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Original Research Paper

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# TRIMESTER SPECIFIC STATUS OF FT3, FT4, TSH AND ANTI- TPO DESCRIPTIVE STUDY IN TERTIARY CARE HOSPITAL

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**ABSTRACT**

**Introduction:-** Thyroid hormone during pregnancy need to be gestational age, method, and population specific and there is need to establish trimester-specific thyroid levels for the different population across the world. The aim of this study was to establish the relationship between trimester-specific maternal thyroid function, AMH and thyroid autoimmunity (anti-TPO Ab), status in pregnant women in a tertiary care in Rajasthan. **Material & Methods:-** A total of 252 pregnant women were recruited for the study. Participants having any history of chronic illness, Cancer, Human immunodeficiency virus (HIV) Syphilis, Thyroidectomy (surgical removal of all parts of the thyroid gland) Tuberculosis (TB) were excluded from the study and reference population was identified to calculate serum free triiodothyronine (FT3), free thyroxine (FT4) and thyrotropin (TSH) AMH and thyroid autoimmunity (anti-TPO Ab), status for each trimester of pregnancy. **Results:-** In first trimester, FT3 Mean (SD) was 3.53 (0.59) which range from 2.54 to 4.52 pg/ml. FT4 Mean (SD) was 1.32 (0.26) which range from 0.88 – 1.77 ng/ml . TSH Mean (SD) was 2.07 (1.01) which range from 0.32 to 4.60 mIU/ml. In first trimester, the mean (SD) serum TSH level were 2.07 (1.01) mIU/ml which range from 2.54 mIU/ml to 4.52 mIU/ml. Anti-Mullerian hormone was tested in the first trimester, the mean (SD) of serum AMH wasfound to be 4.75 (2.79) ng/dl with range from 0.076 ng/dl to 14 ng/dl. Anti-TPO auto antibody was tested in the first trimester, the mean (SD) of serum Anti-TPO was found to be 47.26 (112.84) IU/ml with range from 8.1 IU/ml to 1236.5 IU/ml. **Conclusion:-** Existing findings for trimester-specific reference intervals for thyroid hormones, anti-TPO Ab along with AMH are inconsistent and cannot be extrapolated due to differences in ethnicity, laboratory assay method, and rigor for this study group.

**KEYWORDS :** Pregnancy, Thyroid Function Test, Trimester-Specific, Anti-Müllerian Hormone (AMH)

## INTRODUCTION

Thyroid disease is the second most common endocrine disease to affect women of reproductive age. Thyroid disorders can have adverse reproductive and pregnancy implications. 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.[1,2] In addition to overt thyroid dysfunction, pregnancy may unveil subclinical hyperthyroidism and hypothyroidism.

Women who have been diagnosed with thyroid gland dysfunctions are usually treated and are able to complete a normal pregnancy.

Thyroid-related pathophy siologic changes aggravated by pregnancy, and some obstetric conditions, such as gestational trophoblastic disease or hyperemesis gravidarum, may affect thyroid gland function and impact maternal-fetal thyroid hormone balance. Trimester-specific reference intervals for thyroid function tests are critical for maintaining the delicate balance of thyroid hormones during pregnancy. TSH <2.5 IU/mL and anti-Müllerian hormone (AMH) ≥10pmol/L (1.4 ng/ml) as significant predictors of live births in women with unexplained infertility.[3]

Thyroid hormones can regulate metabolism associated with basal metabolic rate and body composition.[4,5] In general, the thyroid-stimulating hormone (TSH) is positively correlated with BMI, whereas total thyroxine (TT4) and free thyroxine (FT4) are negatively correlated with BMI in adults.[5]

Maternal TSH and FT4 can pass through the placental barrier and affect fetal growth and development, especially before the maturation of the thyroid gland at 18–20 weeks of gestational age.[6] Overt hypothyroidism and hyperthyroidism have a high risk of light birth weight or SGA births (11). Mild thyroid dysfunction, which is more prevalent than overt hypothyroidism, is associated with SGA and LGA according to some studies but not in other studies.[9-12] In euthyroid pregnant women, the effect of thyroid hormones on birth weight remains controversial.[13] In addition, the effects of total

triiodothyronine (TT3) on birth outcomes remain to be confirmed.[14]

Due to the physiological characteristics of pregnancy, thyroid volume increases by 10–40% and is accompanied by a ~50% increase in T3 and T4 levels.[15,16] TSH levels significantly decrease during early pregnancy in response to elevated placental human chorionic gonadotropin (hCG)-stimulating TSH receptors and vary based on geography, ethnicity, BMI, iodine nutritional status, and detection method.[17-23] In 2017, the American Thyroid Association (ATA) recommended the establishment of population-, trimester-, and method-specific reference ranges for thyroid hormones during pregnancy, which is helpful to more scientifically determinate thyroid dysfunction in pregnant women.[24-27]

The objective of our study was to investigate the relationship between trimester-specific maternal thyroid function, AMH and thyroid autoimmunity (anti-TPO Ab), status in pregnant women.

## MATERIAL AND METHODS

The present observational study was conducted on 250 pregnant women in the age group of 20-40 years attending the ANC (antenatal clinic) or maternity wards of obstetrics and gynaecology Department of SMS Medical College and attached groups of hospital, Jaipur (Rajasthan).

### Study Design

The present observational study was conducted in the Department of Biochemistry in association with Department of Gynaecology and Department of Endocrinology.

### Study Area

Tertiary care hospital (Zanana hospital, Jaipur, Rajasthan, 302004). Detailed maternal history of pregnant women was taken, including their mobile no. in consent form to take easy and accessible follow up in each trimester of pregnancy.

### Sample Size:

Sample size was calculated at 95% confidence level, alpha error of 0.05 assuming 11.1 % of autoimmunity positive among pregnant women. As per the reference article "What affects functional ovarian reserve, thyroid function or thyroid autoimmunity?" At an absolute allowable error of 4%, the required sample size was found to be 247 cases of pregnant women in the present study and was further round up to 250 pregnant women for the present study. Considering nearly 10% of lost to follow up 300 pregnant women will be enrolled in the study.

In the study plan we planned to follow the enrolled participants during all three trimesters of pregnancy till the assessment of the outcome. Considering the duration of the study, frequency of follow ups required to record all necessary information and pattern of compliance of pregnant women for frequent and regular ANC checkups in the study area, it is anticipated that there would be considerable instances of loss to follow up till the conclusion of the study.

### Selection Criteria:

### Inclusion criteria:

Those who were willing to participate in the study and able to understand the nature of the study. Primipara/multipara, Age 20-40 years, Hypo/hyperthyroidism, Goiter, Previous Obstetric History, Previous miscarriage, Abortion, Neonatal death, Still births (baby born dead after 24 completed weeks of pregnancy), Full term and preterm deliveries, Pregnancy induced hypertension (PIH), Lower segment caesarean section (LSCS), High risk pregnancy (HRP) etc.

### Exclusion criteria:

Any chronic condition Cancer, Human immunodeficiency virus (HIV) Syphilis, Thyroidectomy (surgical removal of all parts of the thyroid gland) Tuberculosis (TB)

### Detailed Clinical And Obstetrics History:

### Sample Collection:

Blood sample was collected from the anticubital veins of pregnant women at the time of attending the ANC in each trimester. Sample was left standing for one hour; then serum was separated at 2500 rpm in centrifugation machine. Samples were analyzed for thyroid function test ($T_3$, $T_4$ & TSH) and Anti-TPO was done using the electro chemiluminescence (ECL) technique using commercially available kits, serum concentrations of AMH were measured by the ELISA. This experiment uses double-sandwich ELISA technique.

## RESULTS

Total 252 participants were included in the current study. Out of 252 participants, most of the participants (69%) were in the age group of 20 – 24 years. Around a quarter (22%) of the participants were in the age group of 25-29 years, further 8% were in 30-34 years and 1% were in the age group of 35-39 years. (Table.1) Majority of the participants were primigravida (38%) and Secondary gravida (33%), further 16.6% were third gravida, 6% fourth, 4% were fifth, and 1% were of sixth to ninth gravida. (Table.2) More than half of the participants (58%) were having normal weight (BMI between 18.5-24.9), a quarter of them (25%) were underweight (BMI <18.5), 15% were overweight (BMI between 25-29.9) and 2% were obese (BMI>30). (Table.3) Out of the 239 participants who got delivered, there were 201 (84.1%) term deliveries with completed 9 months of gestation. Further, there were 32 (13.4) preterm deliveries out of which 14 (5.9%) delivered at 7 completed months of gestation and 18 (7.5%) delivered at 8 completed months of gestation. Furthermore, there were 6 (2.5%) participants got delivered postdated after their estimated date of delivery. (Table.4). In first trimester, FT3 Mean (SD) was 3.53 (0.59) which range from 2.54 to 4.52 (CI 3.45 – 3.60). FT4 Mean (SD) was 1.32 (0.26) which range from 0.88 – 1.77 (CI 1.29 – 1.35). TSH Mean (SD) was 2.07 (1.01) which range from 0.32 to 4.60 (CI 1.94 – 2.19). In first trimester, the mean (SD) serum TSH level were 2.07 (1.01) mIU/ml which range from 2.54mIU/ml to 4.52mIU/ml (CI 1.94 – 2.19). The mean (SD) serum FT3 level were 3.52 (0.59) pg/ml which ranges from2.54 pg/ml to 4.52 pg/ml(CI 3.45 - 3.60).The mean (SD) serum FT4 level were 1.32 (0.26) ng/ml which ranges from 0.88ng/ml to 1.77ng/ml (CI 1.29 – 1.35) In Second trimester, The mean (SD) serum TSH level were 2.57 (1.18) mIU/ml which range from 0.54mIU/ml to 6.14mIU/ml (CI 2.42 – 2.71). The mean (SD) serum FT3 level were 3.35 (0.82) pg/ml which ranges from 2.02 pg/ml to 4.72 pg/ml (CI 3.25 – 3.45). The mean (SD) serum FT4 level were 1.40 (0.89) ng/ml which ranges from 0.88 ng/ml to 1.77 ng/ml (CI 1.29 – 1.35). In third trimester, The mean (SD) serum TSH level were 2.78 (1.15) mIU/ml which ranges from 0.71 mIU/mlto 4.64mIU/ml (CI 2.64 –2.92). Mean (SD) serum FT3 level were 3.98 (0.58) pg/ml which ranges from 2.02 pg/ml to 4.00pg/ml (CI 2.91 – 3.05). The mean (SD) serum FT4 level were 1.28 (0.27) ng/ml which ranges from 0.83 ng/ml to 1.72 ng/ml (CI 1.25– 1.31). Anti-Mullerian hormone was tested in the first trimester, the mean (SD) of serum AMH was found to be 4.75 (2.79) ng/dl with range from 0.076 ng/dl to 14 ng/dl (CI 4.41 – 5.10). Anti-TPO auto antibody was tested in the first trimester, the mean (SD) of serum Anti-TPO was found to be 47.26 (112.84) IU/ml with range from 8.1 IU/ml to 1236.5 IU/ml (CI 33.33 – 61.19). (Table.5)

### Data Analysis

According to aims and objectives of the study the biochemical parameters data were compiled and entered into MS excel and analysed, using appropriate statistical tests in SPSS (statistical package for social science). For descriptive statistics, frequencies, percentages, standard deviation,

student 't' test, 'p' value, coefficient of correlation(r) and median was calculated. To assess difference between categorical variables 'Chi Square Test' was used.

### Observation Tables

**Table 1. Age wise distribution of the participants**

| Age group (N=252) | Number (%) |
|---|---|
| 20-24 | 173 (68.7) |
| 25-29 | 56 (22.2) |
| 30-34 | 20 (7.9) |
| 35-39 | 3 (1.2) |

**Table 2. Gravida Wise distribution of the participants**

| Gravida | Number (%) |
|---|---|
| 1 | 96 |
| 2 | 82 |
| 3 | 42 |
| 4 | 16 |
| 5 | 10 |
| 6 | 3 |
| 7 | 1 |
| 8 | 1 |
| 9 | 1 |

**Table 3. BMI wise distribution of the participants**

| BMI | Category |
|---|---|
| Normal Weight | 143(58) |
| Obese | 6(2) |
| Overweight | 37(15) |
| Under Weight | 63(25) |
| Total | 249 |

**Table 4. Gestational age at the time of delivery wise distribution of the participants out of those who got delivered**

| Gestation age (in Completed months) | Number (%) |
|---|---|
| 7 | 14 (5.9) |
| 8 | 18 (7.5) |
| 9 | 201 (84.1) |
| Post-dated | 6 (2.5) |
| Grand Total | 239 |

**Table 5. Trimester wise distribution of the FT3, FT4 and TSH**

| Variable | Min | Max | Mean | SD | C I Lower bound | C I Upper bound |
|---|---|---|---|---|---|---|
| 1_trimester_FT3 (pg/ml) | 2.54 | 4.52 | 3.53 | 0.59 | 3.45 | 3.60 |
| 1_Trimester FT4 (ng/ml) | 0.88 | 1.77 | 1.32 | 0.26 | 1.29 | 1.35 |
| 1_TrimesterTSH (mIU/ml) | 0.32 | 4.60 | 2.07 | 1.01 | 1.94 | 2.19 |
| 2_Trimester_FT3 (pg/ml) | 2.02 | 4.72 | 3.35 | 0.82 | 3.25 | 3.45 |
| 2_Trimester_FT4 (ng/ml) | 0.91 | 13.08 | 1.40 | 0.89 | 1.29 | 1.51 |
| 2TrimesterTSH (mIU/ml) | 0.54 | 6.14 | 2.57 | 1.18 | 2.42 | 2.71 |
| 3Trimester FT3 (pg/ml) | 2.02 | 4.00 | 2.98 | 0.58 | 2.91 | 3.05 |
| 3_Trimester_FT4 (ng/ml) | 0.83 | 1.72 | 1.28 | 0.27 | 1.25 | 1.31 |
| 3TrimesterTSH (mIU/ml) | 0.71 | 4.64 | 2.78 | 1.15 | 2.64 | 2.92 |
| ANTI-TPO (IU/ml) | 8.1 | 1236.5 | 47.26 | 112.84 | 33.33 | 61.19 |
| AMH (ng/ml) | 0.076 | 14 | 4.75 | 2.79 | 4.41 | 5.10 |
| | | | | | | |

## DISCUSSION

In this cross-sectional study, we have established gestational age-specific reference intervals for TFT in pregnant women after using rigorous exclusion criteria, i.e. any history of chronic illness, goiter on physical examination, thyroid illness in the past or present, consuming thyroid medications (current and past), family history of thyroid illness, presence of anti-TPO, poor obstetrics history included 3 or more abortions. This current study established the trimester-specific FT3, FT4, and TSH hormones range in pregnant women from a tertiary care center in Jaipur, India. Existing results for trimester-specific reference intervals for thyroid hormones are inconsistent and cannot be extrapolated due to differences in ethnicity, maternal iodine status, laboratory assay method, and rigor for selection of reference population. Thus, the establishment of reference intervals in each region is of great importance

**Rajesh Rajput et al. 2016** evaluated, the 2.5–97.5th percentiles for FT3, FT4, and TSH obtained in this study were 2.53–4.54 pg/ml, 0.88–1.78 ng/ml and 0.37–3.69 μIU/ml in the first trimester, 2.0–4.73 pg/ml, 0.91–1.78 ng/ml and 0.54–4.47 μIU/ml in the second trimester, 2.01–4.01 pg/ml, 0.83–1.73 ng/ml, and 0.70–4.64 μIU/ml in the third trimester of pregnancy. Mean TSH increased and mean FT3 decreased significantly with the progression of gestational period. FT4 decreased from trimester 1–3rd, but the decrease was insignificant from 2nd to 3rd trimester. The present study findings were very close to this study.

**Raghunath Bhattacharyya et al. 2015** evaluated 11.5% of the subjects were positive for anti-TPO-Ab who had mean TSH level of 2.31 μIU/ml, which was significantly (P- 0.0001) higher than pregnant women negative for anti-TPO-Ab (1.73 μIU/ml). Increased incidence of miscarriage was observed in anti-TPO positive mothers when compared to antibody negative mothers. Postpartum thyroid dysfunction developed in 4.7% cases at 12 weeks, among them antibody positivity was observed in 81.25% of subjects. In 18.75% mothers positive for anti-TPO-Ab, the thyroid dysfunction persisted up to 12 months postpartum.

**Zareen Kiran et al. 2021** included, Overall, 146 out 718 cases were included for final analysis. Thyroid peroxidase antibodies were positive in 66.4% and anti-thyroglobulin was positive in 52.1% cases, whereas 43.8% of cases had both antibodies positive. Pregestational diabetes was significantly associated with thyroid autoimmunity. There was a 73% less chance of gestational hypertension for thyroid autoimmune groups. Gestational diabetes and maternal (chronic) hypertension were found to have an independent effect on postpartum hemorrhage. Hypertensive disorders in pregnancy were found to have an independent risk for premature birth. Our observations were found to be same these results significantly.

**Andrea Weghofer et al. 2016** evaluated Mean age of studied women was 38.4 ± 5.0 years; their mean AMH was 1.3 ± 2.0 ng/mL and mean TSH 1.8 ± 0.9 IU/mL. Thyroid autoimmunity was present in 11.1 % of patients. Mean age for study participants was 38.4 ± 5.0 years. Mean AMH levels were 1.3 ± 2.0 ng/mL, mean TSH levels were 1.8 ± 0.9 IU/mL. Body mass index (BMI) and ethnicity were comparable between TSH groups (p = 0.58 and p = 0.49). Thyroid autoimmunity was present in 11.1 % of all patients. TPO antibodies were positive in 11.1 % of patients and TG antibodies in 1.8 % of women.

There were no patients with thyroid receptor antibodies. Patients with low-normal and high-normal TSH levels were of comparable age (i.e. 38.3 ± 5.1 vs. 38.9 ± 4.4 years, P = 0.67). Women with high-normal TSH levels presented with thyroid autoimmunity in 26.9 %, while thyroid autoimmunity was present in only 9.0 % of women with low-normal TSH levels (P = 0.01). Thyroid autoimmunity was mainly attributed to TPO

antibodies. Somaye Gholami et al.2021 evaluated, the mean age of the participants was 28.78 (± 5.86 yr) (range: 15-45 yr). The thyroid hormones reference intervals in the first trimester were TSH (0.2-3.8 mIU/l), T4 (7.45-12.75, g/dl), and T3 (100-217 ng/dl) these observations were found to be similar with our present study.

Deshwal et al. [8] in their study reported that FT3, FT4 decreased, and TSH increased with the progression of the gestational period as is observed in this study. Another study carried out by Kumar et al. from India evaluated 124 pregnant women using radioimmunoassay showed an increase in TSH progressively with each trimester while serum triiodothyronine (T3) and thyroxine (T4) values increased from first to second trimester and declined from second to third trimester. This pilot study showed that the range of T3 was 1.7–4.3 nmol/L in second trimester and 0.4–3.9 nmol/L in third trimester, T4 as 92.2–252.8 nmol/L in second trimester and 108.2–219.0 nmol/L in third trimester, and TSH as 0.1–5.5 $\mu$lU/L in second trimester and 0.5–7.6 $\mu$lU/L in third trimester of pregnancy, respectively. Soldin et al. showed that FT4 decreased from the 1st to 3rd trimester ($P < 0.001$), but there was no significant difference between the second to the third trimester. TSH increased from 1st to 3rd trimester but remains static between 2nd and 3rd trimester. Similar changes were observed in this study where mean TSH increased and mean FT3 decreased significantly with the progression of the gestational period while FT4 decreased from trimester 1st to 3rd but the decrease was nonsignificant from 2nd to 3rd trimester.[29]

Another cross-sectional study by Thevarajah et al.showed that mean TSH levels decreased during the first trimester and then increased significantly ($P < 0.05$) in the second and third trimester similar to our study and mean FT3and FT4 values declined from the first trimester that the decrease was significant in the second and third trimesters ($P < 0.05$). However, the values of TSH reported from these studies are quite different from values reported in the present study as well as from some of the Indian studies. Normal upper limit of TSH in pregnancy has been a subject of debate since a long time. The endocrine society guidelines for thyroid dysfunction in pregnancy published in 2012 have lowered the upper limit of reference range for normal TSH and suggested 0.1–2.5 mIU/L, 0.2–3.0 mIU/L and 0.3–3.0 mIU/L, respectively, in first, second, and third trimester of pregnancy.[29] However, the American Association of Clinical Endocrinology and the Endocrine Society Consensus panel recommended that 4.5 mIU/L should be maintained as the upper limit of normal. They reasoned that although some individuals within the range of 2.6–4.5 mIU/L may have subclinical thyroid disease, there was a lack of evidence of adverse outcome in this group.[30]

Several studies across the globe are available on assessment of TFT among pregnant women with marked heterogeneity like the difference in study design with some being cross-sectional while others being longitudinal, use of rigorous exclusion criteria to define reference population and laboratory method used to estimate T3, T4, and TSH [Table 2]. Soldin et al. showed that FT4 decreased from the 1st to 3rd trimester ($P < 0.001$), but there was no significant difference between the second to the third trimester. TSH increased from 1st to 3rd trimester but remains static between 2nd and 3rd trimester. Similar changes were observed in this study where mean TSH increased and mean FT3 decreased significantly with the progression of the gestational period while FT4 decreased from trimester 1st to 3rd but the decrease was nonsignificant from 2nd to 3rd trimester. These evidences significantly similar to our current observations.[31]

Another cross-sectional study by Thevarajah et al. showed that mean TSH levels decreased during the first trimester and then increased significantly ($P < 0.05$ in the second and third trimester similar to our study and mean FT3and FT4 values declined from the first trimester that the decrease was significant in the second and third trimesters ($P < 0.05$). Our current study results were found to similar with this study significantly.[32]

## CONCLUSIONS

Existing findings for trimester-specific reference intervals for thyroid hormones, anti-TPO Ab along with AMH are inconsistent and cannot be extrapolated due to differences in ethnicity, laboratory assay method, and rigor for this study group.

### Disclosure Statement

This study is investigator initiated. The authors declare that they have no competing/conflict of interests in relation to this article.

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No financial support from an external agency was used for this study.

### Consent For Publication

- Not applicable.
- Ethics approval and consent to participate.
- The study protocol was approved by the medical ethics committee of the Rajasthan University of Health Sciences, Jaipur, Rajasthan (State), India.

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