fifth month.

Data on availing immunization services for child reveals that, during first immunization period (1.5 months) all mothers got their child fully immunized. As time progressed mothers became careless in availing immunization services for their child. During second (2.5 month), third (3.5 month) and fourth (9 month) period of immunization percentage of mothers who got their child immunized was 97.5%, 90.1% and 87.7% respectively. When asked the reason for delay in immunization, mothers reported that they often forget taking MAMTA CARD with them during immunization and hence the nurse refuses to

immunize the child. Few mothers (3 subjects) reported that their elder children have torn the MAMTA CARD so she was not able to produce that during immunization. These reasons reflect that few mothers were careless regarding immunization of their child.

Figure 4.2.35: Child care indicators





[1.5 months-BCG, Polio 1, DPT 1, Hepatitis B-1; 2.5 months- Polio 2, DPT 2, Hepatitis B-2; 3.5 months-Polio 3, DPT 3, Hepatitis B-3 and 9 months-Measles, Vitamin A]

RESULTS

Phase III

Screening of neonates

4.3.1 Characteristics of neonates

Out of the 73 pregnant women, 60 had normal delivery and remaining 13 had a cesarean section (Table 4.3.1). Among neonates percentage of females (58.9%) were more compared to males (41.1%). Mean gestational age at birth was found to be 35.57 (2.3) weeks. Mean birth weight, birth length and head circumference at birth were 2.81 (0.4) kg, 47.59 (2.6) cm and 32.82 (1.2) cm respectively (Table 4.3.2).

Table 4.3.1: Characteristics of neonates

Characteristics		Percentage
Type of delivery	Normal	82.2% (60)
	Cesarean	17.8% (13)
Gender of neonate	Female	58.9% (43)
	Male	41.1% (30)

Figure in parenthesis denote number of subjects

Table 4.3.2: Gestational age at birth and Anthropometric measurements of neonates

Variable	N	Mean (sd)
Gestational age at birth (weeks)	73	35.57 (2.3)
Birth weight (kg)	73	2.81 (0.4)
Birth length (cm)	39	47.59 (2.6)
Head circumference at birth (cm)	39	32.82 (1.2)

When we compared birth weight of males with that of females, a significant difference of 0.23 kg was observed. Male neonates had a mean weight of 2.9 kg while female neonates had 2.7 kg (Figure 4.3.1). Similarly when we compared birth weight of neonates which were born with normal delivery with those born with cesarean section, a difference of 0.2 kg was found (non significant). Mean birth weight was more in neonates born with cesarean section (2.9 kg) compared to neonates born with normal delivery (2.7 kg) (Figure 4.3.2).

Figure 4.3.1: Mean birth weight of male and female neonates

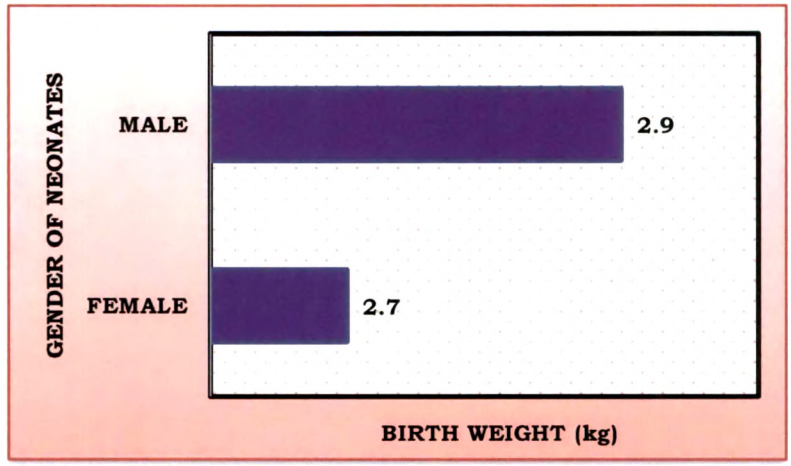


Figure 4.3.2: Mean birth weight of neonates born with normal delivery and with cesarean section

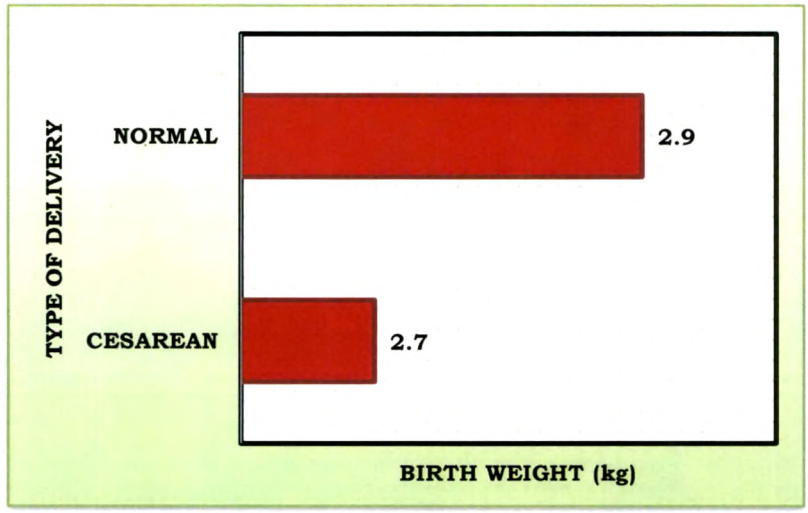


Table 4.3.3: Bio-chemical profile of mothers during pregnancy

Parameter	I trimester	II trimester	III trimester
Hb (g/dl)	9.18	9.31	9.0
TSH (μIU/ml)	2.41	2.33	2.66
FT4 (ng/dl)	0.88	0.77	0.92
TT4 (μg/dl)	7.6	11.0	12.0
TG (ng/ml)	1.6	4.2	11.3
UI* (μg/L)	301	247	275

*median value, (N=32)

Hemoglobin of mothers during pregnancy indicates iron deficiency anemia throughout gestation. Their thyroid hormone profile is given in Table 4.3.3. Median urinary iodine was found to be adequate in all three trimesters.

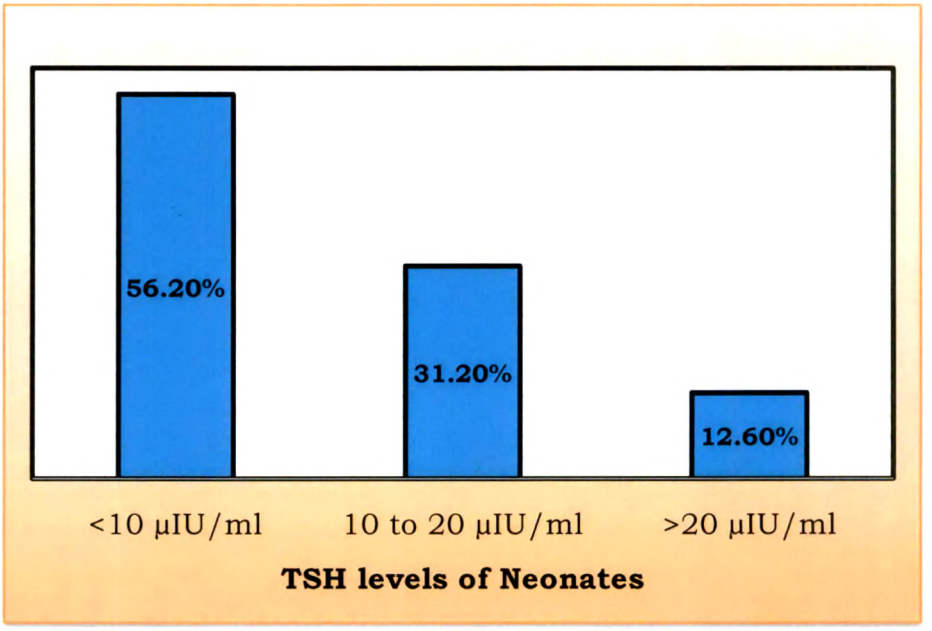
4.3.2 Thyroid profile of neonates

Mean cord blood TSH, FT4, TT4 and TG were 10.23 µIU/ml, 1.25 ng/dl, 9.36 µg/dl and 31.96 ng/ml respectively. Median and 95% CI for thyroid hormones is given in Table 4.3.4. Data on prevalence of raised TSH revealed that 43.8% neonates had CBTSH >10 µIU/ml, among these 31.2% had CBTSH between 10-20 µIU/ml and 12.6% had CBTSH >20 µIU/ml (Figure 4.3.3).

Table 4.3.4: Thyroid hormone level of neonates

Thyroid hormones	N	Mean (sd)	SEM	Median
CBTSH (µIU/ml)	39	10.23 (6.4)	1.13	8.97
CBFT4 (ng/dl)	39	1.25 (0.1)	0.02	1.24
CBTT4 (µg/dl)	39	9.36 (2.5)	0.44	9.45
CBTG (ng/ml)	39	31.96 (13.0)	2.30	33.20

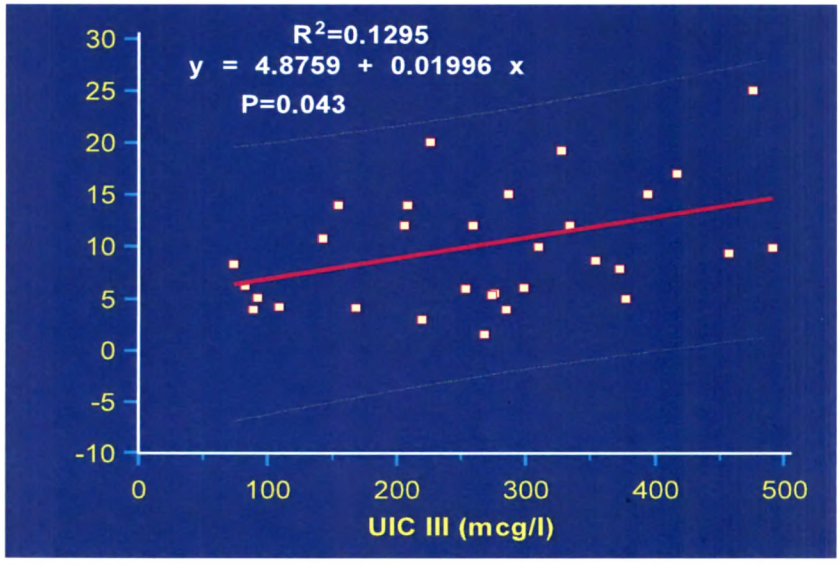
Figure 4.3.3: Prevalence of raised TSH among neonates



Correlation and regression analysis

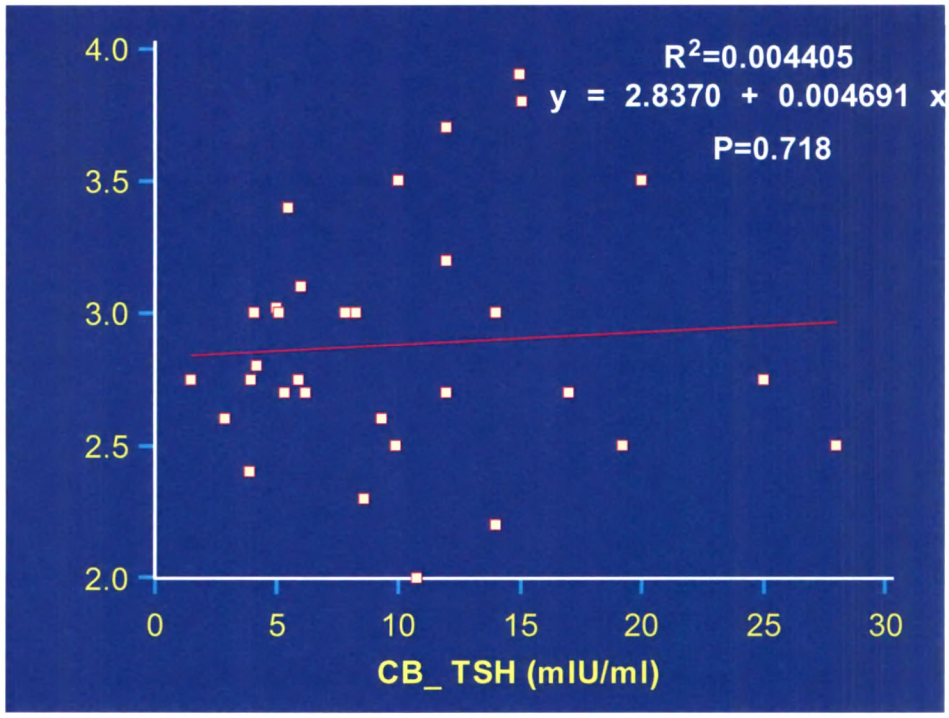
We did not find any significant correlation between CBTSH (r=0.066, p=0.718) and birth weight and between CBFT4 and birth weight (r=-0.091, p=0.619) [Figure 4.3.5]. However, a significant positive association was found between birth weight and birth length (r=0.666, p<0.001) and between birth weight and head circumference at birth (r=0.530, p=0.001) [Figure 4.3.6]. Further significant association was also observed between CBFT4 and FT4 during third trimester (positive, r=0.446, p=0.010) but not between CBTSH and third trimester logTSH (negative, r=-0.092, p=0.616) [Figure 4.3.7]. Additional to this, a significant relation was also found between CBTSH and UI during third trimester (rho=0.360, p=0.043) [Figure 4.3.4]. However, other authors Jaruratanasirikul et al (2009) and Chan et al (2003) did not find a significant association between maternal urinary iodine content and CBTSH. Our results on association between CBTSH and birth weight were echoed in studies of Shields et al (2011) and Jaruratanasirikul et al (2009). Unlike Shields et al (2011) we did not find positive association between CBFT4 and birth weight. Association between maternal FT4 and CBFT4 was also observed by Shield et al (2011).

Figure 4.3.4: Correlation between CBSTH and UI during third trimester

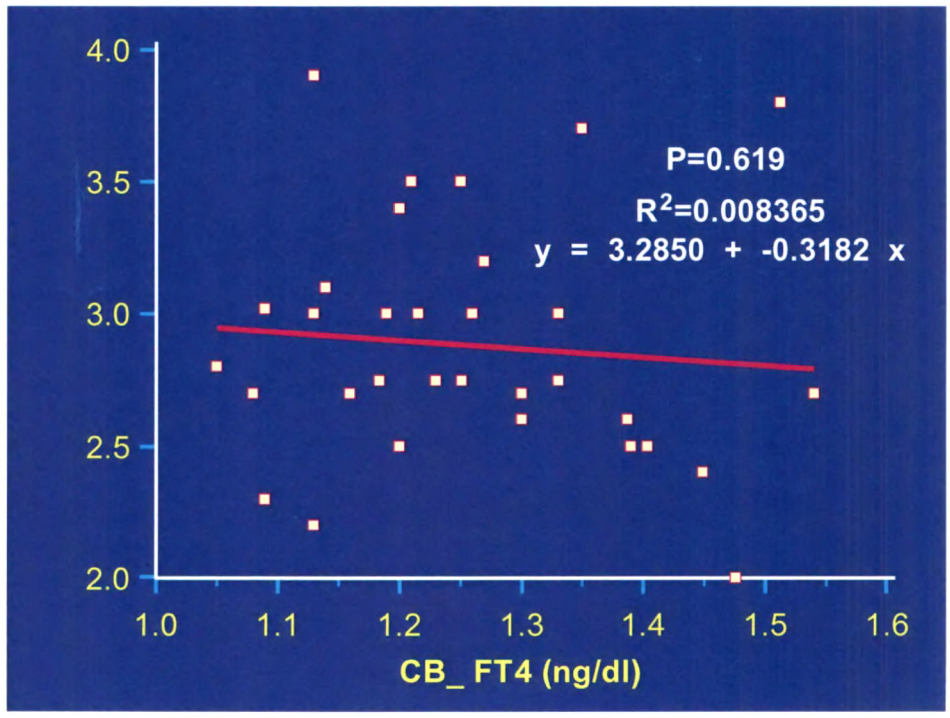


[Pink dotted line denotes 95% prediction interval]

Figure 4.3.5: Correlation between birth-weight and CBTSH and CBFT4 and birth-weight



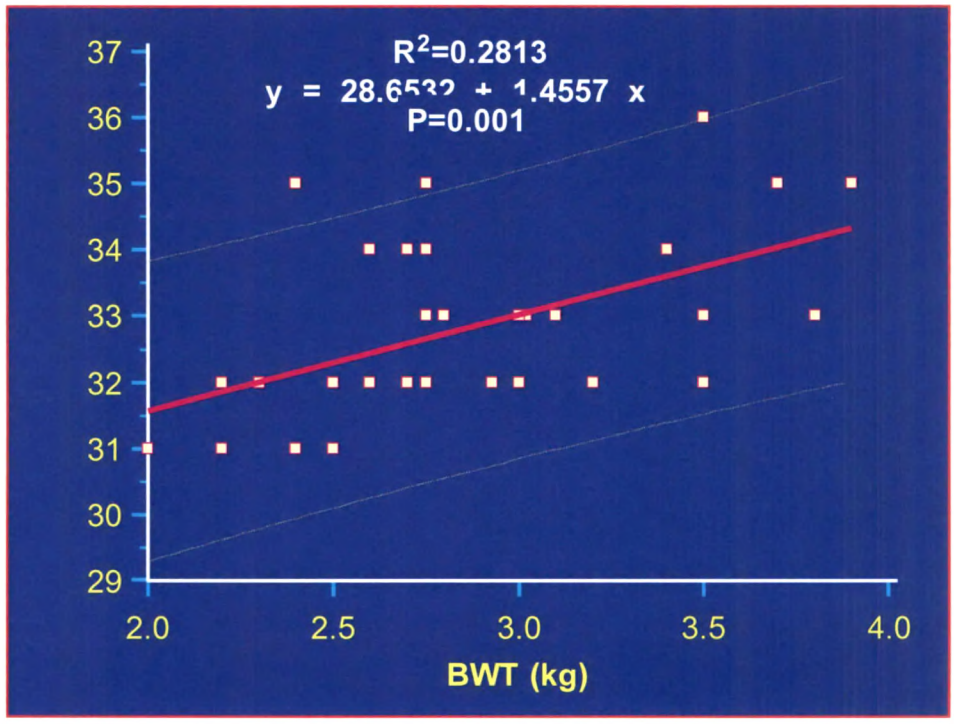
CBTSH and BWt



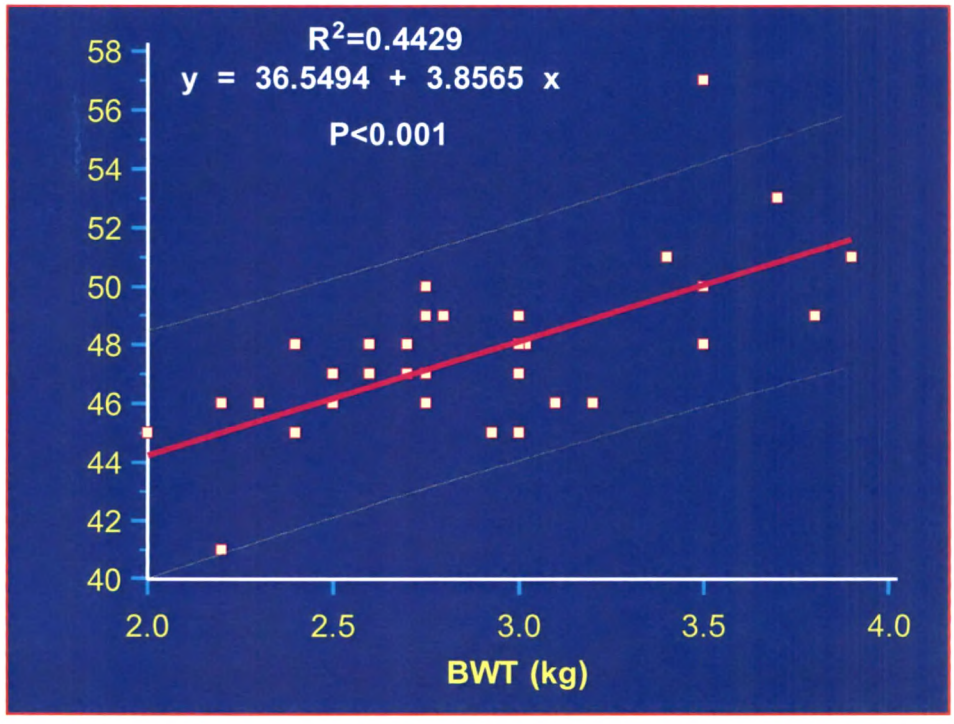
CBFT4 and BWt

Figure 4.3.6: Correlation between birth-weight and head circumference and length and birth-weight

[Pink dotted line denotes 95% prediction interval]



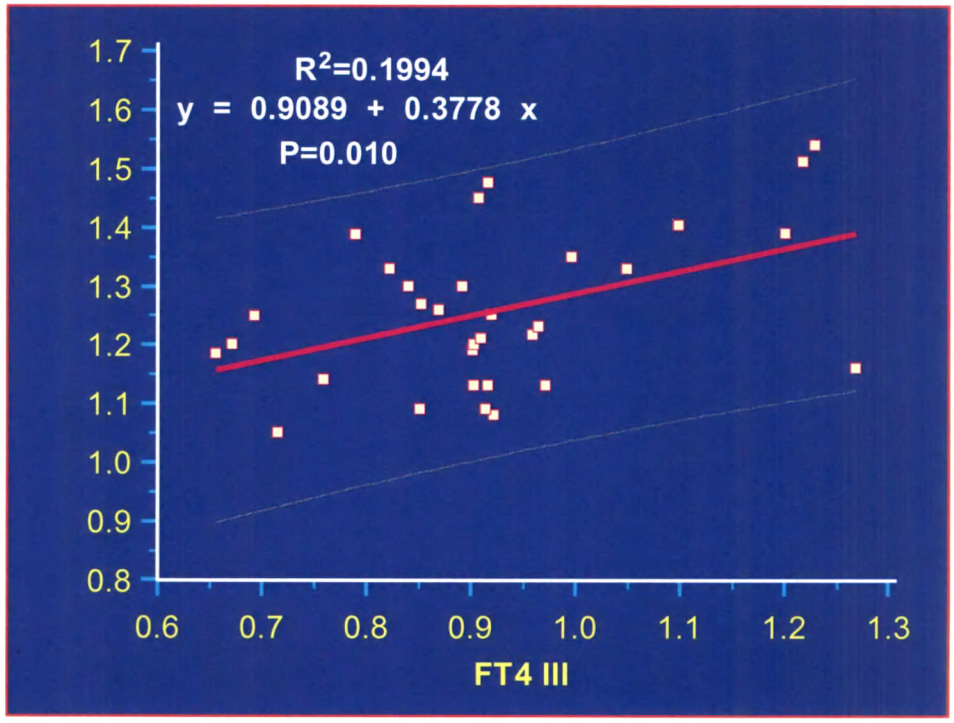
BWt and HC at birth



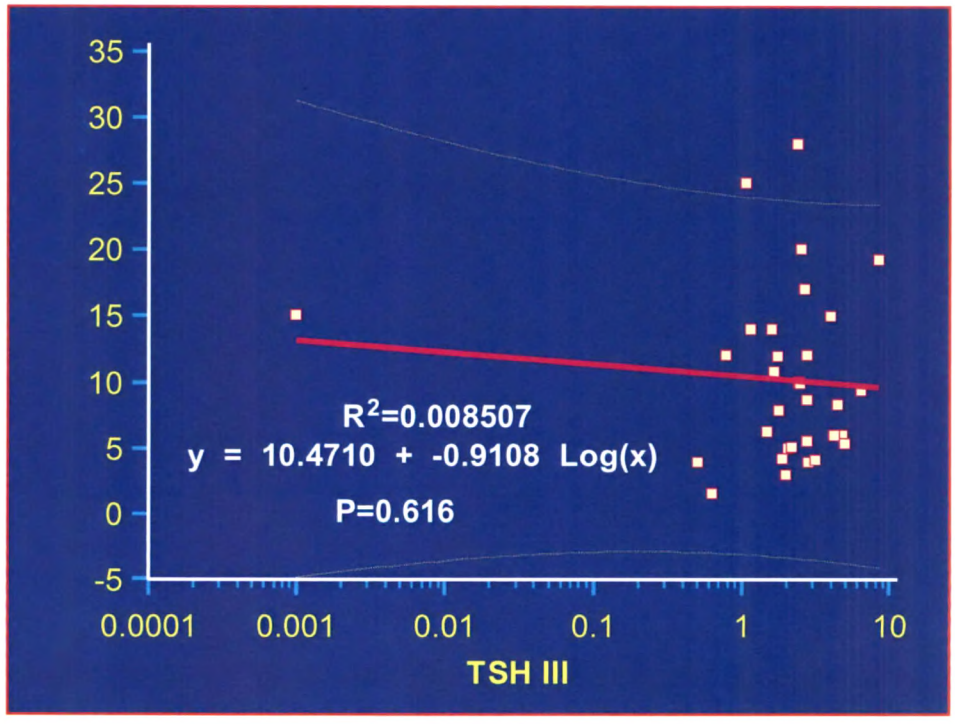
BWt and Length at birth

Figure 4.3.7: Correlation between CBFT4 and FT4 during third trimester and CBTSH and TSH during third trimester

[Pink dotted line denotes 95% prediction interval]



CBFT4 and FT4 during third trimester



CBTSH and TSH during third trimester

4.3.3 Newborn screening

After birth, the term baby experiences a surge of TSH as a physiological response to cold environment. The TSH concentration rises to 60-80 μIU/ml within 30 to 60 minutes after delivery and falls quickly in the first 24 hours to about 20 μIU/ml, followed by a slower decrease to below 10 μIU/ml after the first postnatal week. The rise in TSH initiates increase of T4 and free T4 to peak levels of 17 μg/dl and 3.5 ng/dl, respectively at 24 to 36 hours after birth with a slow decline to adult values over 4-5 weeks.

Congenital hypothyroidism (CH) is a major preventable cause of mental retardation. In most of the screening programs blood samples are collected at 5-6 days of age, but with large number of babies being discharged early, cord blood samples are being used as well (Wu et al, 1999; Ordookhani et al, 2003). In our country, it is very difficult to call back babies once discharged. Also, an effective social system whereby babies could be reached at home is practically non-existent. Thus cord blood remains a very practical alternative for screening purposes, and thus is the practice in some Asian countries (Wu et al, 1999;Ordookhani et al, 2003). Mixed cord blood samples for TSH values have compared well with filter paper samples taken in the first few days of life (Fuse et al, 1991;Walfish, 1976). The Indian Academy of Pediatrics recommends the use of cord blood samples for screening for CH.

Universal newborn screening for CH is currently being done in many parts of the world including Western Europe, North America, Japan, Australia, and parts of Eastern Europe, Asia, South America, and Central America. Three approaches are being used for screening:

1. Primary TSH, back upT4
2. Primary T4, back up TSH
3. Concomitant T4 and TSH

In the first approach, TSH is measured first. T4 is measured only if TSH is >20μIU/ml. This approach is likely to miss central hypothyroidism, thyroid binding globulin deficiency and hypothyroxinemia with delayed elevation of TSH. In the second approach, T4 is checked first and if low TSH is also checked. This is likely to miss milder/subclinical cases of CH in which T4 is initially normal with elevated TSH. Concomitant measurement of T4 and TSH is the most sensitive approach but incurs a higher cost. Screening programs use either percentile based cut-offs e.g., T4 below 10th percentile or TSH above 90th percentile or absolute cut-offs such as T4 <6.5 ug/dl and TSH >20 μIU/ml. In present study absolute cut-offs are used, we have used approach no. 3 (Concomitant T4 and TSH).

Three (9.2 %) neonates out of 32 were found to have low (<6.5 ug/dl) TT4, whereas 4 (20.6 %) neonates had TSH level >20 μIU/ml. Very few reports of cord blood values of TSH or T4 exist in Indian literature. Desai et al (1987) and Khadilkar et al (2002) had reported results on screening of neonates for congenital hypothyroidism. Desai et al screened 12,407 newborns for CH using cord blood TSH measurements, 2.8% babies were called for retesting and the incidence extrapolated was 1: 2481. In 1994, the same group screened 25,244 neonates at 24-94 hours and measured filter paper T4. The babies recalled were 18.91% and the extrapolated incidence was 1:2804. Khadilkar, et al found a mean cord TSH value of 12.3 μIU/ml, which is similar to our mean CBTSH value.

RESULTS

Phase IV

- Postpartum status of women
- Effect of DFS supplementation on maternal Iron and Iodine status
- Effect of early gestation thyroid dysfunction on infant development

4.4.1 Characteristic of women during postpartum

Mean weight of women was 47.5 (10.2) kg. Mean energy, protein and fat intake were 1,115.6 (274) kcal, 35.6 (11.8) g and 24.5 (9.6) g respectively. Mean TSH, FT4, TT4 and TG were 2.13 µIU/ml, 1.08 ng/dl, 9.6 µg/dl and 28.5 ng/ml respectively. Values of all thyroid hormones were falling under normal range. Median urinary iodine was found to be 218.8 µg/L and mean hemoglobin was 10.34 (1.2) g/dl (Table 4.4.1).

Table 4.4.1: Characteristic of women during postpartum (6 months)

Variable	Mean (sd)
Weight (kg)	47.5 (10.2)
Energy intake (kcal)	1115.6 (274)
Protein intake (g)	35.6 (11.8)
Fat intake (g)	24.5 (9.6)
TSH (µIU/ml)	2.13 (1.7-2.6)**
FT4 (ng/dl)	1.08 (0.27)
TT4 (µg/dl)	9.6 (2.3)
TG (ng/ml)	28.5 (12.1)
HB (g/dl)	10.34 (1.2)
UI (µg/L)	218.8 (189.7-273.6)*

** Geometric Mean (95% CI), * Median (95% CI)

Mean weight of women during postpartum period was similar to their early pregnancy weight (45.7 kg). According to RDA for lactating women (sedentary, Indian) calorie, protein and fat intake should be 2,500 kcal, 77.9 g and 30 g respectively. Data on dietary intake reveals that these women were not meeting the RDA for three major macro nutrients. Deficit in calorie, protein and fat intake was -1,384.4 kcal, -42.3 g and -5.5 g respectively; with 9.1% women having calorie intake between 1,500-2,500 kcal, 11.5% women having protein intake between 50-80 g and 26.1% women having fat intake >30 g.

4.4.2 Comparison of dietary intake of subjects during pregnancy and postpartum

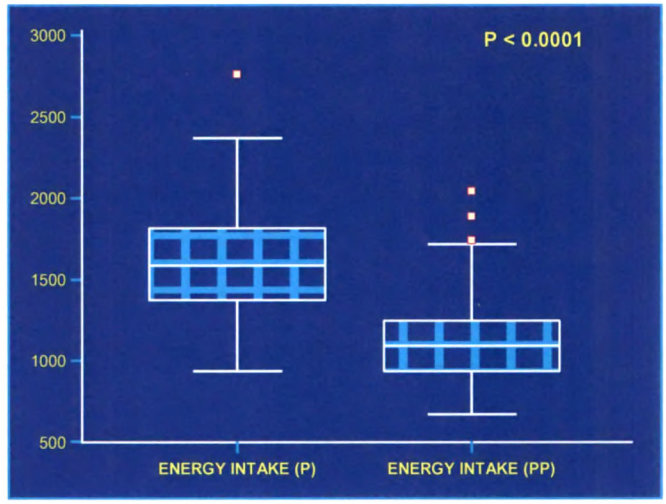
Mean energy, protein and fat intake of women reflected significantly low levels during postpartum period (Figure 4.4.1). The difference was 503.1 kcal in energy intake, 10.99 g in protein and 27.93 g in fat intake (Table 4.4.2).

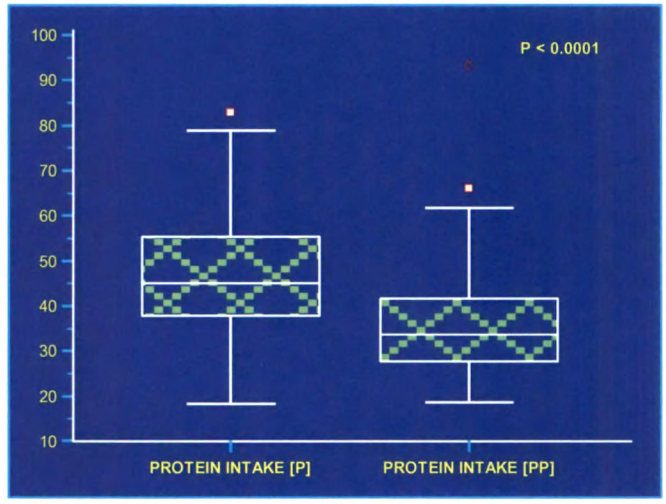
Table 4.4.2: Comparison of maternal dietary intake

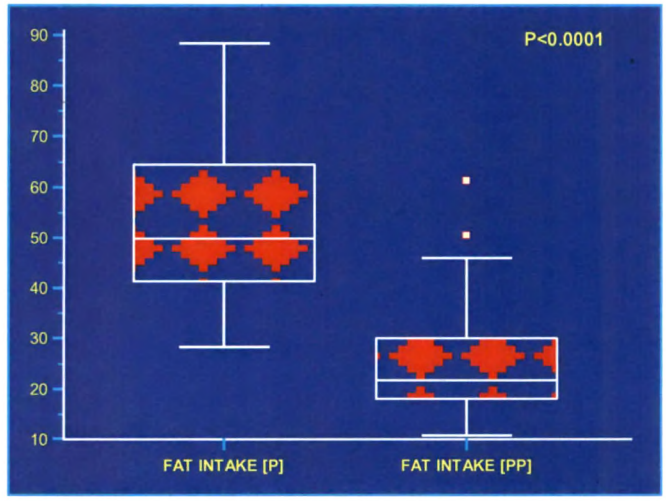
Parameter	Mean (sd) during pregnancy	Mean (sd) during postpartum	
Energy (kcal)	1,617(367)	1115.6 (274)	
Protein (g)	46.5(13.3)	35.6 (11.8)	
Fat (g)	52.3(14.7)	24.5 (9.6)	
Parameter	**Test statistic**	**P**	**Difference**
Energy (kcal)	9.718	<0.0001	503.1
Protein (g)	5.419	<0.001	10.99
Fat (g)	13.65	<0.001	27.93

Only 1.2 % women were meeting the requirements for protein and 25.9 % for fat, whereas none of the women was meeting the requirements for calories according to RDA during lactation. However during pregnancy 1.4 % women were meeting the requirements for energy and protein intake and 97.3 % for fat intake. Hence we can conclude that dietary intake during postpartum period was even poor than during pregnancy. Also it can be stated that dietary intakes of women from LIG are more or less similar during pregnancy and lactation and hence there in widespread maternal malnutrition. Composition of breast-milk depends to some extent on maternal nutrition. In general, even the undernourished mothers can successfully breast-feed. But in the case of severe malnutrition, both the quality and quantity of breast-milk may be affected. Trace element composition of breast-milk, however, is not affected by the mother's nutritional status. Fat content of breast milk is much affected in malnutrition as compared to protein.

Figure 4.4.1: Comparison of energy, protein and fat intake of women during pregnancy and postpartum







4.4.3 Micronutrient deficiency [iodine and iron] during pregnancy and postpartum period

Median UI indicated adequate iodine intake. After categorizing women according to WHO/UNICEF/ICCIDD classification for UI, 18.5% of women were found to be iodine deficient (table 4.31). Mean hemoglobin indicated moderate IDA among these women and 96.3 % of women were anemic. Prevalence of severe, moderate and mild IDA was 1.2%, 29.6% and 65.4% respectively (Table 4.4.3).

Table 4.4.3: Prevalence of IDA and ID

Iron status	**Cut-off (g/dl)**	**Percentage**
Severe	<7	1.2 (1)
Moderate	7-9.9	29.6 (24)
Mild	10-11.9	65.4 (53)
Normal	≥12	3.7 (3)
Iodine status	**Cut-off (µg/L)**	**Percentage**
Inadequate	<100	18.5% (15)
Adequate	>100	81.5% (66)

Figure in parenthesis denote actual number

On comparing prevalence of IDA during pregnancy and lactation, maximum prevalence was found during first trimester and minimum during third trimester (Figure 4.4.2). This highest prevalence during first trimester could be due to low maternal hemoglobin stores and lowest prevalence during third trimester could be due to improvement in hemoglobin stores due to IFA consumption. Similarly on comparing prevalence of ID during pregnancy and lactation, maximum prevalence was found during second trimester and minimum during postpartum period (Figure 4.4.3).

Figure 4.4.2: Prevalence of IDA during pregnancy and lactation

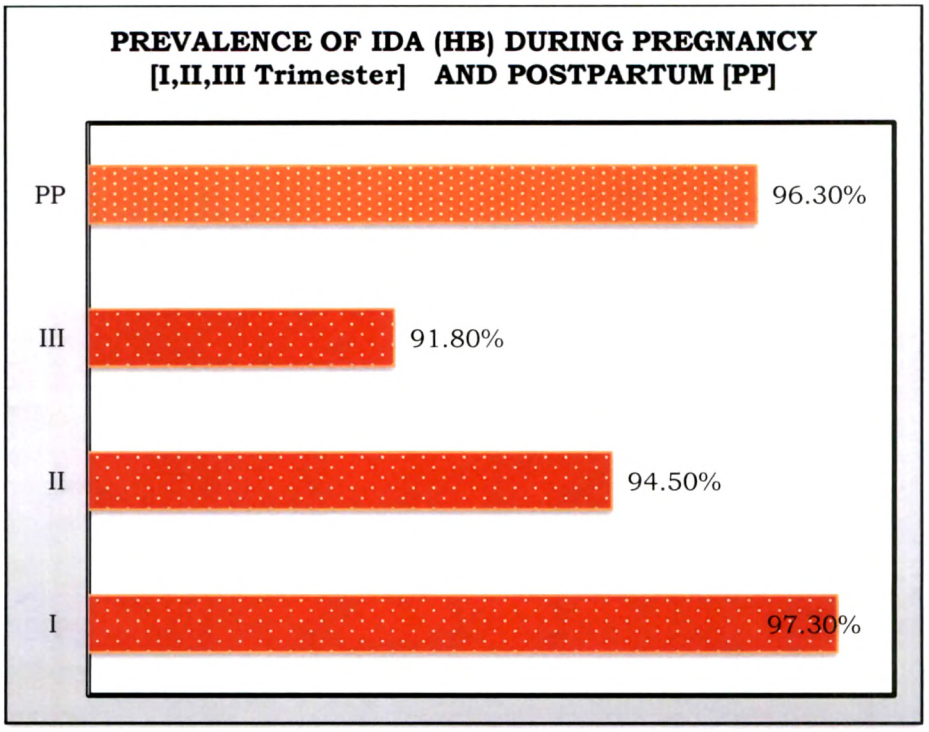
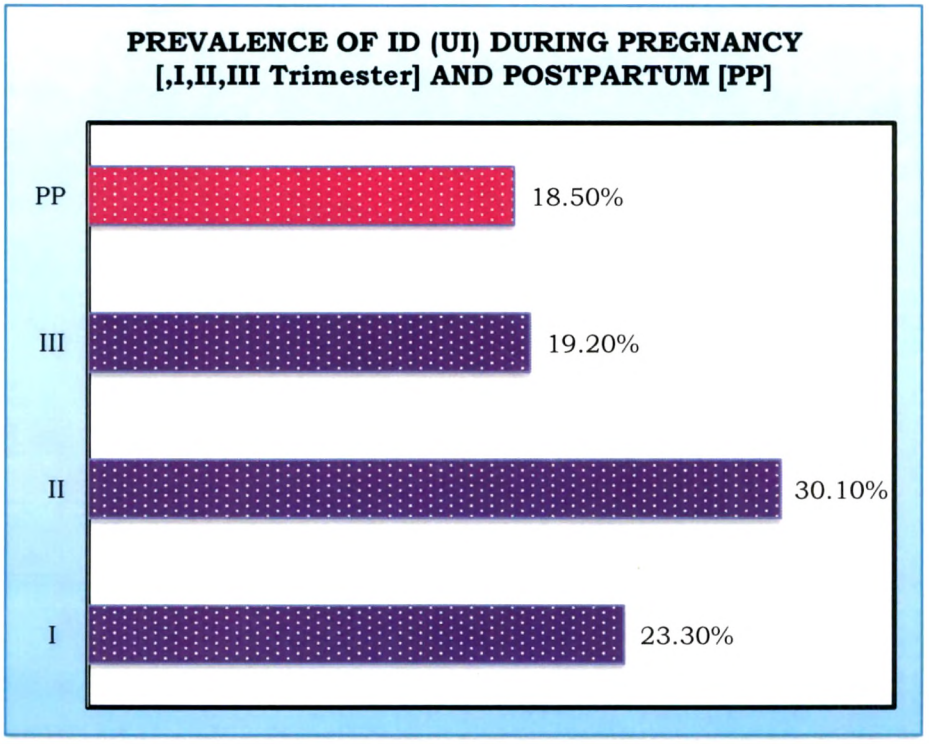


Figure 4.4.3: Prevalence of ID during pregnancy and lactation



4.4.4 Prevalence of thyroid dysfunction during postpartum period

During postpartum period only 9.9% women were found to have thyroid dysfunction. After applying normal (non pregnant) ranges for TSH and FT4, we found 2.5% women having overt hypothyroidism and 7.4% with subclinical hypothyroidism. Not a single woman was found to be hypothyroxinemic during postpartum period (Table 4.4.4).

Table 4.4.4: Comparison of thyroid dysfunction

Thyroid function	Pregnancy [n=73]			Lactation [n=81]
	I Trimester	II Trimester	III Trimester	Postpartum 6 months
Overt hypothyroidism	10.96(8)	5.48(4)	1.37 (1)	2.5 (2)
Subclinical hypothyroidism	17.81(13)	13.7(10)	24.66(18)	7.4 (6)
Hyothyroxinemia	4.11(3)	24.66(18)	5.48(4)	-
Normal	67.12(49)	56.16(41)	68.49(50)	90.1 (73)

Figure in parenthesis denote number

When we compared thyroid function of women during pregnancy and lactation (postpartum) we found that most (90%) of the women became normal after pregnancy (Table 4.4.4). Thus we can state that due to pregnancy there were fluctuations observed in thyroid hormones especially TSH and FT4. A euthyroid state of mother during early pregnancy is very important for proper development and differentiation of the fetal brain. However, most of the women were not able to maintain euthyroid state during pregnancy (Table 4.4.4).

4.4.5 Comparison of diagnostic test

We have evaluated thyroid hormones of our subjects during early gestation (first trimester) using two different upper limits for TSH (μIU/ml), 1) upper TSH cut off as 2.5 and 2) upper TSH cut off as 5.0. After comparing these two diagnostic methods with normal (non

pregnant) thyroid TSH values during postpartum period for same women, we found that the upper TSH cut off as 2.5 was better indicator of thyroid status. Table 4.4.5 shows the comparison of these two diagnostic methods.

Table 4.4.5: Comparison of 2 diagnostic methods for hypothyroidism

	Upper TSH limit as 2.5 μIU/ml	Upper TSH limit as 5 μIU/ml
Sensitivity	73.33%	33.33%
Specificity	55.30%	48.67%
Disease prevalence	18.52%	7.41%
Positive predictive value	27.16%	4.94%
Negative predictive value	90.12%	90.12%

From the above discussion, the need to assess thyroid function during pregnancy is justified. Also proper diagnosis of thyroid dysfunction during pregnancy is important to avoid both fetal and maternal complications. As discussed earlier thyroid activity undergoes many changes during normal pregnancy, including 1) a significant increase in serum TBG, thyroglobulin, TT4, 2) an increase in renal iodine clearance 3) stimulation of the thyroid by hCG. Taken together, these changes can make diagnosis of thyroid dysfunction during pregnancy difficult.

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

Mean FT4, TG and HB during postpartum period were significantly higher than during first, second and third trimester (Table 4.4.6). Difference in FT4 during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 0.23 ng/dl, 0.33 ng/dl, 0.17 ng/dl respectively (Figure 4.4.7).

Difference in Hb during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 1.23 g/dl, 1.11 g/dl, 0.88 g/dl respectively (Figure 4.4.4). Median UI during postpartum period was significantly lower than during first, second and third trimester value. Difference in UI during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was -51.3 µg/L (non significant), -73.9 µg/L, -65.9 µg/L respectively (Figure 4.4.5). However, no significant difference in mean TSH was observed during postpartum period and pregnancy (table 4.34). Difference in TSH during first trimester and postpartum, second trimester and postpartum and third trimester and postpartum was 0.5 µIU/ml, 0.31 µIU/ml, -0.07 µIU/ml respectively (Figure 4.4.6).

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

Parameter	Test statistic (ANOVA)	P	Difference
Hb (g/dl)	F=23.2	<0.001	I,II, III trimester, P<0.05
TSH (µIU/ml)	F=2.25	0.082	NS difference
FT4 (ng/dl)	F=34.9	<0.001	I,II, III trimester, P<0.05
TT4 (µg/dl)	F=104.733	< 0.001	I,II, III trimester, P<0.05
TG (ng/ml)	F=27.195	< 0.001	I,II, III trimester, P<0.05
UI (µg/L)	8.5	0.03	II, III trimester, P<0.05

Mean TT4 was significantly low during postpartum then during pregnancy (Table 4.4.6 and Figure 4.4.8). Mean FT4, TG and Hb were highest during postpartum period (Figure 4.4.4, 4.4.7 and 4.4.9). Median UI (Figure 4.4.5) and mean TT4 (Figure 4.4.8) were low during postpartum period and mean TSH (Figure 4.4.6) was highest during third trimester.

Figure 4.4.4 Mean HB during pregnancy and postpartum

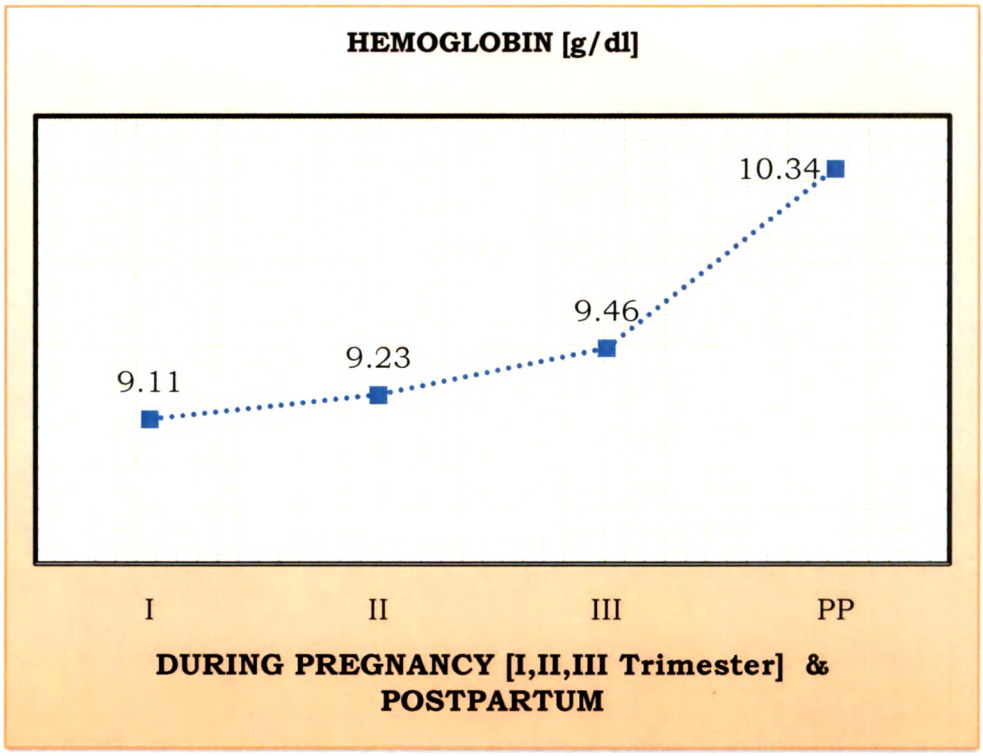


Figure 4.4.5: Median UI during pregnancy and postpartum

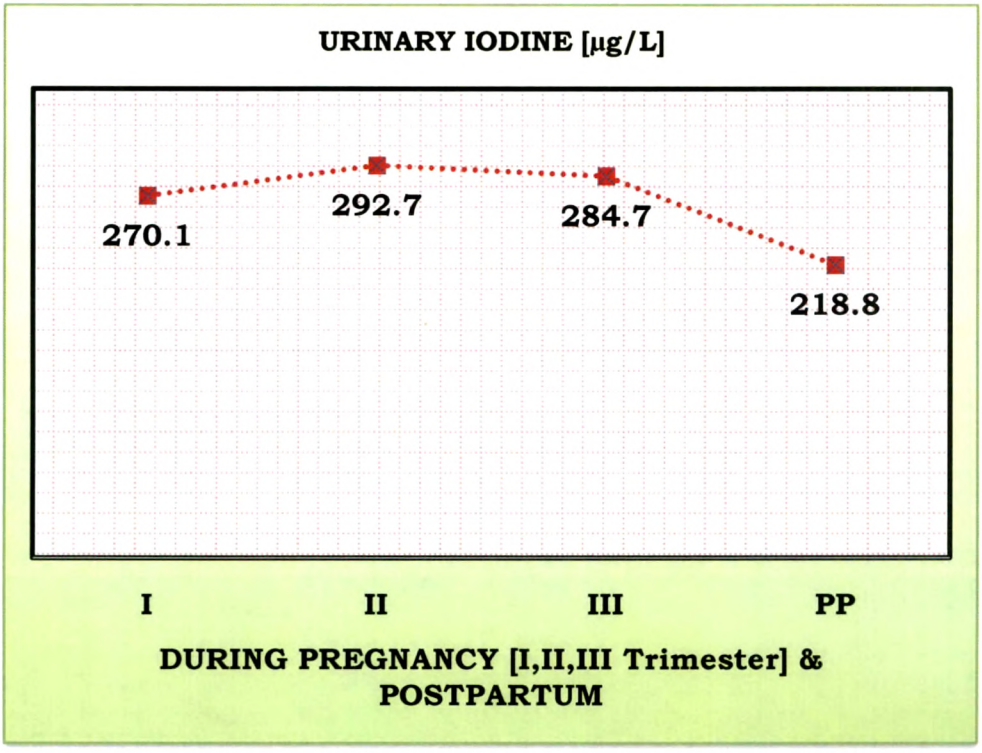


Figure 4.4.6: Mean TSH during pregnancy and postpartum

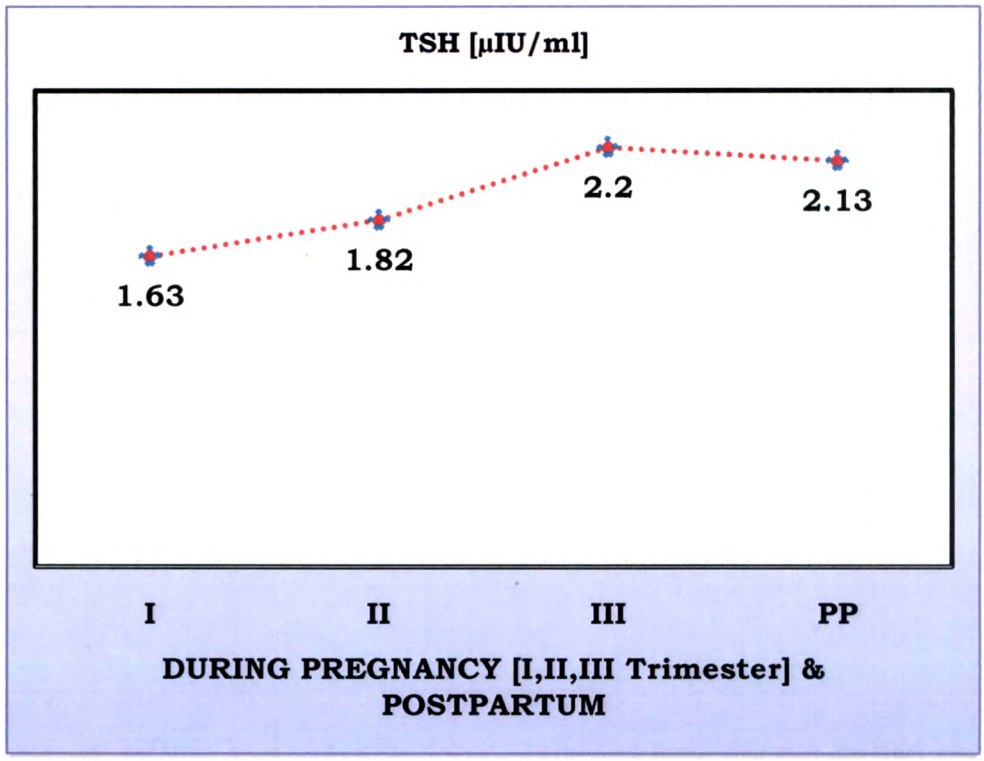


Figure 4.4.7: Mean FT4 during pregnancy and postpartum

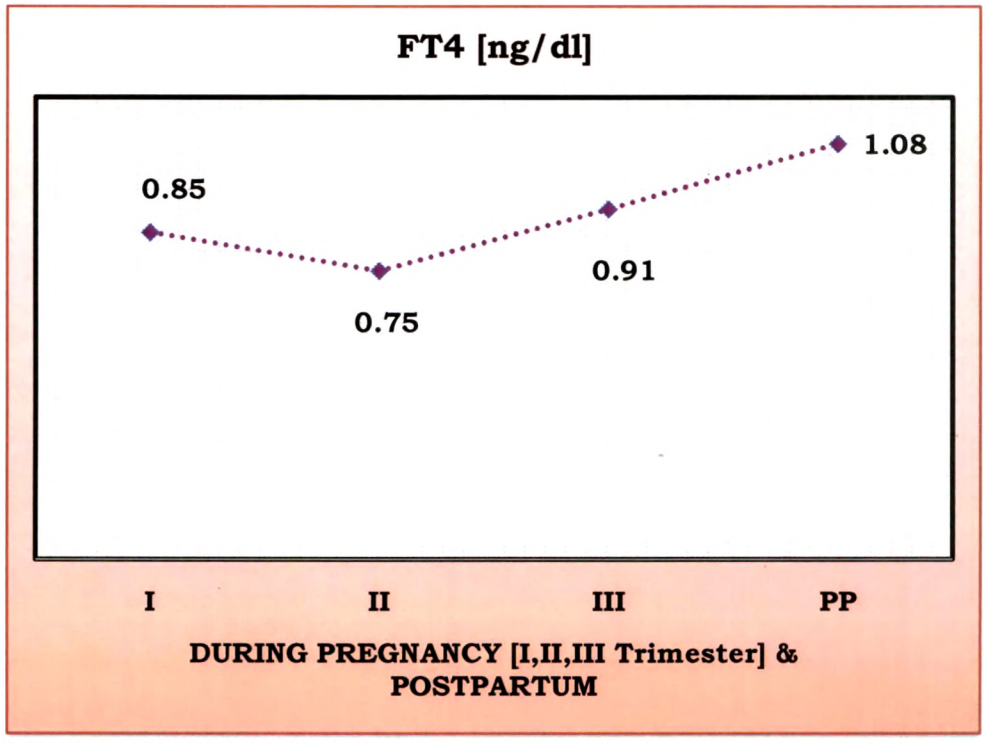


Figure 4.4.8: Mean TT4 during pregnancy and postpartum

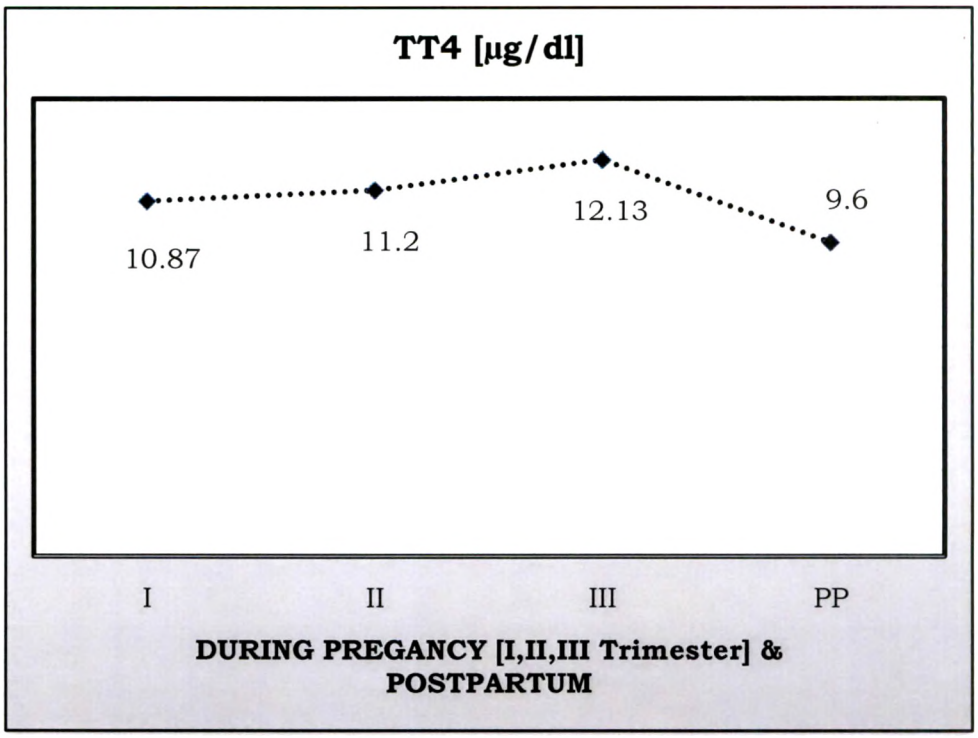
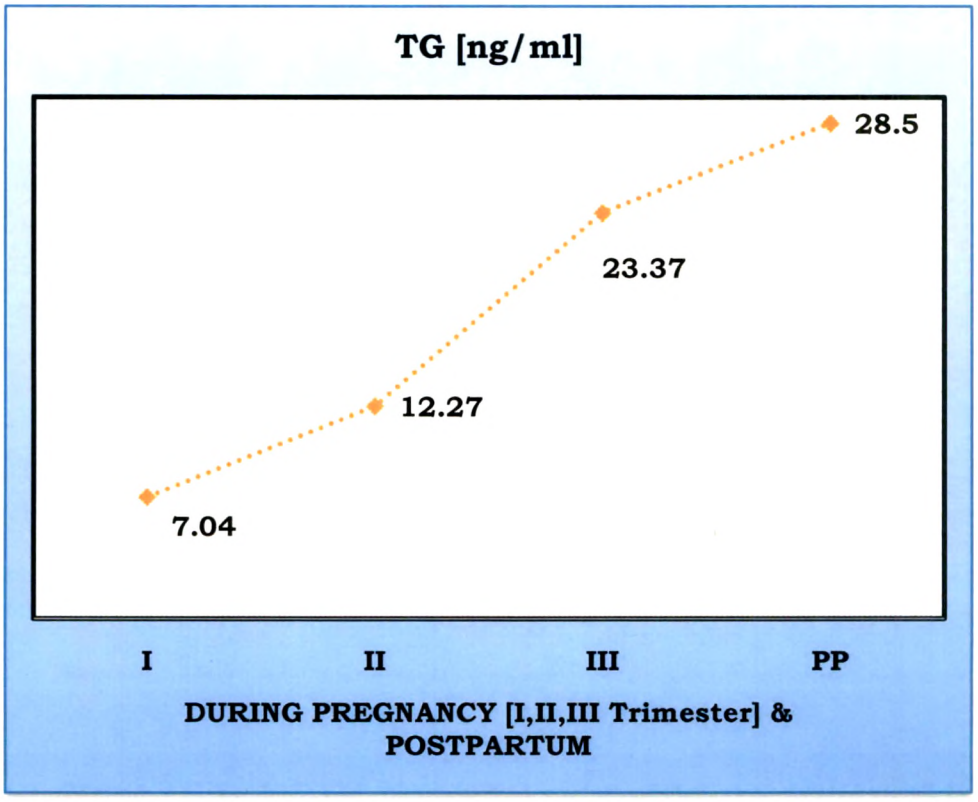


Figure 4.4.9: Mean TG during pregnancy and postpartum



Similar to our results, studies from China (Wang et al, 2009), Sweden (NHNES, 2007) and Hungary (Toldy et al, 2004) had also reported a fall in TSH during postpartum period. A reduction in TT4 was also found by Wang et al (2009), NHNES (2007), Toldy et al (2004) and Kung et al (2000). An increase in FT4 was also reported by NHNES (2007), Dhatt et al (2006), Kurioka et al (2005), Toldy et al (2004), Panesar et al (2001), Kung et al (2000) and Eltom et al (2000). Eltom et al (2000) had also reported an increase in TG from pregnancy to postpartum period. A fall in urinary iodine during lactation (postpartum period) was also mentioned by Eltom et al (2000), Kung et al (2000) and Yeo et al (2001).

The above discussed results confirm the reversibility of pregnancy induced changes in the iodine status and thyroid function of subjects. Glinoer el at (1997) found that the restoration of thyroid function to the pre-pregnancy state occurred at about six months after delivery, with exception of TG, which persisted in some of the cases till 1 year post-natally.

The organ most vulnerable to iodine and thyroid hormone is the central nervous system. Iodine is also necessary during the first few months of life for neurological development and myelination in order to achieve optimum intellectual development. Elevated levels of TSH and reduced level of FT4 and UI indicate deterioration of maternal iodine status. This deterioration during the post natal period may be due to breastfeeding, which may increase the demand for extra iodine intake (Delange et al, 1988). Since we have not found any deterioration in iodine status of women during post natal period we can assume that these women would have met the extra requirements of iodine during lactation and hence they delivered adequate iodine to their infants via breast milk. In case when lactating women could not meet the extra requirements, they are expected to lose some of their iodine in breast milk, this may lead to a reduced maternal iodine pool and consequently reduced thyroid hormone production.

4.4.7 Characteristics of Infants

Mean birth weight of infants was 2.8 (0.4) kg. Median urinary iodine value was 370.6 µg/l. Mean weight, length and head circumference during six months was 6.6 (0.8) kg, 66 (2.6) cm and 41.6 (1.3) cm respectively. During twelve months weight, length and head circumference increased to 7.4 (0.7) kg, 70.9 (2.8) cm and 44.2 (1.3) cm respectively. Mean BDSTI at six and twelve months were found to be 17.8 (0.5) and 34.7 (0.6) (Table 4.4.7).

Table 4.4.7: Characteristic of infants

Variable	Mean (sd)
Birth Weight (kg)	2.8 (0.4)
UI (µg/l)	370.6 (251.9-438.7)*
Weight at 6 M (kg)	6.6 (0.8)
Weight at 12 M (kg)	7.4 (0.7)
Length at 6 M (cm)	66.0 (2.6)
Length at 12 M (cm)	70.9 (2.8)
Head circumference at 6 M (cm)	41.6 (1.3)
Head circumference at 12 M (cm)	44.2 (1.3)
BDSTI at 6 M	17.8 (0.5)
BDSTI at 12M	34.7 (0.6)

*median (95% CI), M-months

Despite a low weight gain by the mother during pregnancy, mean birth weight was >2.5 kg. Median UI indicated optimal iodine status among infants. None of the infant had UI <100 µg/L. BDSTI at six month and twelve month were less than normal. Nutritional status of infants at six months and twelve months of age is given in Table 4.4.8.

Table 4.4.8: Nutritional status of infants (at six and twelve months)

Nutritional status of infants at 6 months based on z- scores							
Weight-for-age				Height-for-age			
<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD
4 (9.4%)	13 (16%)	64 (79%)	-	1 (1.2%)	9 (11.1%)	70 (86.4%)	1 (1.2%)
Weight-for height				Head circumference-for-age			
<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD
5 (6.2%)	13 (16%)	62 (76.5%)	1 (1.2%)	1 (1.2%)	7 (8.6%)	72 (88.9%)	1 (1.2%)
Nutritional status of infants at 12 months based on z- scores							
Weight-for-age				Height-for-age			
<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD
2 (2.5%)	25 (30.9%)	52 (64.2%)	2 (2.5%)	7 (8.6%)	23 (28.4%)	51 (63%)	-
Weight-for height				Head circumference-for-age			
<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD	<-3SD	>-3SD to <-2SD	-2SD to +2SD	≥2SD
2 (2.5%)	11 (13.6%)	64 (695)	4 (4.9%)	1 (1.2%)	11 (13.6%)	68 (84%)	1 (1.2%)

Figure in parenthesis denote percentage

4.4.8 Nutritional status of infants

Weight for age (under nutrition)

Percentage of severely undernourished (<-3SD) infants was 9.4 % at six months which reduced to 2.5% at twelve months. Percentage of moderately undernourished (>-3SD to <-2SD) infants was increased from 16 % to 30.9% from six months to twelve months. Hence, we can conclude that there was shift from severe to moderate category from six to twelve months. None of the infant was falling in overweight category at six months, however a few (2.5%) infants were found to be overweight (>2SD) at twelve months.

Weight for height (stunting)

Cases of stunting (severe and moderate) increased from six months to twelve months. Percentage of infants who were severely stunted increased from 1.2 % to 8.6% and of moderately stunted increased from 11.1% to 28.4%. At six months a few (1.2%) infants were having length as above average (>2SD). However at twelve months none of the infant had length above average.

Weight for height (wasting)

Percentage of infants who were wasted was reduced. Severe wasting reduced from 6.2% at six months to 2.5% at twelve months and moderate wasting reduced from 16% at six months to 13.6% at twelve months. Percentage of infants who were above average increased from 1.2 % at six months to 4.9% at twelve months.

Head circumference for age

Percentage of infants who were <-3SD and >+2SD remained same at six months and at twelve months. Percentage of infants who were <-2SD were increased from 8.6% at six months to 13.6% at twelve months.

Overall nutritional status of infants with respect to under nutrition and wasting was improved. However, recovery in case of stunting and head circumference for age was not observed (Table 4.4.8). According to recent WHO/UNICEF statements for SAM, these infants are more prone to be affected by various morbidities and hence later mortality. Appropriate interventions at this time will cure these infants.

4.4.9 DFS supplementation to combat anemia among lactating women

There was no significant difference in energy, protein, fat and dietary iron intake in both experimental and control groups (Table 4.4.9). There was no significant difference in median UI of infants born to mothers who were given DFS and who received IS.

Table 4.4.9: Comparison of maternal and infant characteristics in 2 groups

Variable	Mean (sd)		P value
	Experimental group[DFS]N=48	Control group[IS]N=33	
Infant Characteristics			
UI	*385.7	*319.5	P = 0.513
Maternal Characteristics			
Energy intake (kcal)	1115.1 (268.5)	1116.5 (286.0)	P = 0.982
Protein intake (g)	36.2 (10.7)	34.7 (13.2)	P = 0.58
Fat intake (g)	24.0 (9.2)	25.1 (10.4)	P = 0.611
Dietary iron (mg)	8.5 (4.1)	8.5 (3.5)	P = 0.994
UI at postpartum 6 M	*198.5	*252.8	-
UI at postpartum 12 M	*281.6	*265.5	-
Hb at postpartum 6 M	10.44 (1.2)	10.19 (0.9)	-
Hb at postpartum 12 M	10.66 (1.3)	10.02 (0.9)	-

*median; M-months; UI-(µg/L); Hb-g/dl, RDA for iron=21mg; RDA for iodine=250µg; normal value for UI >100 µg/L

DFS supplementation

Double Fortified Salt (DFS) is an edible salt fortified with iodine and encapsulated iron. At 10 g/day of daily consumption of salt, a person can receive 10 mg of iron per day – that's around one third of their daily requirement of iron. Today, DFS has emerged as an exciting

intervention that works complementarily with other approaches to tackle iron and iodine deficiencies, which affect half the world's population. The technology is available in India and is transferable. Thus it is suggestive that supplementation of DFS is a powerful new solution which India and other nations can use to address anemia especially in developing countries.

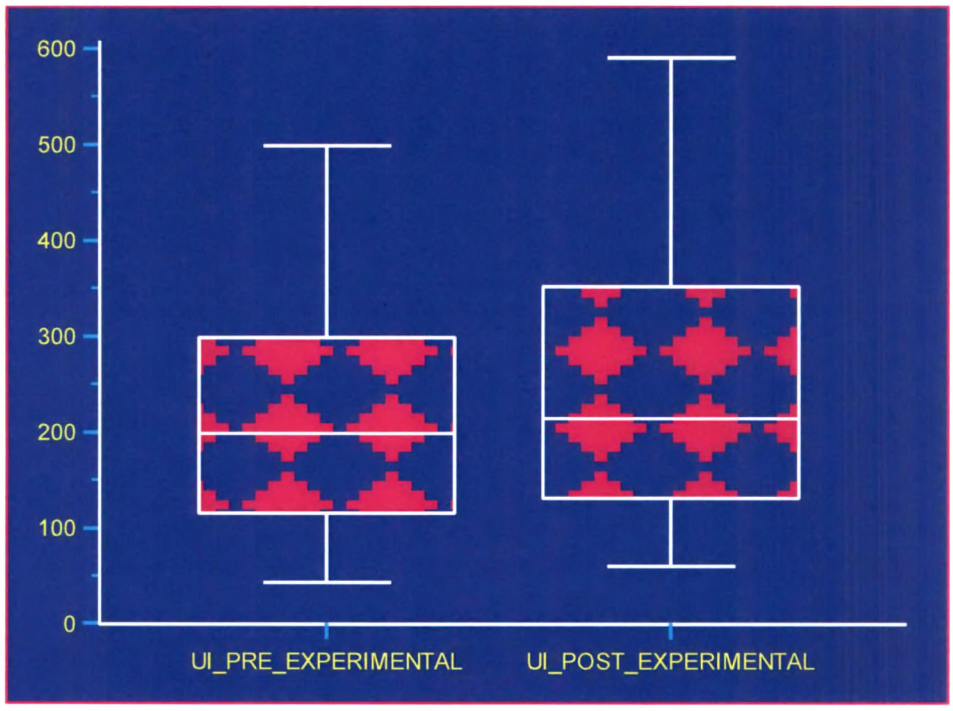
After a six months supplementation of DFS during lactation period, there was a significant increase (Figure 4.4.11) in hemoglobin of 0.22 g/dL [($p<0.05$), 10.66±1.3 (final)- 10.44±1.2 (initial)] in experimental group and in control group, there was a significant decrease in hemoglobin of 0.17 g/dL [($p<0.05$), 10.02±0.9 (final)- 10.19±0.9 (initial)]. Median urinary iodine increased (Figure 4.4.10) by 78 mcg/L [($p<0.05$), 274 (final) - 199 (initial) in experimental group and in control group it decreased by 16 mcg/L [($p=0.964$), 265 (final) - 281 (initial)]. Mean energy, protein fat and iron in experimental group was 1,115 kcal (±268), 36.2 g (±10.7), 24 g (±9.2) and 8.5 mg (±4.1) respectively. In control group mean energy, protein fat and iron was 1,116 kcal (±286), 34.7 g (±13.2), 25.1 g (±10.4) and 8.5 mg (±3.5) respectively (Table 4.4.9). Prevalence of IDA and ID before and after supplementation during lactation is recorded in Table 4.4.10.

Table 4.4.10: Prevalence of IDA and ID before and after 6 months supplementation of DFS

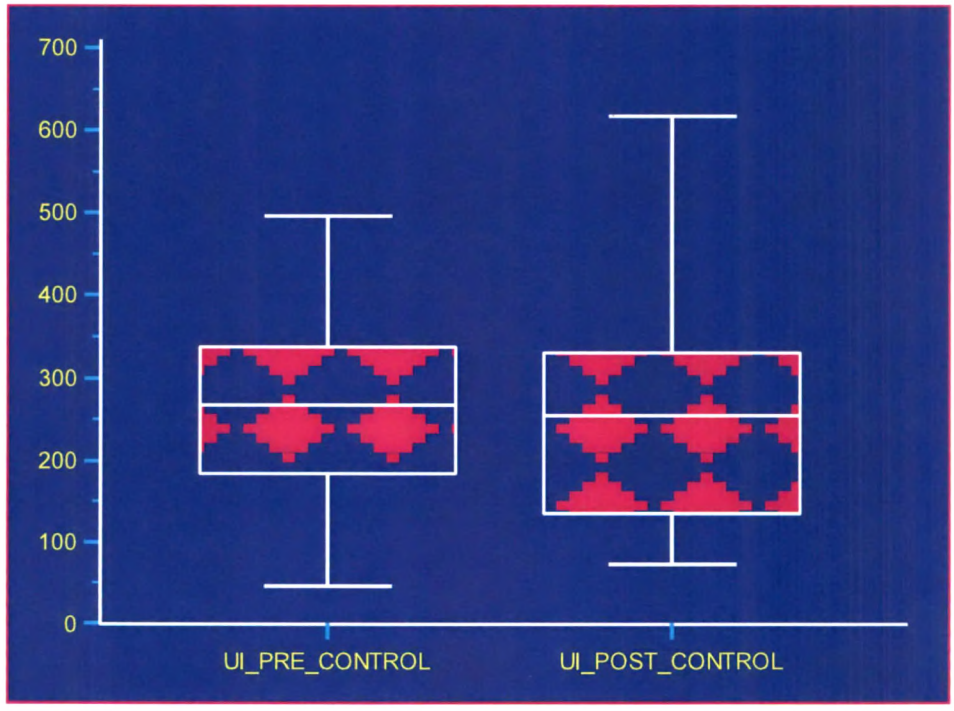
Indicator	Category	Experimental group (n=48)		Control group (n=33)	
		Pre	Post	Pre	Post
Hb (g/dl)	<7	2.1% (1)	2.1 % (1)	-	-
	7-9.9	20.8 % (10)	22.9 % (11)	42.4 % (14)	51.5 % (17)
	10-11.99	72.9 % (35)	54.2 % (26)	54.5 % (18)	48.5 % (16)
	>12	4.2 % (2)	20.8 % (10)	3.0 % (1)	-
UI (µg/L)	<100	20.8 % (10)	6.3 % (3)	15.2 % (5)	6.1 % (2)
	>100	79.2% (28)	93.7 % (45)	84.8 % (28)	93.9 % (31)

Figure in parentheses denote actual number

Figure 4.4.10: Median urinary iodine (UI) before (Pre) and after (Post) DFS supplementation in experimental group and control group

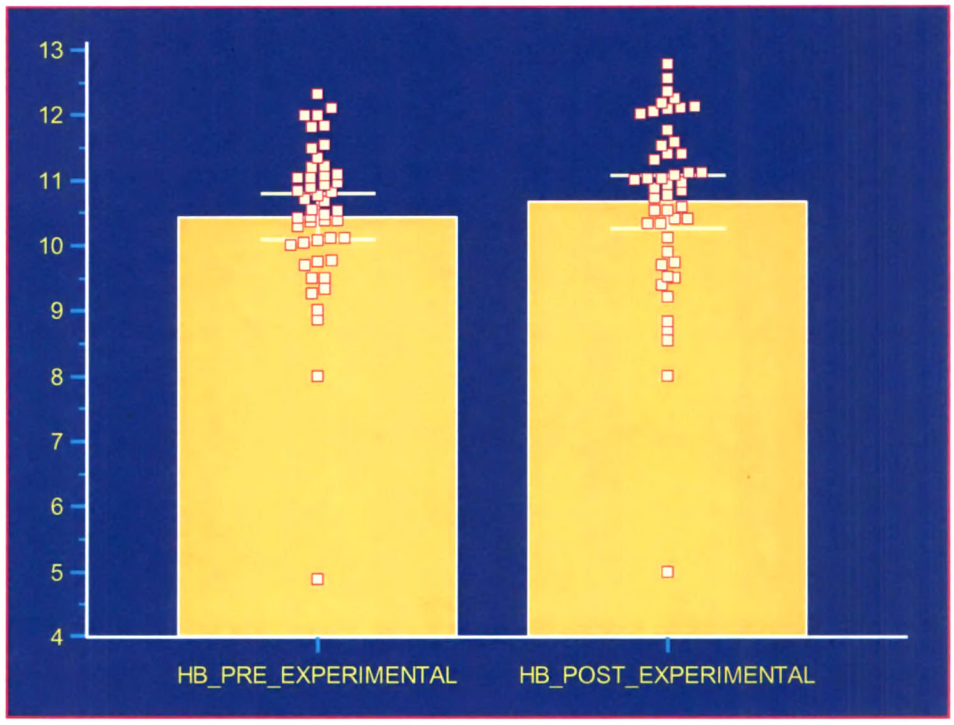


↑ 78 µg/L [274 (final)-199 (initial), p=0.006*]

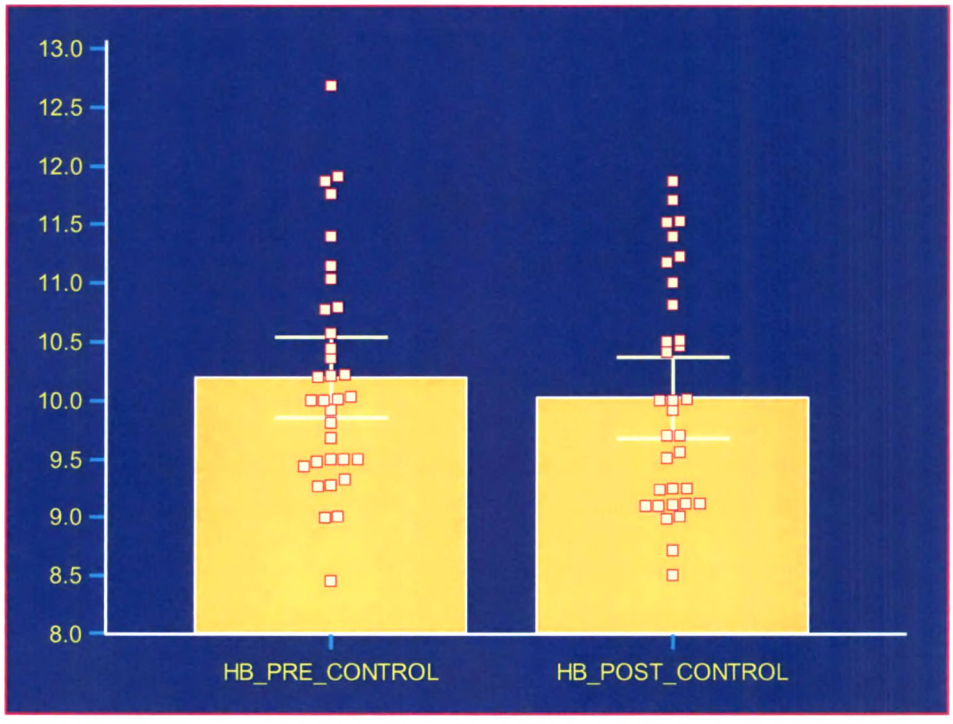


↓16 µg/L [265 (final)-281 (initial), p=0.964^{ns}]

Figure 4.4.11: Mean hemoglobin before (Pre) and after (Post) DFS supplementation in experimental and control group



↑0.22 g/dl [10.66±1.3 (final)-10.44±1.2 (initial), p=0.020*]



↓ 0.17 g/dl [10.02±0.9 (final)-10.19±0.9 (initial), p=0.039*]

NIN conducted an efficacy trial from 1989 to 1992 in the tribal areas of East Godavari (Andhra Pradesh), which is endemic for goitre as well as with a high prevalence of IDA. Four blocks were randomly selected; three blocks were allocated to experimental group (DFS supplementation for 2 years), while the fourth block served as control (IS for 2 years). There was a significant reduction in the prevalence of total goitre from an initial 28% to 14% after intervention of DFS in tribal areas in 2 years. Median urinary iodine excretion increases from 116 to 155 μg/L in DFS group and from 59 to 160 μg/L in IS group. Overall prevalence of anemia decreased from 78 % to 55 % in DFS group. The results demonstrated that the hemoglobin levels increased significantly in anemic subjects and there was a marginal or no improvement in non-anemic subjects (NIN, 2005).

In a multicentric study in India, the bio-efficacy of DFS was assessed in communities covering three states of the country. Over a period of one year, there was an increase of 1.98 g/dL of hemoglobin in the experimental group and 0.77 g/dL of hemoglobin in the control group; the latter increase may have been due to deworming. The median urinary iodine changed from 200 μg/L at baseline to 205 μg/L at the end of the study in the experimental group and from 225 mcg/L to 220 mcg/L in the control group (Vinodkumar et al, 2007).Zimmerman et al (2003) also studied the efficacy of DFS [containing 25 mcg iodine/g salt (as potassium iodide) and 1 mg iron/g salt (as ferrous sulfate hydrate encapsulated with partially hydrogenated vegetable oil)] supplementation to that of iodized salt in a 9-months, randomized, double-blind trial in iodine-deficient, 6–15-y-old children (n=377) in Morocco (Zimmermann et al, 2003). During the efficacy trial, urinary iodine levels and thyroid volumes improved significantly ($p<0.001$ and <0.05, respectively) from baseline in both groups. At 40 weeks, mean hemoglobin concentrations in the DFS group had increased by 1.4 g/dL ($p<0.01$). The prevalence of iron deficiency anemia in the DFS group decreased from 35% at baseline to 8% at 40 weeks ($p< 0.001$).

Concurrent to results in above mentioned studies, our study supplementation of DFS showed an improvement in both iron and iodine status. DFS 10 g/day provided 10 mg iron (40 % RDA for lactating women) and 200 µg iodine (80% RDA for lactating women).Six months supplementation of DFS (providing 10 mg of daily iron) could bring an increase of 0.22 g/dL in hemoglobin during lactation as compared to an increase of 0.44 g/dL in hemoglobin during pregnancy after six months supplementation of 60 mg of elemental iron. The prevalence of IDA in the DFS group decreased from 96% (before supplementation) to 79% (after supplementation) ($p<0.001$).DFS delivered less but crucial amount of iron to lactating women, which significantly contributed for sustained release of iron and iodine during breastfeeding and postpartum period.

This amount of iron from DFS was 1.5 mg more than what they were getting from their daily diet [8.5 mg (34% RDA)]. When experimental and control groups were compared, an increase in mean hemoglobin was found in experimental group and a decrease in control group. Hence we can state that, though the amount of iron in DFS is less but it could help lactating women to sustain their hemoglobin levels.

Median urinary iodine in experimental group increased after supplementation; however in control group a decrease in median urinary iodine was observed. The prevalence of iodine deficiency in the DFS group decreased from 21% (before supplementation) to 6% (after supplementation) ($p< 0.05$). As mentioned earlier iodine requirements during pregnancy and lactation are higher during non-pregnant and non-lactating state. Single iodized salt provides 150 µg of iodine/day and the requirement is 250 µg/day. DFS contains 400 µg iodine/g salt and it can provide 200 µg (80% RDA) iodine/g salt at consumer level, whereas single iodized salt contains 300 µg iodine/g salt and it can provide 150 µg (60% RDA) iodine/g salt at consumer level.

4.4.10 Effect of thyroid dysfunction during early gestation on infant development

For determining the effect of early gestation thyroid function on infant development, pregnant women were categorized into two groups- group-I [women with thyroid dysfunction] and group-II [women with normal thyroid function].

Table 4.4.11: Mean BDSTI scores, anthropometric measurements and thyroid hormones in both groups

Variable	Mean (sd)		P value
	Group IN=39	Group IIN=42	
Maternal Characteristics			
TSH (μIU/ml)	3.08* (2.18-4.34)	1.56* (1.25-1.94)	P=0.001*
FT4 (ng/dl)	1.043 (0.28)	1.122 (0.25)	P=0.194^{ns}
Infant Characteristics			
BDSTI at 6 months	17.7 (0.7)	17.9 (0.1)	P = 0.032
BDSTI at 12 months	34.5 (0.9)	34.9 (0.3)	P = 0.028
Birth weight (kg)	2.8 (0.4)	2.7 (0.4)	P = 0.354 ^{ns}
Weight at 6 M (kg)	6.7 (0.9)	6.6 (0.7)	P = 0.858 ^{ns}
Weight at 12 M (kg)	7.48 (0.8)	7.47 (0.5)	P = 0.963 ^{ns}
Length at 6 M (cm)	66.1 (2.6)	65.9 (2.6)	P = 0.715 ^{ns}
Length at 12 M (cm)	71.1 (3.0)	70.8 (2.6)	P = 0.631 ^{ns}
HC at 6 M (cm)	41.68 (1.5)	41.63 (1.0)	P = 0.862 ^{ns}
HC at 12 M (cm)	44.09 (1.6)	44.39 (1.0)	P = 0.333 ^{ns}

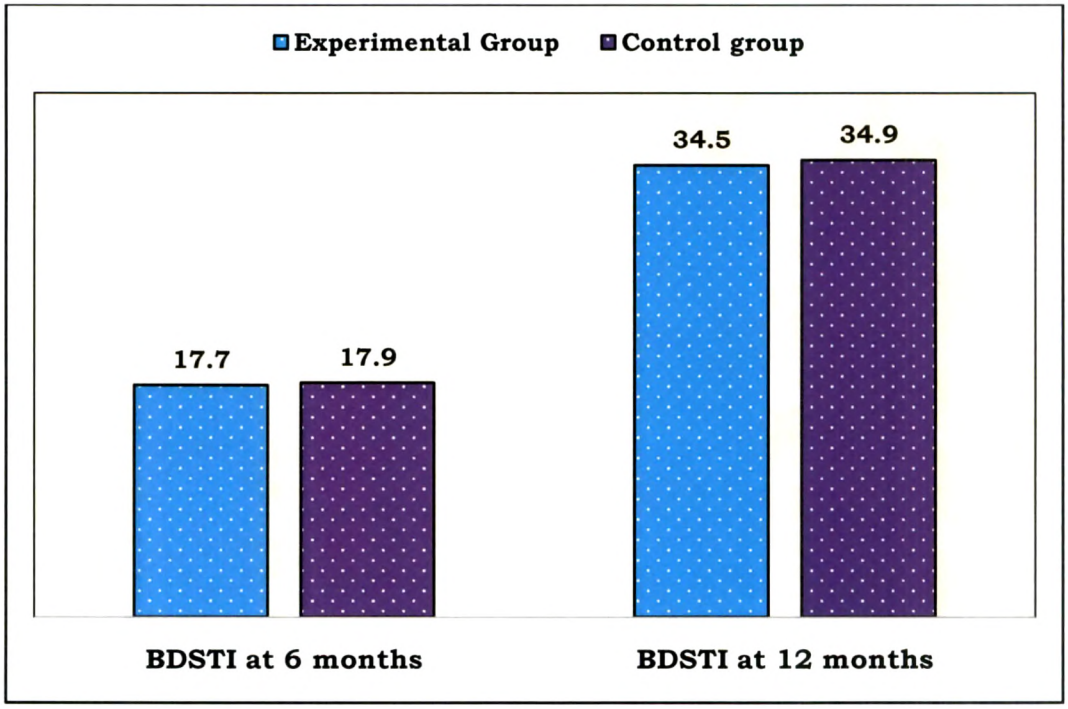
*Geometric Mean (95%CI), M –months, HC-head circumference

Mean TSH in both the groups was found to be significantly different. In group I mean TSH was higher (3.08) than in group II (1.56).

However no significant difference was found in mean FT4 values in both groups. Significant difference in both groups were not found with respect to anthropometric indices (birth weight, weight at 6 and 12 months, length at 6 and 12 months and head circumference at 6 and 12 months) (Table 4.4.11).

BDSTI at 6 months in Group I and Group II was found to be 17.7 (0.7) and 17.9 (0.1) respectively and BSDTI at 12 months in Group I and group II was 34.5 (0.9) and 34.9 (0.3) respectively. A significant difference of 0.2 and 0.4 was found between mean scores of both groups at 6 months and 12 months respectively. This difference is an indicator of effect of early gestation thyroid dysfunction on infant mental and psychomotor development (Figure 4.4.12).

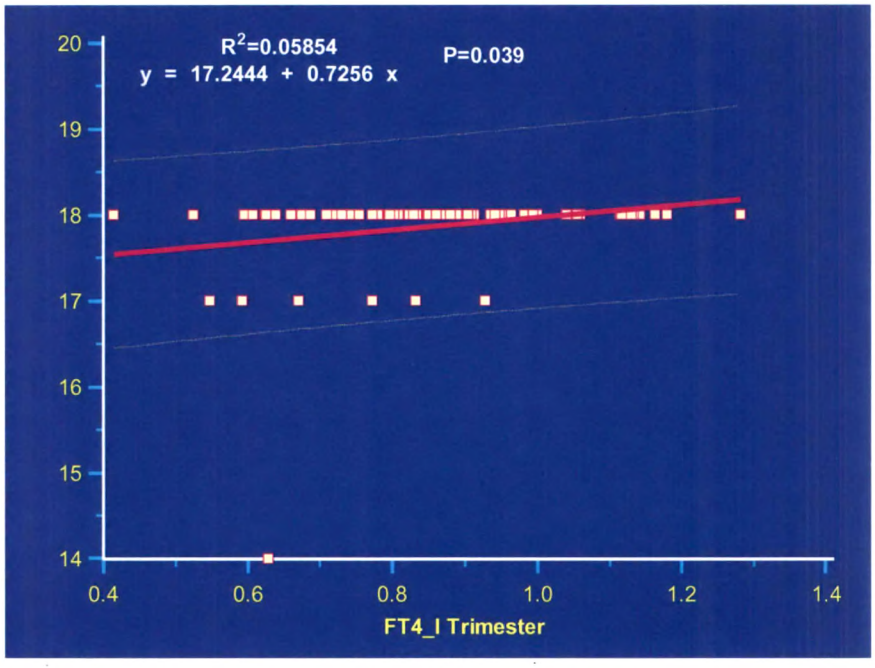
Figure 4.4.12: Mean BDSTI in group I [experimental] and Group II [control]



A positive significant (r=0.241, p=0.039) association was found between first trimester FT4 and BDSTI at six months (Figure 4.4.13) but not between first trimester TSH and BDSTI at six months (r=-0.06, p=0.588). BDSTI at twelve months was also not significantly

associated with first trimester TSH (r=-0.037, p=0.751) and FT4 (r=0.163, r=0.167).

Figure 4.4.13: Association between first trimester FT4 and BDSTI at 6 months



[Pink dotted line denotes 95% prediction interval]

Thyroid hormones and brain development

Thyroid hormones are essential for normal brain development. Extremely low levels of thyroid hormones during gestation result in mental retardation. In early pregnancy the embryo depends entirely on maternal thyroid hormone that crosses the placenta, and by about 12-14 wk gestation, fetal thyroid function begins (de Escobar et al 2004 and 2007). Even after the onset of fetal thyroid secretion, maternal transfer constitutes a fraction of circulation fetal T4, and continues to have a protective role in fetal neurodevelopment until birth. Mothers with hypothyroidism during first trimester of pregnancy, and even those with low-normal T4 levels or mild serum TSH elevations, have children with poorer neurocognitive function (Haddow et al 1999; Pop et al 1999 and 2003).

The development of fetal thyroid function is dependent on the embryogenesis, differentiation, and maturation of the thyroid gland. This is coupled with evolution of the hypothalamic-pituitary-thyroid axis and thyroid hormone metabolism, resulting in regulation of thyroid action, production, and secretion. Throughout gestation there is a steady supply of maternal thyroxine which has been observed in embryonic circulation as early as 4 weeks post-implantation. This is essential for normal early fetal neurogenesis. T4 concentrations are highly regulated to maintain low concentrations, essential for protecting the fetus and reaching key neurological sites such as the cerebral cortex at specific developmental stages.

Thyroid hormones primarily regulate genes involved in myelination and neuronal glial cell differentiation (Bernal, 2005). Delivery of thyroid hormones to the fetal brain is a complex process requiring, at different times, expression of brain thyroid hormone receptors, maternal-fetal thyroid hormone and iodine transport, an intricate system of endocrine feedback (HTP axis) and thyroid hormone metabolism by liver and brain deiodinase enzymes (D2) and D3 to ensure basal levels are sustained (Zoller et al, 2007).

In 1969, Man and Jones suggested that mild maternal hypothyroidism alone was associated with lower IQ levels in the offspring. In 1990, Matsuura and Konishi documented that fetal brain development is adversely affected when both the mother and fetus have hypothyroidism caused by chronic autoimmune thyroiditis.

Pop et al (1999) reported that low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Neurodevelopment was assessed in 220 healthy infants at 10 months of age using Bayley Scales of Infant Development. Children of women with FT4 levels below 5th (0.76 ng/dl) and 10th (0.80 ng/dl) percentiles at 12 weeks gestation had significantly lower scores on Bayley Psychomotor Development Index (PDI) at 10 months of age, compared to children of mothers

with higher FT4 levels. At 32 weeks gestation, no significant differences were found.

Kooistra et al (2006) confirmed that maternal hypothyroxinemia constitutes a serious risk factor for neurodevelopmental difficulties that can be identified in neonates as early as 3 weeks of age. The group examined 108 neonates who were born to mothers with low FT4 levels (<10th Percentile) at 12 weeks gestation and 96 neonates who were born to women whose FT4 values were between 50th to 90th percentiles. Newborn development was assessed using Neonatal Behavioral Assessment Scale (NBAS). Infants of women with low FT4 at 12 weeks had significantly lower scores on the NBAS orientation index compared to other infants.

In 2011, Su et al (China) studied 1027 serum samples of women with singleton pregnancy for TSH and FT4 during first 20 weeks of gestation. Clinical hypothyroidism was associated with congenital circulation system malformations; the adjusted odds ratio (95% (CI) was 10.44 (1.15–94.62). Subclinical hypothyroidism was associated with poor vision development, and neurodevelopment delay; the adjusted odds ratios (95% CI) were 5.34 (1.09–26.16), and 10.49 (1.01–119.19), respectively. Isolated hypothyroxinemia was related to musculoskeletal malformations; the adjusted odds ratios (95% CI) was 9.12 (1.67–49.70). Wang et al (2011) reported that maternal thyroid disorders during early pregnancy can influence pregnancy outcome and fetal development.

From the above discussion and from results of present study it is evident that thyroid hormones during early gestation play an important role in brain development. It is indicative from the data, that these hormones being a major metabolite hormone have its influence in regulating the system. The data further suggests, all pregnant women should be subjected to thyroid screening at the onset of pregnancy and government should take initiative in implementing the same.

General discussion

The high global prevalence of iodine deficiency and thyroid disorders, the mental and physical consequences of these disorders create a huge human and economic burden that can be prevented by early detection and therapeutic measures. Over the past several years it has been proved that maternal thyroid disorders influence the outcome of both mother and fetus, during pregnancy and after pregnancy. However, currently there are no recommendations for universal screening for thyroid disorders in women before or during pregnancy.

In present study we have made an attempt to justify early screening of pregnant women for thyroid disorders. We have screened pregnant women using two TSH cut-offs [2.5 and 5.0 μIU/ml]. Screening results indicated that, 28% women were at low risk (TSH >2.5) and 5.5% women were at high risk (TSH >5.0) of developing hypothyroidism.

We have followed (sub sample) these pregnant women till delivery and one year postpartum. TSH cut-off of 2.5 (first trimester) and 3.0 (second and third trimester) μIU/ml, with FT4 cut-off of 0.65 ng/dl (first, second and third trimester) was used for defining thyroid dysfunction during pregnancy. However, during postpartum period when thyroid status of these women was again tested, normal adult reference range for TSH (0.25-5.0 μIU/ml) and FT4 (0.65-2.10 ng/dl) was used. Results indicated that, mean values for all four thyroid hormones (TSH, FT4, TT4 and TG) were normal, but thyroid dysfunction was found in 82.1%, 86.7% and 75.3% women during first, second and third trimester respectively. Thyroid hormones were compared using different trimester specific reference intervals. Results of comparison revealed that, use of different trimester specific reference interval resulted in different prevalence of thyroid dysfunction. Hence, we concluded that choosing a right method is very essential.

During postpartum period only 9.9% (2.5% overt hypothyroidism and 7.4% subclinical hypothyroidism) women were found to have thyroid

dysfunction. Not a single woman was found to be hypothyroxinemic during this period. After comparing thyroid function of women during pregnancy and lactation (postpartum period) we found that, 90% women become normal after pregnancy. Thus we concluded, due to pregnancy there were fluctuations observed in thyroid hormones especially TSH and FT4. A euthyroid state of mother during early pregnancy is very important for proper development and differentiation of fetal brain. However, most of them were not able to maintain euthyroid state during pregnancy.

After obtaining thyroid hormone status of women during postpartum period (when there was no effect of pregnancy induced changes on thyroid gland), we compared our two diagnosis methods (TSH >2.5 and TSH >5.0 μIU/ml). Our result revealed that, TSH cut-off >2.5 μIU/ml was proved to be a better indicator of thyroid status with high sensitivity and specificity as compared to TSH cut-off >5.0 μIU/ml.

Hence, considering our results of pregnancy and postpartum period, the need to asses thyroid function during pregnancy is justified.

We have also observed the effect of thyroid dysfunction during early gestation on infant development at 6 and 12 months. A significant difference of 0.2 and 0.4 was found between mean BDSTI scores of both groups (with thyroid dysfunction and with normal thyroid function) at 6 and 12 months respectively. This difference is an indicator of effect of early gestation thyroid dysfunction on mental and psychomotor development of infant.

Iodine Deficiency is the world's leading cause of preventable intellectual disability and Iron Deficiency Anemia is the most common and wide-spread nutritional disorder in the world. Iodine plays a critical role in the neuropsychological development of the fetus throughout gestation and in the first two years of life. Iron is critical for cognitive and motor development in childhood and for physical activity in all humans. The requirements for these two micronutrients are increased during pregnancy and lactation as compared to non

pregnant state. These increased requirements are higher to meet the physiological changes and increased nutritional needs during pregnancy and lactation.

In present study, double fortified salt (DFS) was considered as an additional strategy (along with IFA supplementation) to combat IDA and ID during lactation. During lactation, women were randomized into experimental group (DFS supplementation for 6 months) and control group (single iodized salt for 6 months).

We found a significant increase of 0.22 g/dl in hemoglobin in experimental group compared to a significant decrease of 0.17 g/dl in hemoglobin in control group. Median urinary iodine level significantly increased by 78 µg/l in experimental group, while in control group it decreased by 16 µg/l (non significant). DFS delivered crucial amount of iodine and iron to these (experimental group) women through their diets. Hence we conclude that, DFS helps in sustaining iron and iodine levels in lactating women.