



MATERNAL THYROID PROFILE PATTERNS IN THE THREE TRIMESTERS OF HEALTHY PREGNANT WOMEN IN JODHPUR DISTRICT OF RAJASTHAN

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ABSTRACT

Pregnancy is associated with significant but reversible changes in thyroid function tests results, which are among the most profound seen as a result of a normal physiologic state. During pregnancy the thyroid is hyperstimulated, resulting in changes in thyroid hormone concentrations. Accurate assessment of thyroid function during pregnancy is critical, for both the initiation of thyroid hormone therapy, adjustment of thyroid hormone dose in those already receiving thyroid hormone and neonatal development. The present study was carried out to find out alterations in thyroid function tests in each trimester in normal pregnant women as compared to non-pregnant women. The study was designed with two groups of women as followed; 100 healthy pregnant women randomly selected from the first (40 samples), the second (30 samples), and the third (30 samples) trimesters, and fifty (50) randomly selected non-pregnant healthy female controls. Thyroid function tests were carried out by measuring the serum levels of thyroid stimulating hormone (TSH), free and total thyroxine (FT4, T4), and free and total triiodothyronine (FT3, T3) by commercially available radio-immunoassay kits. Results: We found that mean T4 Increased progressively during pregnancy. Serum levels of T3 increased in the second trimester and then declined during the third trimester compared with non-pregnant women. We also found that FT4 strongly decreased during the third trimester. Free T3 declined in the second and third trimesters. The mean TSH did not significantly differ in the three trimesters compared to the levels of non-pregnant women. Thyroid function tests in pregnancy should be interpreted against gestational age-related reference intervals to avoid misinterpretation of thyroid function during pregnancy.

KEYWORDS: Thyroid Hormone, Total T4, Total T3, Free T4, Free T3, TSH and Pregnancy.

1.0 INTRODUCTION

Pregnancy is associated with profound anatomical, physiological, biochemical and endocrine changes that affect multiple organs and systems. These changes are essential to help the woman to adapt to the pregnant state and to aid fetal growth and survival. However, such anatomical and physiological changes may cause confusion during clinical examination of a pregnant woman. Similarly, changes in blood biochemistry during pregnancy may create difficulties in interpretation of results. Conversely, clinicians also need to recognise pathological deviations in these normal anatomical and physiological changes during pregnancy to institute appropriate action to improve maternal and fetal outcome.^[1] Advancement of pregnancy is accompanied by the extra demand of energy. As pregnancy progresses, a well-integrated metabolic shift occurs to ensure an adequate supply of nutrients to a constantly feeding fetus from an intermittently fasting and feeding mother.

During early pregnancy, maternal metabolic environment is modified by a rise in serum levels of estrogen and progesterone, thyroid hormone, pancreatic beta-cell hyperplasia occurs and there is an increase in the secretion of insulin.^[2] Hyperinsulinemia leads to an increase in peripheral glucose utilization, a decline in fasting plasma glucose levels, increased tissue storage of glycogen, increased storage of fats and decreased lipolysis.^[2]

Pregnancy is a physiological state, associated with significant but reversible changes in thyroid function.^[3] Thyroid is a small endocrine gland found in the front of the neck. The hormones are essential for proper growth, proliferation, differentiation, apoptosis, development, neurotransmission, behaviour and metabolic homeostasis.^[3] Thyroid gland generates the hormones thyroxine (T4), 3,5,3-triiodothyronine (T3), calcitonin and secretes them into the bloodstream. All thyroid

hormone actions are mediated by the binding of 3,5,3-triiodothyronine (T3) to specific nuclear receptors and T3 is the chief bioactive form of Thyroid, whereas T4 acts as a prohormone to T3.^[3] The role of the hormone in embryogenesis and fetal development during pregnancy is well known.

During the first trimester of pregnancy, the fetus is reliant on transplacental passage of maternal thyroxine, as the fetal thyroid is not fully functional until about 16 weeks of gestation.^[17] The production of hCG (human chorionic gonadotropin) by placenta during first trimester is known to stimulate thyroid gland because of its structural similarity to thyrotropin (TSH). This leads to reduced serum thyrotropin levels during first trimester because of negative feedback effect.^[17] Alterations in iodine metabolism, production of β -chorionic gonadotropin (β -hCG), and increases in both thyroid hormone-binding proteins and thyroid hormones per se are some of the physiologic changes that occur during normal pregnancy.^[12] At the same time, thyroid hormones play a critical role in neonatal and child neurodevelopment and maternal thyroid disorders which can lead to obstetric complication and irreversible effect on the fetus^[18]. During pregnancy there are complex changes in circulating maternal steroid hormones and in thyroid binding globulin (TBG) concentrations. In addition to the stimulatory effects of estrogen on TBG synthesis, a major contribution to the increased TBG concentration during pregnancy is the reduced plasma clearance of the protein caused by changes in TBG glycosylation induced by estrogen.^[11] It is commonly thought that total thyroxine (TT4) and total triiodothyronine (TT3) concentration increase in the setting of pregnancy-induced increases in serum TBG concentrations. The concentrations of TBG double by weeks 16 to 20 of gestation. In addition to the 2- to 3-fold increase in serum TBG, modest decreases in both serum transthyretin and albumin are commonly found in pregnancy.^[11] Free T3 (FT3) and free T4 (FT4) levels are slightly lower in the second and third trimesters. Thyroid-stimulating hormone (TSH) levels are low-normal in the first trimester, with normalization by the second trimester.^[7] Thyroid disease is the second most common endocrine disease to affect women of reproductive age. Thyroid disorders can have adverse reproductive and pregnancy implications.^[13] Although gestational hyperthyroidism is uncommon (0.2%), gestational hypothyroidism occurs in higher prevalence (2.5%) and can lead to neonatal and child neurodevelopmental deficits and maternal obstetric complications. In regions where the iodine supply is borderline or low, the situation is clearly different and significant changes occur during pregnancy.^[13] Thyroid disorders are common in pregnancy. There is an increasing interest in identifying changes in thyroid profile during pregnancy due to the role of thyroid function in maintaining a viable pregnancy and contributing to healthy offspring.^[13] Thus, the present study was aimed at evaluation of thyroid profile level in

1st, 2nd and 3rd trimesters of healthy pregnant women and compared it with control subject (non-pregnant women)

2.0 MATERIAL AND METHOD

2.1 study population

The study was carried out between the months of October 2015 to May 2016 at Vasundhra hospital and fertility research centre Jodhpur, Rajasthan, India. A total of 150 healthy pregnant and non-pregnant women were enrolled in the study attending Vasundhra hospital and fertility research centre, Jodhpur, India. They study groups are as follows.

2.2.0 Experimental Design and Sample Collection

2.2.1 Experimental Design

The control Group: comprised of 50 healthy non-pregnant women, having normal menstrual function with no evident hormonal deficiency (infertility), aged (25 $\pm$ 15) years.

The Study Group: Comprised of 100 pregnant women and their gestational aged (28 $\pm$ 10) years.

2.2.2 Sample collection

Serum TT3, TT4, FT4, FT3, and serum TSH concentrations levels were assessed in both groups; to do this, 5ml of Blood was in turn collected from the antecubital vein. Samples were collected with all aseptic precautions, using sterile needles and syringes in plain sterile vials. The controls samples were obtained from healthy volunteers, while the study group samples were taken during nine months of pregnancy period. Samples were kept undisturbed for 30 minutes and centrifuged at 400 rpm for 10 minutes. Serum was separated then stored in deep freezer at (-20C) until for use of monoclonal antibody in ELISA Test, which eliminates cross reactivity with other hormones. The study was approved by the corresponding ethical committees, and all participants provided written informed consent.

2.3 Hormone analysis

The levels of serum thyroid stimulating hormone (TSH), total triiodothyroxine (T3), free thyroxine (FT4), and free triiodothyronine (FT3) were measured by a chemiluminescence Immunoassay (CLIA) on the LUMACE System (Abbott Laboratories, Abbott Park, USA), while serum total thyroxine (T4) was measured by the Fluorescence Polarization Immunoassay (FPIA) method on the AXSYM System using standard laboratory methods.

2.4 statistical analysis

The analyses were performed using SPSS software. Data were presented as mean $\pm$ standard deviation using Microsoft excel 2010. The student t- test was used for analyses of Differences in thyroid function tests between each trimester and the control group. A two-sided p value <0.05 was the level of statistical significance.

3.0 RESULT AND DISCUSSION

3.1 RESULTS

Table 1 shows the mean $\pm$ SD of the measured maternal thyroid profile results of pregnant and non-pregnant women. We found that in the first and second trimesters, the mean TT4 levels of pregnant women were not significantly lower. However, in the third trimester, the mean TT4 increased significantly ($p < 0.01$) relative to the mean of non-pregnant women.

In the first trimester, the mean TT3 values were found to be somewhat significant between the two groups of subjects. However, in second trimester, the mean TT3 value significantly increased ($p < 0.05$) compared to that of the control group. In the third trimester, the mean TT3 of pregnant women decreased again and did not significantly differ from that of non-pregnant women.

The mean FT4 levels in the first and the second trimesters were non-significantly lower than that of the non-pregnant subjects. But in the third trimester, the

mean FT4 was significantly decreased ($p < 0.01$) relative to the mean for non-pregnant women. The pregnant groups' mean FT3 values declined over the trimesters. In the second and third trimesters, the mean levels of pregnant women were significantly lower ($p < 0.01$) than the mean of non-pregnant women.

In each trimester, the mean TSH levels of pregnant women were lower than the mean level of non-pregnant. However in the first and third trimesters, the TSH levels of pregnant women were not statistically significant, but in the second trimester, pregnant women's mean TSH were found to be significantly lower ($p < 0.05$). On the other hand, we compared the mean values of each thyroid function test between pregnant and non-pregnant women. We found the following: The mean TT4 of pregnant women was significantly higher ($p < 0.01$), the FT4 was significantly lower ($p < 0.01$), the FT3 was significantly lower ($p < 0.05$), and the mean TSH was significantly lower ($p < 0.05$).

Table 1: Shows Comparative Result of Maternal Thyroid Profile Status of Healthy Pregnant and Non-Pregnant In 3 Trimesters of Pregnancy.

Subjects	TT4 (nmol/L)	TT3 (nmol/L)	FT4 (pmol/L)	FT3 (pmol/L)	TSH (mIU/ml)
Non-pregnant Women N= 50	93.75 $\pm$ 30.12	2.80 $\pm$ 1.07	14.75 $\pm$ 11.01	5.73 $\pm$ 2.99	2.23 $\pm$ 1.20
pregnant women					
First Trimester N= 40	84.38 $\pm$ 40.32	2.49 $\pm$ 1.47	14.91 $\pm$ 4.22	6.98 $\pm$ 2.18	1.82 $\pm$ 1.46
Second Trimester N= 30	93.80 $\pm$ 42.65	3.28 $\pm$ 1.60*	12.92 $\pm$ 3.61	3.97 $\pm$ 1.68**	1.99 $\pm$ 1.42*
Third Trimester N= 30	125.65 $\pm$ 51.50**	2.90 $\pm$ 1.57	6.96 $\pm$ 3.45**	3.06 $\pm$ 1.49**	2.43 $\pm$ 0.84

Results are presented as mean $\pm$ SD where N stands for number of sample in a particular group

* Significant difference at p value less than 0.05 ($P > 0.05$)

** Significant difference at p value less than 0.01 ($P > 0.01$)

Table 2: Shows characteristics and biochemical changes in pregnant and Non-pregnant subjects.

Parameter	Non-pregnant women	Pregnant Women
Age (years)	25 $\pm$ 15	28 $\pm$ 10
Sex-Females (%)	50 [100%]	100 [100%]
Body mass index (kg/m ²)	26 $\pm$ 3.4	32 $\pm$ 6.2 *
Fasting blood glucose (mg/dl)	92 $\pm$ 13	113 $\pm$ 27 **
Urea (mg/dl)	21 $\pm$ 15	28 $\pm$ 17 ^{NS}
Creatinine (mg/dl)	0.6 $\pm$ 0.3	0.7 $\pm$ 0.4 ^{NS}
Total cholesterol (mg/dl)	165 $\pm$ 15	164 $\pm$ 17 ^{NS}
Values are given as mean $\pm$ S.D from Seventy-five subjects in each group Pregnant subjects compared with non-pregnant subjects (* $P < 0.05$, ** $p < 0.001$). NS- Not significant		

3.2 DISCUSSIONS

During pregnancy several hormonal changes and metabolic demands occur, resulting in complex effects on thyroid function.^[10] Alterations in the pituitary-thyroid axis include an increase in thyroid hormone-binding globulin along with increases in total T4, T3 as well as serum thyroglobulin (TG). Additionally, iodine clearance by the kidneys is enhanced during gestation, while the mild thyrotropic effects of rising β -hCG may exert negative feedback on TSH secretion.^[20]

The results of our study shows elevated level of TT4 and TT3 in healthy pregnant women as compared to control group. TT4 increased progressively in all the trimesters of pregnancy which was statistically significant whereas TT3 increased significantly in second trimester. Free T4 strongly declined during the third trimester, whereas FT3 decreased in the second and the third trimesters. Mean TSH was seen to rise progressively through the three trimesters of pregnancy, but was not significant compared with non-pregnant women. Overall mean of each thyroid function test showed significant increase in TT4 and declining of FT4 and FT3. But, TSH and TT3 did not show significant difference in pregnant women compared with non-pregnant women.

Similar results were obtained by Mujawar *et al.* He found significantly high elevation of hCG, TT3 and TT4. But he also found elevated level of TSH in these subjects. In a study carried out by Kumar *et al.*, they also found rise in TT3 and TT4 in both first and second trimester of pregnancy. Our results are in agreement with WohllkN *et al.*, who also found significant elevation of TT3, TT4 and suppression of TSH in 6-14 weeks of pregnancy. In our study we also found TSH levels to be declined in first and second trimesters as compared to control subject, but are not statistically significant. In another study by Zarghami *et al.*, TSH levels are suppressed in First and second trimesters and also their difference is not statistically significant.

The plausible rationale behind the increase in the thyroid hormones might be as result of, increased concentration of plasma thyroxine binding globulin (TBG) which occur during pregnancy.^[9] Another proposed mechanism for this increase in total thyroid hormone concentration is production of type III deiodinase from placenta. This enzyme converts T4 to reverse T3 and T3 to diiodotyrosine (T2), which has extremely high activity during fetal life. Increased demand for T4 and T3 has been suggested to increase production of these hormones with, ultimately increased concentration in circulation.^[9] Increased sialylation, mediated by oestrogens, reduces the clearance of TBG, resulting to increase levels of T4 and T3 as well. In the third trimester, usually there is high concentration of Total T4 which mainly binds to TBG, results in decline in FT3 and FT4 levels in this trimester.^[14, 15] and thereby a rise in serum TSH levels. It means, not every pregnant women which demonstrate elevated

TT4, is really in a state of hyperthyroidism but it is the new physiological condition and the metabolic regulation of pregnant women which realized and on that base, extra thyroxine requirement is requested, T4 is elevated, not for the sake of hyperthyroidism, but for the sake growing fetus demand and the maternal new physiological condition, therefore the establishment of reference interval and the methodology of thyroid hormone measurements in each region is great importance.^[14,15]

Khandakar *et al.* performed a case-control study to find out alteration in serum thyroid hormones levels in normal pregnant women as compared to non-pregnant women in Dhaka City. For this purpose, they randomly selected 35 pregnant women during third trimester and 21 non-pregnant women of childbearing age as control.

Their study showed that serum TT4 level of non-pregnant women was normal, but in pregnant women during third trimester was significantly higher compared to non-pregnant. This finding was somewhat similar to our study. Khandakar *et al.*, also found that TT3 levels in both groups were within normal range without significant difference between them. This result was in contrast to our finding. Their finding about TSH was closely similar to our study too. However, a significant limitation of Khandakar *et al.* investigation was small sample size. Panesar NS *et al.*, carried out a prospective study with 343 healthy pregnant women (5-41 weeks) and 63 non-pregnant controls to establish gestation-related reference intervals for thyroid hormones in pregnant Chinese women. They found that FT3 decreased during pregnancy, whereas FT4 initially increased, peaking between 9-13 weeks and then decreased, the decline becoming significant by week 21, and TSH changes was similar to FT4. We also found declining in FT3 over the pregnancy. FT4 changes during pregnancy in our study were nearly similar to their study. In contrast to Panesar NS *et al.*, we did not find any significant change in TSH in each trimester compared to non-pregnant women.

McElduff reports that the serum concentration of FT4 and FT3 during the three trimesters of pregnancy to decreased as compared to non-pregnant women, and this may result for the need that each laboratory to develop its own reference range for FT4 levels in pregnancy. Our study also showed a progressive lowering of mean FT4 and FT3 levels with each trimester during the pregnancies. Kumar A *et al.*, measured serum levels of T3, T4, and TSH in 124 pregnant women. The women were apparently normal, healthy young primigravidas with no known metabolic disorders and normal carbohydrate gestational intolerance test and report that the mean FT3 level decreased during the second trimester and third trimester compared to the first trimester, which was in agreement with the result in our study.

Erem C, *et al.*, investigated maternal thyroid function in 29 pregnant women with goiter and 51 pregnant women without goiter. The location of the women was the eastern black sea region of Turkey, which is an endemic goiter area.

They found that TT4, FT4, TT3, FT3, and thyroxine binding globulin increased during pregnancy, they also found that serum TSH levels declined in pregnant women without goiter compared with non-pregnant women without goiter. In our study, changes in the serum levels of TT4, TT3, and TSH in pregnant women were closely similar to those reported by Erem C, *et al.* But in contrast to their findings, we found that serum levels of FT4 and FT3 in pregnant women decreased compared to those of non-pregnant women the possible reason behind this variation might due to geographical location or their physiological condition of being goiter positive.

CONCLUSION

Physiologic changes associated with pregnancy require an increased availability of thyroid hormones by 40% to 100% to meet the needs of a mother and fetus during pregnancy. Pregnancy has an effect on other thyroid functions with significant changes in iodine metabolism and clearance, serum thyroid binding proteins, and the development of maternal goiter and other thyroid disorders, especially in areas with various levels of iodine deficiency. For the maternal thyroid gland to meet the demands of pregnancy, it must have sufficient supplies of iodine.

Thyroid autoimmunity is common and may contribute to miscarriages and hypothyroidism. The fetus is totally dependent on maternal thyroxine supply during the first trimester of gestation and up to mild gestation for normal neurologic development and nervous system maturation. Because the progression of pregnancy and fetal, neonatal and child health are dependent on adequate thyroid hormone supplementation throughout pregnancy, as such trimester specific reference intervals for thyroid functions can be crucial for both maternal and fetal health and the result of this study could decline the possibility of the misinterpretation of thyroid function in pregnant women in our research area.

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