# Reference intervals in evaluation of maternal thyroid function of Manipuri women

### Felix K. Jebasingh, Ranabir Salam, Trinity Laishram Meetei<sup>1</sup>, Premchand Thangjam Singh, Nabakishore Naorem Singh<sup>1</sup>, Lallan Prasad

Departments of General Medicine and <sup>1</sup>Obstetrics and Gynaecology, Regional Institute of Medical Sciences, Imphal, Manipur, India

#### ABSTRACT

**Context:** The population of Manipur is of different ethnic background from the rest of the country. Several authors have suggested population/ethnic and laboratory specific reference range of maternal thyroid profile of different trimesters. **Aims:** To find the reference range of thyroid stimulating hormone (TSH), total thyroxine (TT4) and total tri-iodothyronine (TT3) levels for normal pregnant women of native Manipur descendants. **Settings and Design:** The cross-sectional study was conducted at a teaching Institute after ethical clearance was obtained. **Subjects and Methods:** A reference populations of 375 normal pregnant women were established after screening about 600 pregnant women. The study excluded patients with hyperemesis gravid arum, past history or family history of thyroid disorders as well as the connective tissue disorders, WHO grade 1 or 2 goiter, or any medications that alter thyroid functions. The serum levels of TSH, TT4, and TT3 were measured using chemiluminescence assay. **Statistical Analysis Used:** Data for TT3 and TT4 were expressed as mean ± standard deviation, median and 5–95<sup>th</sup> percentiles. **Results:** The mean TSH in the three trimesters was 1.06 + 0.45, 1.23 + 0.30, and 1.25 + 0.36, respectively. The normal reference range thus was different from that of the kit reference range. On comparing to the Indian normative reference for the pregnant women, our results were not similar. However, the values were near similar to that of the American Thyroid Association guidelines. **Conclusions:** We conclude our study results with a new reference range for the pregnant population in Manipur and also emphasis the use of trimester-specific reference range of thyroid hormone.

Key words: Pregnancy, reference range, thyroid hormones

#### INTRODUCTION

During pregnancy, there is a profound physiological change in the mother, which will complicate the interpretation of maternal thyroid function tests.<sup>[1-6]</sup> Human chorionic gonadotropin (hCG) have similar alpha-subunit to thyroid stimulating hormone (TSH) and a unique beta-subunit. As a result, hCG can stimulate the thyroid gland during the first trimester.<sup>[7]</sup> During

#### Corresponding Author: Prof. Lallan Prasad, Department of General Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India. E-mail: Iallan.imp@gmail.com

Access this article online			
Quick Response Code:			
	Website: www.ijem.in		
	<b>DOI:</b> 10.4103/2230-8210.176354		

pregnancy, the serum thyroxine-binding globulin concentrations rise almost two-fold and thyroid hormone production increase. Hence, total T4 and T3 concentrations rise during the first half of pregnancy followed by a plateauing at approximately 20 weeks of gestation. Fetal brain development in its early stage depends on the maternal thyroid hormones.<sup>[6-12]</sup> Reference range were studied extensively across various countries and also American Thyroid Association (ATA) had published their guidelines.<sup>[13-22]</sup> Although several studies are available from different regions of the world,

For reprints contact: reprints@medknow.com

Cite this article as: Jebasingh FK, Salam R, Meetei TL, Singh PT, Singh NN, Prasad L. Reference intervals in evaluation of maternal thyroid function of Manipuri women. Indian J Endocr Metab 2016;20:167-70.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

it is essential to develop norms for different Indian population due to the ethnic variations.<sup>[23-25]</sup>

#### **SUBJECTS AND METHODS**

The study primarily aimed at establishing the trimesterspecific reference ranges for TSH and thyroid hormones (total tri-iodothyronine [TT3], total thyroxine [TT4]) from healthy pregnant women of the Manipuri descendants. We also compared the results with the Indian normative reference range and the ATA guidelines reference for thyroid disease in pregnancy. This was a cross-sectional study that was conducted after approval from the Institutional Ethics Committee. The women were recruited after a prior informed consent. The sample size was calculated with an absolute error of 0.5. The serum levels of TSH, TT4, and TT3 were measured using SIEMENS ADVIA third generation chemiluminescence assay which has an intra-assay variability of <10% for all the three parameters.

Healthy women with uncomplicated single intrauterine gestations consuming iodized salt in any trimester were consecutively recruited. A detailed obstetric history such as duration of gestation, parity and number of abortions and also history related to any thyroid-related illness were obtained. Physical examination included anthropometry, goiter grading<sup>[25]</sup> and general and systemic examinations. Venous samples for the serum were drawn; serum separated and sent for analysis of thyroid hormones (TSH, TT3, and TT4). The following are the exclusion criteria to cone down the population for the reference range:

- History of hyperemesis gravid arum, thyroid illness or use of thyroid medications, connective tissue disorder and abortions
- Family history of thyroid illness
- Presence of goiter, WHO grade 1 or 2<sup>[25]</sup>
- Overt hypothyroidism (OH) or hyperthyroidism.

The data were analyzed using the SPSS 17 (Chicago, SPSS Inc.). Data for TT3 and TT4 were expressed as mean  $\pm$  standard deviation (SD), median, and 5<sup>th</sup> to 95<sup>th</sup> percentiles. TSH was normalized using log transformation as it did not follow normal distribution. The 5<sup>th</sup> and 95<sup>th</sup> percentile values of serum TSH, TT3, and TT4 from this group were used to derive reference intervals for pregnant women.<sup>[26]</sup>

#### RESULTS

A total of 600 normal pregnant women were examined, and 375 normal pregnant women were taken as the reference population after applying the exclusion criteria. Of the 375 women, 109, 148, and 118 were in the first, second, and third trimester, respectively. Using the kit reference values, OH was found in 2 (0.5%) women (1 in the first trimester, 1 in the second trimester). The mean age groups of the women were 25.9 + 5.1, 27.3 + 5.6 and 27.1 + 5.1 in first, second, and third trimester, respectively. The basic characteristics of the women are shown in Table 1. The mean  $\pm$  SD, median and 5<sup>th</sup> and 95<sup>th</sup> percentiles for TT3, TT4, and TSH in each trimester of pregnancy are given in Table 2. The reference intervals for each trimester of our study population are shown in Table 3.

#### DISCUSSION

The International Federation of Clinical Chemistry recommends that the determination of the normal reference values have to be described in such a way that the reference population should be appropriately described, and criteria for the selection should be clearly made.<sup>[26,27]</sup> The National Health and Nutrition Examination Survey of USA had used the exclusion criteria such as the personal or family history of thyroid illness, goiter but not ultrasound for an adult thyroid hormone reference population.<sup>[15]</sup> Marwaha et al., in their study had included the anti-thyroid antibodies and ultrasound in the Indian normative reference for the pregnant women.<sup>[13]</sup> The reference population should be as exclusive as possible as the purpose of reference intervals is to decide on thyroid status of a pregnant woman, wherein the thyroid functions cannot be compromised. This is because the adverse outcomes in fetus arising from thyroid dysfunction during pregnancy are very well-established.<sup>[5-8]</sup> In the Indian thyroid hormone reference study by Marwaha et al., a total of 331 women were studied with 107 in the first trimester, 137 in the second trimester and 87 in the third trimester. From the 5<sup>th</sup> and 95<sup>th</sup> percentile, the trimester-specific TSH values were 0.6-5.0, 0.44-5.78, and 0.74-5.7 µIU/ml, respectively.<sup>[13]</sup> Unlike the ATA guidelines for the thyroid diseases in pregnant women the TSH in Marwaha et al. study were similar to that of the normal adult population reference range [Table 4]. However, the reference range of the ethnic Manipuri population was near similar to that of the ATA guidelines.<sup>[14]</sup> Furthermore, in our study, we found a gradual increase in the median TSH levels as the trimester proceeds forwards. Another study from India

Table 1: The basic characteristics of the study population				
	Patient recruited	First trimester	Second trimester	Third trimester
Number of patients (%)	375	109 (29)	148 (39.5)	118 (31.5)
Number of primigravida	215	64	83	68
Number of multigravida	160	45	65	50
Age (mean±SD)	375	25.9±5.1	27.3±5.6	27.1±5.1
Gestational age (mean±SD)	375	9.3±2.5	20.8±4.1	35±3.1

SD: Standard deviation

Table 2: Thyroid hormone levels of our study population					
	Mean±SD	Median	5 <sup>th</sup> percentile	95 <sup>th</sup> percentile	Reference range
First trimester					
TSH (μlU/ml)	1.06±0.45	1.08	0.21	1.82	0.21-1.82
TT3 (ng/dl)	124.11±26.66	119.17	88.2805	176.80	88.28-176.80
TT4 (μg/dl)	9.98±2.18	6.49	6.49	13.75	6.49-13.75
Second trimester					
TSH (μIU/ml)	1.23±0.30	1.23	0.71	1.71	0.71-1.71
TT3 (ng/dl)	159.25±37.77	152.94	102.24	233.27	102.24-233.27
TT4 (μg/dl)	11.47±2.21	11.47	7.9	16.06	7.9-16.06
Third trimester					
TSH (μlU/ml)	1.25±0.36	1.23	0.7	1.93	0.70-1.93
TT3 (ng/dl)	153.20±34.19	154.42	88.46	205.50	88.46-205.50
TT4 (μg/dl)	11.48±2.35	11.80	7.77	15.30	7.77-15.30

TT4: Total thyroxine, TT3: Total tri-iodothyronine, TSH: Thyroid stimulating hormone, SD: Standard deviation

Table 3: Comparison of the manufacturer's reference range and the trimester specific range of our study population				
	Nonpregnant range	First trimester	Second trimester	Third trimester
	(manufacturer's range)	range (median)	range (median)	range (median)
TSH (μIU/ml)	0.35-5.5	0.21-1.82 (1.07)	0.72-1.71 (1.23)	0.69-1.93 (1.25)
T4 (μg/dl)	4.50-12.60	6.49-13.75 (9.53)	7.9-16.06 (11.46)	7.77-15.3 (11.21)
T3 (ng/dl)	60-181	88.28-176.8 (124.11)	102.24-233.27 (159.25)	88.46-205.49 (153.2)

TT4: Thyroxine, TT3: Tri-iodothyronine, TSH: Thyroid stimulating hormone

## Table 4: Comparison of our TSH reference range with the proposed Indian normative range and the ATA quidelines reference range

•	<b>U</b>		
	Our reference range (µIU/mI)	Indian normative range (µIU/mI) <sup>[13]</sup>	ATA guidelines (µIU/mI) <sup>[14]</sup>
First trimester	0.21-1.82 (1.07)	0.6-5.0 (2.42)	0.1-2.5
Second trimester	0.72-1.71 (1.23)	0.44-5.78 (2.49)	0.2-3
Third trimester	0.69-1.93 (1.25)	0.74-5.7 (2.6)	0.3-3

TSH: Thyroid stimulating hormone, ATA: American Thyroid Association

evaluated 124 pregnant women using radioimmunoassay also showed an increase in TSH progressively with each trimester. When mean serum TT3 and TT4 were analyzed, both values increased from first to the second trimester but declined from second to the third trimester.<sup>[16]</sup> Similarly, in our study, the TT3 and TT4 increased from first to the second trimester, followed by a decline during the third trimester. A longitudinal study by Price et al., also arrived at the trimester-specific reference range. They found the serum TSH levels increase from first to the second trimester, while FT4 decreased with progress in the pregnancy. However, the serum FT3 did not change between the first and second trimesters. Furthermore, the study did not find any difference in the thyroid hormone changes between pregnant Asian and pregnant Caucasian women.<sup>[17]</sup> In a longitudinal study by Panesar et al., on pregnant Chinese women, the authors found a decrease in FT4 and FT3 by about 25% with gestational age from peak to nadir. Whereas, TSH, which was suppressed in the first trimester and increased subsequently with the advancing pregnancy.<sup>[18]</sup> A longitudinal study by Soldin et al., through the trimesters from a group of pregnant women used

immunoassay and mass spectrometry to analyze the thyroid functions. The mean TSH increased with advancing gestational age from first to the second trimester, while it remained stable from second to the third trimester.<sup>[19]</sup> In an another cross-sectional study of 522 pregnant women from Japan, the authors showed a significant decrease in both free T3 (FT3) and free T4 (FT4) and increase in the TSH with advancing pregnancy.<sup>[20]</sup> Our study showed a TSH reference range, which is different from that of the proposed Indian normative range for pregnant women [Table 4]. In the study by Marwaha et al., the author suggests that the Asian pregnant women are more prone for gestational thyrotoxicosis.<sup>[13]</sup> However, in our study, we did not find women with overt hyperthyroidism. A study on thyroid function that was carried out only in the second trimester by La'ulu and Roberts showed a higher prevalence of raised TSH in whites compared with Asians and Blacks.<sup>[21]</sup> Hence, the ethnic variations still remain a concern, and the studies that address this aspect are very scant. Hence, we can overview that there is a definite difference in the thyroid reference range across various countries and also within the Indian population due to its ethnic origin. The limitations in our study are that we did not measure the anti-thyroid antibodies as well as the free thyroid hormone levels.

This study concludes the following reference intervals for TT3, TT4, and TSH for the each trimester of pregnancy (TT3 [88.28–176.8, 102.24–233.27 and 88.46–205.49 ng/dl], TT4 [6.49–13.75, 7.9–16.06 and 7.77–15.3  $\mu$ g/dl] and TSH [0.21–1.82, 0.72–1.71 and 0.69–1.93 IU/ml]) for

the pregnant Indian women of Manipur state. We also emphasize, there is a definite difference in the trimesterspecific reference range of thyroid hormones within country like India due to its ethnic variation.

Financial support and sponsorship Nil.

#### **Conflicts of interest**

There are no conflicts of interest.

#### REFERENCES

- Man EB, Jones WS. Thyroid function in human pregnancy. V. Incidence of maternal serum low butanol-extractable iodines and of normal gestational TBG and TBPA capacities; retardation of 8-month-old infants. Am J Obstet Gynecol 1969;104:898-908.
- Davis LE, Leveno KJ, Cunningham FG. Hypothyroidism complicating pregnancy. Obstet Gynecol 1988;72:108-12.
- Leung AS, Millar LK, Koonings PP, Montoro M, Mestman JH. Perinatal outcome in hypothyroid pregnancies. Obstet Gynecol 1993;81:349-53.
- Allan WC, Haddow JE, Palomaki GE, Williams JR, Mitchell ML, Hermos RJ, et al. Maternal thyroid deficiency and pregnancy complications: Implications for population screening. J Med Screen 2000;7:127-30.
- Glinoer D, Soto MF, Bourdoux P, Lejeune B, Delange F, Lemone M, et al. Pregnancy in patients with mild thyroid abnormalities: Maternal and neonatal repercussions. J Clin Endocrinol Metab 1991;73:421-7.
- Morreale de Escobar G, Obregón MJ, Escobar del Rey F. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab 2000;85:3975-87.
- Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999;341:549-55.
- Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, et al. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol (Oxf) 1999;50:149-55.
- Klein RZ, Haddow JE, Faix JD, Brown RS, Hermos RJ, Pulkkinen A, et al. Prevalence of thyroid deficiency in pregnant women. Clin Endocrinol (Oxf) 1991;35:41-6.
- Burrow GN, Fisher DA, Larsen PR. Maternal and fetal thyroid function. N Engl J Med 1994;331:1072-8.
- Glinoer D. The regulation of thyroid function in pregnancy: Pathways of endocrine adaptation from physiology to pathology. Endocr Rev 1997;18:404-33.
- Mandel SJ, Spencer CA, Hollowell JG. Are detection and treatment of thyroid insufficiency in pregnancy feasible? Thyroid 2005;15:44-53.

- 13. Marwaha RK, Chopra S, Gopalakrishnan S, Sharma B, Kanwar RS, Sastry A, *et al.* Establishment of reference range for thyroid hormones in normal pregnant Indian women. BJOG 2008;115:602-6.
- Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, *et al.* Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 2011;21:1081-125.
- Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87:489-99.
- 16. Kumar A, Gupta N, Nath T, Sharma JB, Sharma S. Thyroid function tests in pregnancy. Indian J Med Sci 2003;57:252-8.
- 17. Price A, Obel O, Cresswell J, Catch I, Rutter S, Barik S, *et al.* Comparison of thyroid function in pregnant and non-pregnant Asian and western Caucasian women. Clin Chim Acta 2001;308:91-8.
- Panesar NS, Li CY, Rogers MS. Reference intervals for thyroid hormones in pregnant Chinese women. Ann Clin Biochem 2001;38(Pt 4):329-32.
- Soldin OP, Tractenberg RE, Hollowell JG, Jonklaas J, Janicic N, Soldin SJ. Trimester-specific changes in maternal thyroid hormone, thyrotropin, and thyroglobulin concentrations during gestation: Trends and associations across trimesters in iodine sufficiency. Thyroid 2004;14:1084-90.
- Kurioka H, Takahashi K, Miyazaki K. Maternal thyroid function during pregnancy and puerperal period. Endocr J 2005;52:587-91.
- La'ulu SL, Roberts WL. Second-trimester reference intervals for thyroid tests: The role of ethnicity. Clin Chem 2007;53:1658-64.
- 22. Quinn FA, Gridasov GN, Vdovenko SA, Krasnova NA, Vodopianova NV, Epiphanova MA, et al. Prevalence of abnormal thyroid stimulating hormone and thyroid peroxidase antibody-positive results in a population of pregnant women in the Samara region of the Russian Federation. Clin Chem Lab Med 2005;43:1223-6.
- Marwaha RK, Tandon N, Gupta N, Karak AK, Verma K, Kochupillai N. Residual goitre in the postiodization phase: Iodine status, thiocyanate exposure and autoimmunity. Clin Endocrinol (Oxf) 2003;59:672-81.
- Gopalakrishnan S, Singh SP, Prasad WR, Jain SK, Ambardar VK, Sankar R. Prevalence of goitre and autoimmune thyroiditis in schoolchildren in Delhi, India, after two decades of salt iodisation. J Pediatr Endocrinol Metab 2006;19:889-93.
- WHO, UNICEF, ICCIDD. Indicators for Assessing Iodine Deficiency Disorders and Their Control Through Salt Iodization. WHO/ NUT/94.6. Geneva, Switzerland: World Health Organization; 1994.
- 26. Solberg HE. International federation of clinical chemistry. Scientific committee, clinical section. Expert panel on theory of reference values and international committee for standardization in haematology standing committee on reference values. Approved recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. Clin Chim Acta 1987 29;165:111-8.
- Demers LM, Spencer CA. Laboratory medicine practice guidelines: Laboratory support for the diagnosis and monitoring of thyroid disease. Clin Endocrinol (Oxf) 2003;58:138-40.