ORIGINAL ARTICLE

# Establishing center-based first trimester specific reference range of thyroid stimulating hormone in healthy pregnant women visiting a tertiary care hospital in Bengaluru, Karnataka, India

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## Abstract

*Background:* In India, in the current scenario, largely, non-pregnant reference intervals of thyroid hormones are being used for diagnosis of gestational thyroid dysfunction thus having direct impact on clinical decision making. International guidelines strongly recommend using local trimester-specific Thyroid Stimulating Hormone (TSH) reference range for assessing the thyroid status during pregnancy. However, till date region oriented and trimester specific data for thyroid profile hormones in Indian pregnant women has not been established. The present study therefore aimed to establish a centre-based first trimester specific reference range of TSH in pregnant women who come for ante-natal visit at a tertiary care centre of South Bengaluru. *Aim and Objectives:* The primary objective was to estimate TSH, free T3 (fT3), total T3 (TT3), free T4 (fT4), total T4 (TT4) and antithyroid peroxidase (anti- TPO) antibodies in the subject population. The secondary objective of the study was to establish 1st trimester specific reference ranges of TSH. *Material and Methods:* A total of four hundred eighty-eight women comprising the reference population were recruited into the project based on strict inclusion and exclusion criteria. Blood samples were collected and serum levels of thyroid hormones and anti-TPO antibodies were estimated by chemiluminescence immunoassay. Results obtained were analysed to obtain first trimester specific Reference Intervals (RI) of thyroid hormones. *Results:* The RI were calculated by percentile method as recommended by International Federation of Clinical Chemistry. The RI of TSH: 0.26 - 4.36 µIU/ml, fT3: 2.78 – 4.39 pg/ml; TT3: 0.89 -1.82 ng/ml; fT4: 0.69 – 1.24 ng/dl; and that of TT4 was 7.41 - 18.01µg/dl. *Conclusion:* The incorporation of population, assay and trimester specific reference range of TSH to screen and detect thyroid disorder in pregnancy will eradicate the perpetual errors caused by unempirical reporting methodologies.

**Keywords**: First trimester, Reference interval, Thyroid hormones, TSH, Trimester specific reference range

## Introduction

The two principal hormones, the Triiodothyronine (T3) and Thyroxine (T4) secreted by the thyroid gland are vital during pregnancy as they are responsible for the normal growth of the foetus. These two thyroid hormones regulate organogenesis, primarily the fetal brain development [1]. Subtle variations in thyroid function have been associated with maternal and foetal morbidity raising

concerns. The recent global statistics of thyroid disorders during pregnancy reveals an alarming 5 to 7% prevalence which is unnerving. Overt and subclinical hypothyroidism in pregnant women has a prevalence of about 2-3% and 3–15% respectively and 0.4–1.7% of pregnant women across the world are hyperthyroid [2]. An epidemiological survey involving eleven major cities of India has disclosed 13.31% prevalence of hypothyroidism with subclinical hypothyroidism being the predominant thyroid condition among pregnant women [3].

Pregnancy then again is associated with significant physiologic and metabolic alterations in thyroid gland, resulting in variations in maternal thyroid profile. The maternal thyroid gland is stimulated to secrete up to one and half times more thyroid hormones fostered by circulating estrogen, β-human chorionic gonadotropins (β-HCG) and also to meet the increased demand by the growing foetus [4]. Study by Munde *et al.* (2022) reveal that a slight increase in thyroid stimulating hormones in women was associated with metabolic syndrome [5]. Similar research conducted by Anwar *et al.* (2024) report that thyroid dysfunction and metabolic syndrome are interlinked more so in subclinical hypothyroidism [6].

These studies emphasize the fact that thyroid fluctuations are associated with cardiovascular disease risk and close monitoring of these parameters with a well-defined reference range is imperative [5,6]. This results in subsequent suppression of release of thyroid Stimulating Hormone (TSH) from the anterior pituitary. Consequently, the reference range of thyroid profile parameters applicable to the non-pregnant population does not hold good to pregnant women.

The estimation of serum TSH is regarded as the reliable parameter to diagnose thyroid dysfunction during pregnancy [7]. Therefore, it is obligatory to determine pregnancy specific reference range of TSH which will aid to appropriately diagnose thyroid diseases in pregnant women. In the year 2011, American Thyroid Association (ATA) recommended the trimester specific reference ranges for TSH values of 0.1-2.5 mIU/L (first trimester), 0.2-3.0 mIU/L (second trimester), and 0.3-3.5 mIU/L (third trimester) [8]. However, in the 2017, the ATA revised first trimester TSH reference range upper limit to 4.0 mIU/L [9].

The use of international guidelines for the assessment of thyroid status in the local population is highly debatable. In addition, multifaceted ethnicity, geographic and racial diversity in India does not permit the use of universal trimester specific cut off values of TSH established by ATA and other international scientific societies. Further the extrapolation of the few existing Indian data as a unified pan India normative range and 'one size fit all' concept does not seem to apply to India. International guidelines strongly recommend using local trimester-specific TSH reference range for assessing the thyroid status during pregnancy [7].

Incidentally, there is no data available till date which reports to have established trimester specific reference interval for thyroid profile hormones in pregnant women in the entire region of Karnataka, a Southern state of India. With the urge to fill this lacuna, the current study aimed to establish a centre-based first trimester specific reference range of TSH in pregnant women who come for ante-natal visit at this tertiary care centre of South Bengaluru.

## Material and Methods

This is a cross-sectional study that was conducted at Rajarajeswari Medical College and Hospital, Bengaluru for a duration of three years from April

2021 to March 2024. Department of Biochemistry and Department of Obstetrics and Gynaecology collaborated to undertake this project. Before commencement of the project, approval was obtained from Institutional Ethics Committee. The pregnant women in first trimester of pregnancy who conformed to our inclusion criteria and gave an informed written consent were recruited as subjects. A detailed history recording and clinical examination of these pregnant women ensured their compliance to be participants of this research project. The pregnant women between 18 to 35 years of age with singleton intrauterine first trimester of pregnancy (4–12 weeks gestation) confirmed by last menstrual period and ultrasonography were orally enquired about adequate iodine intake in their diet by use of packaged salt or unpacked salt routinely. Women with history of consumption of packaged salt routinely were included in the present study.

Pregnant women with any history of or current thyroid illness, bad obstetric history, multiple pregnancy, history of medical, surgical or psychiatric illness were excluded. Six hundred and seven pregnant women enrolled to be part of the study, after stringent scrutinization. Five ml venous blood sample was drawn under all aseptic precautions from these women and the thyroid parameters, TSH, free Triiodothyronine (fT3), Total Triiodothyronine (TT3), free Thyroxine (fT4), total Thyroxine (TT4), and anti-Thyroid Peroxidase (anti-TPO) assays were performed. Twenty-one samples were rejected due to pre-analytical errors and another eighty-five subjects' samples were expelled at this stage from the study as they tested positive for anti-TPO. Thirteen samples with grossly abnormal TSH (TSH $\leq$ 0.03 and $\geq$ 10.0 mIU/L) values were dropped-out of the study here. Finally, four hundred eighty-eight women constituted the reference population. These details are depicted in the flow chart below (Figure 1).

The study was undertaken to estimate the values of TSH, free T3, TT3, free T4, TT4 and anti-TPO antibodies in the subject population and to establish 1st trimester specific reference ranges of TSH based on their 2.5th and 97.5th percentile values as per International Federation of Clinical Chemistry (IFCC) guidelines [10].

All thyroid assays were performed by Chemiluminescence (CLIA) method on Beckman Coulter-Access- 2 analyzer using commercially available kits from the same company. Quality Control (QC) serum from Biorad (Lyphocheck Immunoassay) was used to assess performance quality. Before analysis, the instrument was calibrated and quality - controls (two levels) were run and QC values were recovered. TSH and anti-TPO assays were based on "Sandwich" immunoassay principle, while fT3, TT3, fT4 and TT4 were based on "Competitive" immunoassay. The Access TSH (3rd IS) assay had a functional sensitivity of 0.01 – 0.02µIU/ml and anti-TPO assay offered a cut-off of $\leq$ 9 IU/ ml. fT3, fT4, TT3 and TT4 had analytical sensitivity of 0.88pg/ml, 0.25ng/dl, 0.1 ng/ml and 0.5µg/dl respectively.

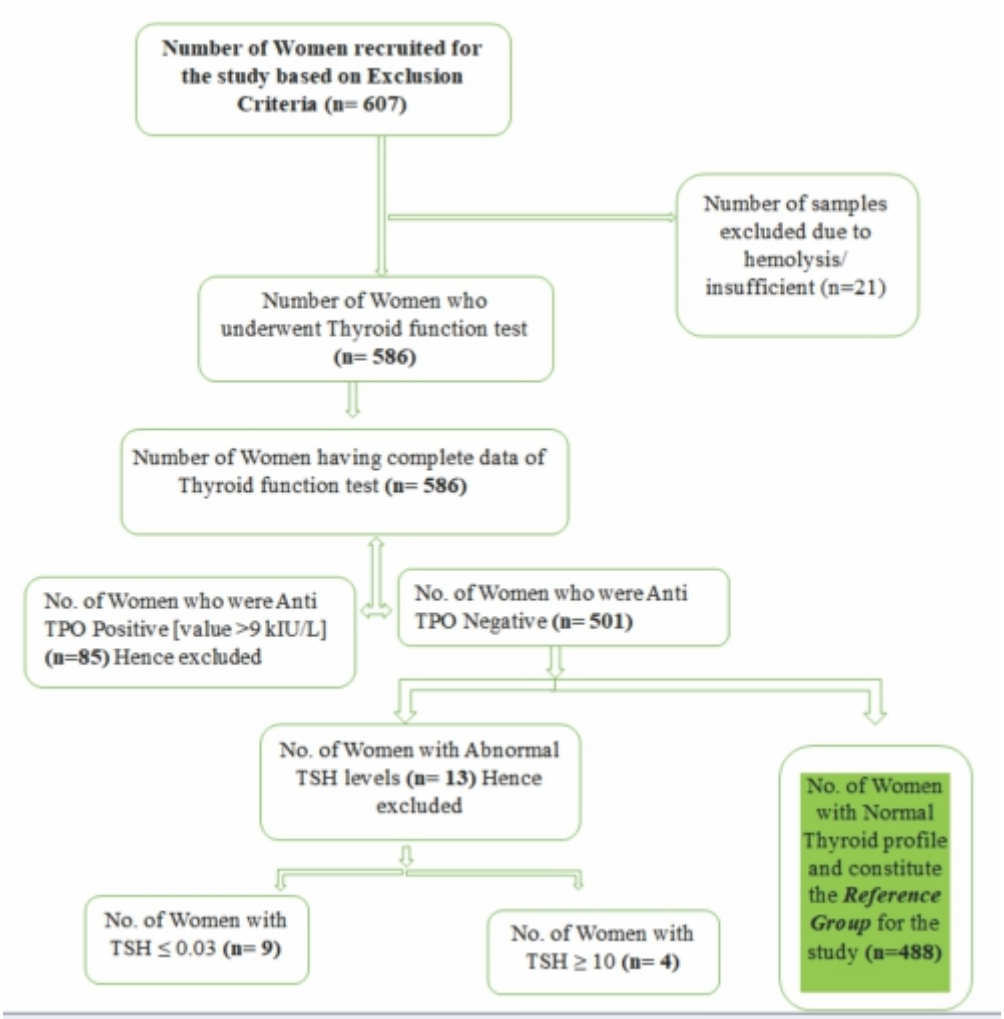


**Figure 1: Flow chart depicting the selection of reference population of the study**

*Anti-TPO – Anti-thyroid Peroxidase; TSH – Thyroid Stimulating Hormone*

**Table 1: Demographic characteristics of the study subjects**

| Parameter | Value (n=488) |
|---|---|
| Mean ± standard deviation of age in years | 24.88 ± 4.14 years |
| Mean ± standard deviation of gestational age at presentation | 8.27 ± 2.20 weeks |
| Mean Blood pressure | 110/75 mm Hg |

**Table 2: Description of histographical representation of TSH distribution in the study population**

| Groups | TSH |
|---|---|
| Sample size (n) | 488 |
| Mean ($\bar{x}$) | 2.057525 |
| Skewness | 1.530924 |
| Skewness Shape | Asymmetrical |
| Excess kurtosis | 2.98403 |
| Tails Shape | Leptokurtic |
| Outliers | 5.04, 5.07, 5.18, 5.19, 5.25, 5.31, 5.33, 5.34, 5.34, 5.35, 5.56, 5.56, 5.7, 5.8, 5.95, 5.96, 6, 6.36, 6.74, 6.84, 7.08, 7.24, 7.36, 7.78, 8.75, 9.04 |

*TSH – thyroid stimulating hormone*

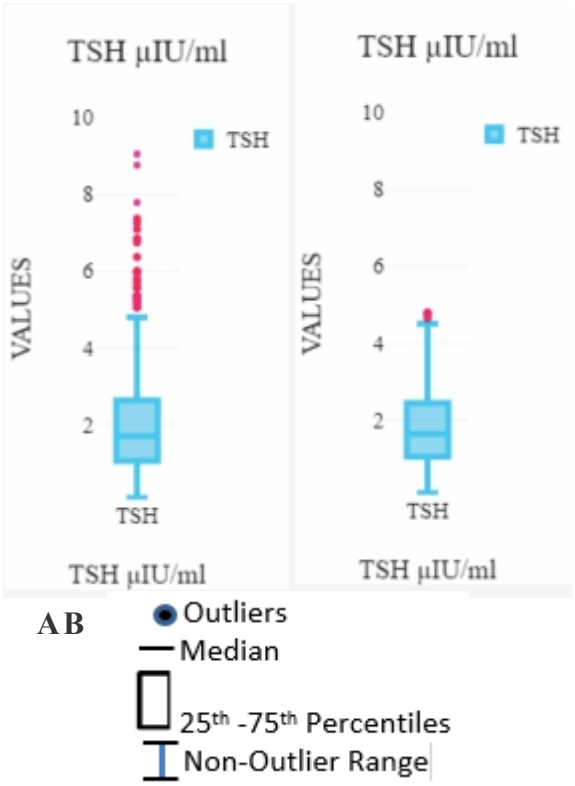


**Figure 2: Box plots of TSH: Box plot A showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers and outliers represented as dots. Box plot B showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers with few outliers very close to max value**

**Table 3: Description of histographical representation of fT3 distribution in the study population**

| Groups | fT3pg/ml |
|---|---|
| Sample size (n) | 488 |
| Mean ($\bar{x}$) | 3.530287 |
| Skewness | 0.659338 |
| Skewness Shape | Asymmetrical |
| Excess kurtosis | 4.841506 |
| Tails Shape | Leptokurtic |
| Outliers | 4.75, 4.72, 4.67, 5.07, 5.82, 5.76, 5.37, 1.97, 5.29, 5.9, 4.82, 0.52, 5.58, 4.83, 4.75 |

*Interpretation: fT3 is slightly right skewed with few outliers*
*fT3 – free T3*

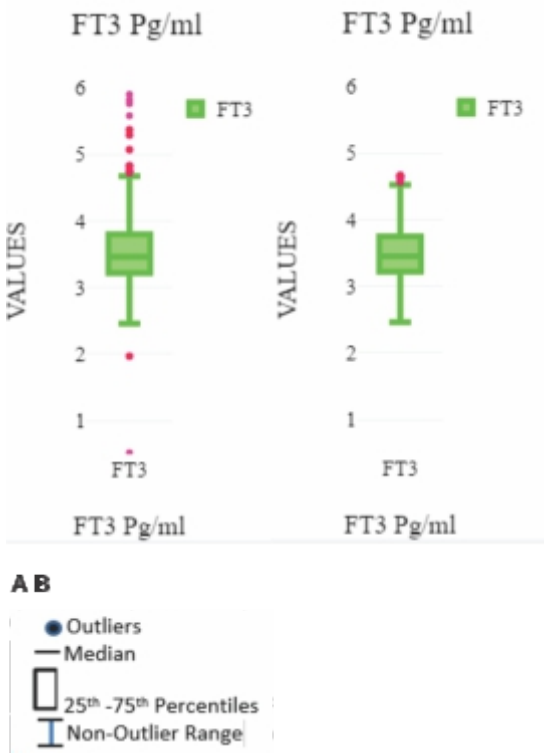


**Figure 3: Box plots of fT3: Box plot A showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers and outliers represented as dots. Box plot B showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers with few extreme values**

*fT3 – free T3*

**Table 4: Description of histographical representation of fT4 distribution in the study population**

| Groups | fT4ng/dl |
|---|---|
| Sample size (n) | 488 |
| Mean ($\bar{x}$) | 0.931025 |
| Skewness | 5.191304 |
| Skewness Shape | Asymmetrical |
| Excess kurtosis | 67.374502 |
| Tails Shape | Leptokurtic |
| Outliers: | 1.43, 1.39, 1.4, 1.49, 1.38, 0.22, 3.56 |

*Interpretation: fT4 is slightly skewed with few outlier*
*fT4 – free T4*


**Figure 4: Box plots of fT4. Box plot A showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers and outliers represented as dots. Box plot B showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers without outliers**

*fT4 – free T4*

**Table 5: Description of histographical representation of TT3 distribution in the study population**

| Groups | T3 |
|---|---|
| Sample size (n) | 488 |
| Mean ($\bar{x}$) | 1.332705 |
| Skewness | 0.749897 |
| Skewness Shape | **Asymmetrical** |
| Excess kurtosis | 1.094596 |
| Tails Shape | **Leptokurtic** |
| Outliers | 2.21, 2.13, 2.05, 2.07, 2.16, 2.01, 2.16, 2.28, 2.5, 2.09 |

*Interpretation: TT3 is slightly right skewed with few outliers.*
*TT3 – Total T3*

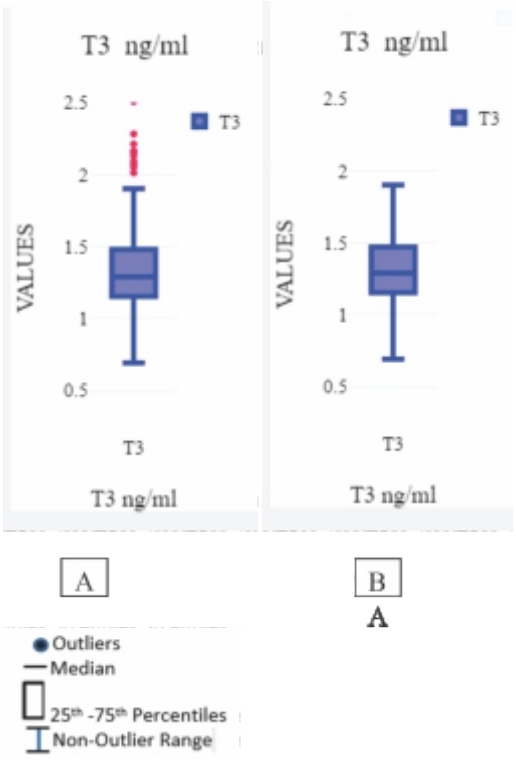


**Figure 5: Box plots of Total T3. Box plot A showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers and outliers represented as dots. Box plot B showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers without outlier**

**Table 6: Description of histographical representation of total T4 distribution in the study population**

| Groups | T4 |
|---|---|
| Sample size (n) | 488 |
| Mean ($\bar{x}$) | 12.409262 |
| Skewness | 0.683233 |
| Skewness Shape | Asymmetrical |
| Excess kurtosis: | 1.500985 |
| Tails Shape: | Leptokurtic |
| Outliers: | 20.57, 20.95, 22.92, 20.25, 23.21, 26.57 |

*Interpretation: TT4 is slightly right skewed with few outliers.*
*TT4 – Total T4*

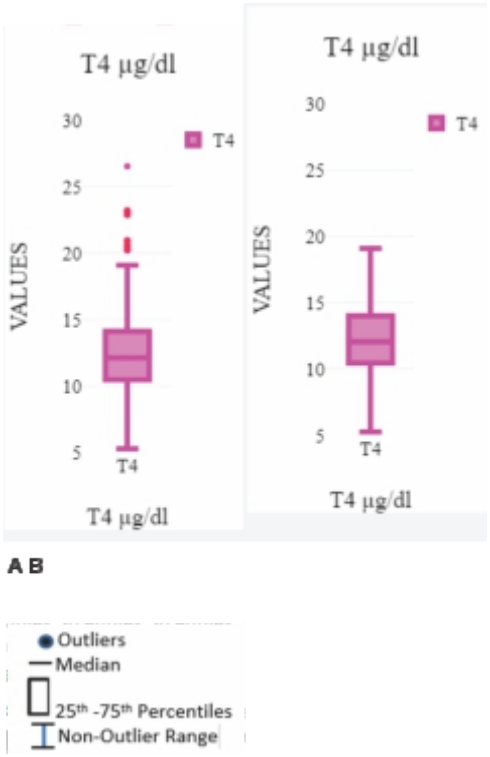


**Figure 6: Box plots of Total T4. Box plot A showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers and outliers represented as dots. Box plot B showing the 25th, 50th (median) and 75th percentile, non-outlier range within whiskers without outliers**

**Table 7: Summary statistics of thyroid parameters**

| Statistic | T3 ng/ml | T4 µg/dl | TSH IU/ml | fT3 pg/ml | fT4 ng/dl |
|---|---|---|---|---|---|
| **Mean** | 1.31 | 12.28 | 1.83 | 3.49 | 0.92 |
| **Standard Deviation** | 0.24 | 2.64 | 1.08 | 0.41 | 0.14 |
| **Standard Error of Mean** | 0.0109 | 0.1202 | 0.0502 | 0.0188 | 0.0064 |
| **Median** | 1.29 | 12.05 | 1.63 | 3.45 | 0.91 |
| **Max** | 1.9 | 19.11 | 4.79 | 4.66 | 1.30 |
| **Min** | 0.69 | 5.24 | 0.08 | 2.46 | 0.56 |
| **Range** | 1.21 | 13.87 | 4.71 | 2.2 | 0.74 |
| **2.5$^{th}$ percentile** | 0.89 | 7.41 | 0.26 | 2.78 | 0.69 |
| **97.5$^{th}$ percentile** | 1.82 | 18.01 | 4.36 | 4.39 | 1.24 |
| **95% CI for the mean** | 1.288-1.331 | 12.044-12.516 | 1.732-1.928 | 3.453-3.527 | 0.907-0.933 |
| **95% CI for 2.5$^{th}$ percentile** | 0.868-0.911 | 7.174-7.645 | 0.161-0.358 | 2.743-2.816 | 0.677-0.702 |
| **95% CI for 97.5$^{th}$ percentile** | 1.798-1.841 | 17.774 – 18.246 | 4.261-4.458 | 4.353-4.423 | 1.227-1.253 |

*CI – Confidence Interval; TSH – Thyroid Stimulating Hormone; fT3 – free T3; fT4 – free T4*

**Table 8: Comparison of TSH RI established by various studies from across India**

| Author and year of publication | City | Assay method | Number of study subjects | TSH Values µIU/ml | | |
|---|---|---|---|---|---|---|
| | | | | Mean | Median | 2.5 to 97.5 percentile |
| Marwaha *et al*. (2008) [16] | New Delhi | ECLIA | 107 | 2.42 ± 1.65 | 2.1 | 0.6-5.0 (5-95 percentile) |
| Maji *et al*. (2014) [17] | Kolkata | ELISA | 125 | 1.81 ± 0.79 | 1.80 | 0.25-3.35 |
| Rajput *et al*. (2016) [18] | Haryana | ECLIA | 301 | 1.63 ± 1.02 | 1.40 | 0.37-3.69 |
| Jebasingh *et al*. (2016) [19] | Manipur | CLIA | 109 | 1.06 | 1.08 | 0.2-1.82 (5-95 percentile) |
| Mankar *et al*. (2016) [20] | Nagpur | CLIA | 50 | 1.65 ± 0.85 | NA | 0.24-4.17 (5-95 percentile) |
| Sekhri *et al*. (2016) [21] | New Delhi | ECLIA | 86 | 2.07 ± 1.46 | 1.89 | 0.09-6.65 |

*Continued...*

| | | | | | | |
|---|---|---|---|---|---|---|
| Chakrabarty *et al*. (2017)[22] | Ranchi | ELISA | 191 | 2.55 ± 1.98 | NA | 2.26-2.84 |
| Rani *et al*. (2018) [23] | Nellore | CLIA | 47 | 1.11± 0.57 | 1.12 | 0.08 to 2.24 |
| Pramanik *et al*. (2020) [24] | Kolkata | CLIA | 80 | NA* | NA* | 0.19-4.34 |
| Nanda *et al*. (2021)[25] | Chattisgarh | CLIA | 341 | 2.04 ± 1.30 | 1.82 | 0.25-4.97 |

*(* data not available)*

*ECLIA - Electrochemiluminescence Immunoassay; ELISA - Enzyme-Linked Immunosorbent Assay; CLIA - Chemiluminescence Immunoassay; TSH – Thyroid Stimulating Hormone; RI – Reference Interval*

**Statistical analysis**

All analyses were performed with Statistical Package for the Social Sciences software version 23 and MS Excel; continuous data variables were expressed as the mean ± standard deviation / median, non-parametric percentiles according to whether they had a normal or skewed distribution respectively. The nature of distribution of TSH, fT3, TT3, fT4 and TT4 for the first trimester was examined by histogram and confirmed by Kolmogorov Smirnov test. Aberrant values were identified using box plots; identified probable outliers were reconfirmed by applying Tukey's method and the confirmed outliers were removed from the reference sample group after which reference interval was determined. Reference Interval (RI), according to ISO15189, is the interval between and including 2 reference limits. Most often, the central 95% distribution between 2.5 and 97.5 percentiles are used to calculate RIs. In this study, RIs for all five parameters were calculated from the central 95% of the distribution of the data between the lower reference limit of 2.5 percentile and upper reference limit of 97.5 percentile values.

**Results**

In all the five-histogram presented above, it can be noted that the graph distribution is leptokurtic, with more data points concentrated around the mean (average) and heavier tails compared to a normal distribution. The frequency of distribution of data set points of all five parameters of the study do not follow normal distribution which can be appreciated from the histograms above. The statistical experts recommend that the data that do not follow a normal distribution should be presented as median and the Interquartile Range (IQR) and analysed with non-parametric tests [11]. Thus, in the present study we have chosen median over mean to establish RI of TSH and the same is seen depicted in the box-plots of thyroid analytes.

**Discussion**

TSH is the single sensitive assay highly endorsed by the obstetricians to screen and detect thyroid dysfunction in pregnant women across the globe. The onus is on the clinical biochemist to provide an accurate TSH assay report of the pregnant woman with the precise reference range for the interpretation of her thyroid status. A narrow reference range may over-diagnose and a wider reference range can under-diagnose thyroid status which may have an adverse impact on the growing foetus. This can happen when clinical laboratories

either follow the reference range suggested in the kit inserts of the reagents used or incorporate the ranges recommended by standard authorities or boards. An explicit reference range for the given population established by the laboratory alone can give a true interpretation of the measured thyroid parameter value.

In the present study we have estimated five thyroid profile hormones, TSH, fT3, TT3, fT4 and TT4. Reference intervals of these hormones in the first trimester along with 95% CI for both 2.5$^{th}$ and 97.5$^{th}$ percentile is represented in Table 7. The study design, objective and focus however was laid on to establish the reference range of the most important hormone TSH. The reference range of TSH of women in first trimester of pregnancy in the study cohort was 0.26 - 4.36 µIU/ml. The mean ± SD and median values were 1.83 ± 1.08 and 1.63 µIU/ml respectively. The endeavour to put-forth trimester specific RI of TSH has been made by many researchers across the globe. Mehran *et al*. in 2013 conducted a study involving 152 healthy Iranian pregnant women and defined RI of TSH in first trimester of pregnancy with 5$^{th}$ to 95$^{th}$ percentile cut-off as 0.2–3.9mIU/L [12]. Moon *et al.* in a similar study in 2015 involving 265 Korean women stated a RI of TSH in first trimester of pregnancy was 0.01-4.10 mIU/L. They estimated TSH by Electrochemi-luminescence (ECLIA) and non-parametric analysis between 2.5-97.5 percentile was perform-ed [13]. Matyja *et al*. have reported TSH reference range of 0.009–3.1mIU/L in a reference population of 152 pregnant women in Poland in research published in the year 2017 [14].

A recent study in Italy on 150 Caucasian pregnant women conducted by Dorizzi *et al*. in 2022 have mentioned that RI of TSH in first trimester was 0.34-3.81 mU/L [15]. The studies quoted here demonstrate different upper limit of the reference range of TSH. Centre-based studies to determine trimester specific reference range for TSH in Indian pregnant women from different regions of our country in the recent past are compiled and presented in Table 8. No consensus can be drawn with respect to TSH RI in any of the project cited in the table with our study. The one that comes close to RI of the present study is the study conducted by Pramanik *et al*. (2020) where the TSH reference interval was 0.19–4.34 mIU/L [24]. Rest of the studies divulge diverse reference intervals of TSH in their population. It is quite evident from the above quoted international and national research projects that the reference interval of TSH differ from region to region and therefore establishment of region and population specific TSH RI is fundamental.

There exists a log-linear rather than a linear relation between fT3, fT4 and TSH. A minor alteration in fT3 and fT4 leads to large change in TSH, on the contrary, narrow change in TSH causes only slight variation fT3 and fT4 levels in the body [26]. This proves that TSH is sensitive and highly responsive to slight change in circulating thyroid hormones and thus to thyroid function. Indeed, TSH is the primary and best test to evaluate the thyroid status and more so during pregnancy where TSH along with other partial-structurally analogous anterior pituitary hormones like luteinizing hormone, follicular stimulating hormone and Human Chorionic Gonadotropin (hCG) dominates the circulation and dictate the maternal physiology [27].

The hCG secreted initially by the embryo and thereafter by the syncytiotrophoblasts has structural similarity with TSH. hCG, thus has mild thyrotropic activity which it mediated through TSH receptors. As hCG level peaks between 9$^{th}$ and 11$^{th}$ week of gestation, there is a corresponding TSH decrease in

circulation [28]. Also increase in plasma volume, thyroid binding proteins and circulating estrogen levels further modify thyroid function during pregnancy [29]. All these factors limit the application of RI of TSH and thyroid hormones of the non-pregnant population to first trimester pregnant women population. The reference interval of TSH in the current study is higher than that of kit insert which is 0.05-3.70 µIU/ml. Use of the upper cut off of TSH in kit insert (3.7 µIU/ml) in place of the newly established upper cut-off (4.36µIU/ml) could misclassify 5.8% of study subjects as hypothyroid, leading to misdiagnosis.

In studies done by Pramanik *et al.* (2020) and Chandrasekhar *et al.* (2019) the reference range for TSH in first trimester of pregnancy was 0.19–4.34 µIU/ml and 0.43-4.5 mU/L (µIU/ml) respectively which are on par with our obtained reference ranges. Apart from iodine status, ethnicity, locality and regional food habits may also influence the reference range in pregnant women as in both West Bengal and Andhra Pradesh the staple food is rice, similar to Karnataka [24,30]. As fortification is done with iodine to almost all brands of packed salt in and around Bengaluru as per Government regulations, iodine status may not vary among individuals significantly. The newly established reference range of TSH will now replace the previously operative non-conforming range in the thyroid profile reports of the Clinical Biochemistry laboratory of our institute. The strength of this study is the sample size (n = 488) in accordance with IFCC guidelines and stringent criteria used in recruiting reference population.

**Limitations of the study**

Thyroid ultrasound was not done. Iodine excretion test to assess iodine status was not done. This is a mono-centric study.

**Conclusion**

The newly established reference range of TSH is optimized and conforming to the local population which will aid in factual interpretation of thyroid status of pregnant women in first trimester of pregnancy. The laboratory validated reference range will enhance quality of the patient-care and contribute to overall improvement in the health status of the society.

**Acknowledgement**

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**Disclosure**

This descriptive study proposal was designed and submitted to Indian Council of Medical Research on their notification inviting research projects under extramural research program 2020 across the country. The study was then approved and funded by **ICMR (Unique ID: 2020-1225).**

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