

## Determination of reference intervals of thyroid markers during pregnancy in Urban area of District Rawalpindi Pakistan

Mehwish Gilani,<sup>1</sup> Naveed Asif,<sup>2</sup> Ammad Akram,<sup>3</sup> Mehreen Gilani,<sup>4</sup> Aamir Ijaz,<sup>5</sup> Saima Shakil Malik<sup>6</sup>

### Abstract

**Objective:** To determine the reference values for thyroid stimulating hormone, free tetra-iodothyronine and total tri-iodothyronine for healthy pregnant women.

**Method:** This cross sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January 2016 to June 2017. Pregnant women with normal, single intrauterine, uncomplicated pregnancy were recruited from the local population. Blood sample was taken to analyse thyroid stimulating hormone, free tetra-iodothyronine and total tri-iodothyronine using chemiluminescence immunoassay. For thyroid hormone levels during each trimester 5th and 95th percentiles were calculated as reference intervals. Data was analysed using SPSS 24.

**Result:** Out of 384 subjects, 188(48.95%) were in their first trimester and 196(51.04 %) females were in their second trimester. There were 109(57.97%) primigravida in the first trimester and 137(69.9%) in the second trimester. Mean age of subjects presenting in the first and second trimester was  $25.37 \pm 3.78$  years and  $26.54 \pm 4.65$  years respectively. Reference intervals for those in the first trimester for thyroid stimulating hormone was 0.05-2.8uIU/ml, for free tetra-iodothyronine 14.4-22.7pmol/l and total tri-iodothyronine 1.5-3.3nmol/l. For those in second trimester the corresponding values were 0.16-3.3 uIU/ml, 14.2-24.6.0 pmol/l and 1.6-3.1nmol/l.

**Conclusion:** Laboratories should adopt trimester-specific reference intervals for thyroid function tests in pregnancy.

**Keywords:** Pregnancy, Normal reference intervals, Pakistan, Thyroid stimulating hormone, Total tri-iodothyronine, Free tetra-iodothyronine. (JPMA 68: 1488; 2018)

### Introduction

Pregnancy prompts a huge load on thyroid, resulting in hypothyroidism or postpartum thyroiditis. During pregnancy, since most of the thyroid function diseases are symptomatic, laboratory investigations are crucial for diagnosis.<sup>1</sup> To meet the demand for increased metabolic challenges in pregnancy, profound physiological changes invariably take place in mother. All these endocrinologic and metabolic changes ensure an ample supply of fuel and nutrients to the foetus. Thyroid gland also adapts accordingly by alteration in its output and changes in hypothalamic pituitary thyroid axis regulation.<sup>2</sup> Thyroid gland has a known contribution to foetal development.<sup>3</sup> Foetal thyroid starts significant functioning around the 20th week of gestation, until which it entirely depends on maternal supply of thyroid hormones.<sup>4,5</sup>

Important changes that occur in thyroid hormones during pregnancy include increase in total thyroid binding globulins (TBGs), increased thyroid stimulation owing to increased thyroid stimulation hormone (TSH)

levels, and increased renal iodide clearance. Due to structural resemblance of human chorionic gonadotropin (hCG) with TSH, hCG stimulates thyroid gland, resulting in an increase in its secretion of tetra-iodothyronine (T4) and tri-iodothyronine (T3) during the early phase of pregnancy followed by a plateauing at approximately 16 weeks of gestation.<sup>6</sup> Thyroid hormone is degraded by placental type 3 iodothyronine deiodinase, necessitating increased production of maternal thyroid hormone.<sup>4</sup>

Thyroid abnormalities are not very unheard of during pregnancy. Adverse outcomes by thyroid abnormalities include intra-uterine growth retardation (IUGR), repeated miscarriages, preterm delivery, hypertensive disorders, decreased cognitive and neurological development of foetus, including decreased intelligence quotient (IQ) in the child.<sup>3,7,8</sup> Overt hypothyroidism is found in 0.2% while sub-clinical hypothyroidism is found in 2.3% of pregnant women.<sup>9</sup> Maternal hypothyroidism and subclinical hypothyroidism have been associated with increased risk of placental abruption.<sup>10</sup> Mild or sub-clinical hyperthyroidism in pregnancy account for approximately 1.7% and has not been associated with any significant adverse pregnancy outcome.<sup>9</sup>

Asian population has a preponderance of gestational

<sup>1,2,5,6</sup>Armed Forces Institute of Pathology, <sup>3</sup>Military Hospital, Rawalpindi, <sup>4</sup>Army Medical College (NUMS), Rawalpindi.

**Correspondence:** Mehwish Gilani. Email: drmehwishgilani@hotmail.com

thyrotoxicosis compared to the western Caucasian women.<sup>11</sup> Therefore, American Thyroid Association (ATA), European Thyroid Association (ETA) and the Endocrine Society (ES) recommend that each centre should establish its own trimester-specific reference intervals for thyroid profile for timely and appropriate detection of thyroid abnormalities and early interventions that would prevent foeto-maternal complications.<sup>4,8</sup>

Many laboratories follow reference values for thyroid markers that have been established in healthy men and women, but there is limited data available on the reference intervals for pregnant women. Without establishing the reference intervals during pregnancy, there is an increased chance of misinterpreting normal changes as pathological and also missing important events due to pathological changes.<sup>12</sup> The current study was planned to establish gestational specific reference intervals of thyroid markers in pregnant women.

## Subjects and Method

This cross sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from January 2016 to June 2017. Subjects were selected using non-probability consecutive sampling technique. Sample size was calculated by setting level of significance at 5% with a confidence interval of 95% and assuming a population of 100,000. After approval from institutional review board, healthy women with uncomplicated single intrauterine gestation consuming iodised salt during pregnancy were recruited from the local population. A thorough history regarding gestational age of subject, parity, iodine intake, any thyroid-related illness or history of any abortion in the past was taken. Those with history

of hyperemesis gravidarum (HG) or use of thyroid medications, those with connective tissue disorder, a positive family history of thyroid illness, presence of goitre World Health Organisation (WHO) grade 1 or 2<sup>13</sup> and those with overt hypothyroidism (OH) or hyperthyroidism were excluded. All the study participants were of Pakistani origin. A detailed physical examination including general and systemic physical examination, anthropometry and goitre grading was done.

Serum total T3, free T4 (FT4) were analysed on competitive immunoassay (ADVIA Centaur® XP Random access Immunoassay System, Siemens Healthiness) using direct chemiluminescent technology and TSH by third-generation assay employing anti-fluorescein isothiocyanate (FITC) monoclonal antibody for chemiluminescent detection. For serum T3, T4 and TSH, 3ml blood sample was collected in gel vacutainers after taking informed consent from the subjects.

Data was analysed using SPSS 24. Data for TSH, FT4 and total T3 (TT3) were expressed as mean  $\pm$  SD and median. Reference intervals were derived from the 5th and 95th percentile values of TSH, FT4 and TT3.

## Result

Out of 384 subjects, 188 (48.95%) were in their first

**Table-1:** Basic characteristics of study population.

Characteristics	Patients recruited (n)	First trimester	Second trimester
No. of patients (%)	384	188 (48.9)	196 (51.04)
Primigravida	246	109	137
Multigravida	138	72	66
Age (Years) (mean $\pm$ SD)	384	25.37 $\pm$ 3.78	26.54 $\pm$ 4.65

SD: Standard Deviation.

**Table-2:** Thyroid Hormone levels of our study population.

	Mean $\pm$ SD	Median	Confidence Interval (CI)	5th percentile	50th percentile	95th percentile	Reference Interval
<b>1st Trimester</b>							
TSH (uIU/ml)	1.1065 $\pm$ 0.866	0.90	0.98-1.23	0.05	0.9	2.8	0.05-2.8
TT3 (nmol/l)	2.275 $\pm$ 0.542	2.20	2.19-2.35	1.5	2.2	3.3	1.5-3.3
FT4 (pmol/l)	18.739 $\pm$ 2.688	18.5	18.35-19.12	14.4	18.5	22.7	14.4-22.7
<b>2nd Trimester</b>							
TSH (uIU/ml)	1.351 $\pm$ 0.947	1.0	1.21-1.48	0.16	1.0	3.3	0.16-3.3
TT3 (nmol/l)	2.441 $\pm$ 0.461	2.50	2.37-2.50	1.6	2.5	3.1	1.6-3.1
FT4 (pmol/l)	19.091 $\pm$ 3.557	18.9	18.58-19.59	14.2	18.9	24.6	14.2-24.6

SD: Standard Deviation

TSH: Thyroid stimulating hormone

TT3: Total tri-iodothyronine

FT4: Free tetra-iodothyronine.

**Table-3:** Comparison of non-pregnant reference interval and trimester specific range of our study population.

Parameter	Non-Pregnant range	First Trimester (median)	Second Trimester (median)
TSH (uIU/ml)	0.4-4.5	0.05-2.8(0.9)	0.16-3.3(1.0)
TT3 (nmol/l)	1.1-2.7	1.5-3.3(2.20)	1.6-3.1(2.5)
FT4 (pmol/l)	8-21	14.4-22.7(18.5)	14.2-24.6(18.9)

TSH: Thyroid stimulating hormone

TT3: Total tri-iodothyronine

FT4: Free tetra-iodothyronine.

**Table-4:** Comparison of Our reference interval with medians for TSH with Indian normative range and ATA guidelines.

Trimester	Our reference interval (median)	Indian normative range <sup>18</sup> (median)	ATA Guidelines <sup>7</sup>
<b>TSH (uIU/ml)</b>			
1st Trimester	0.05-2.8(0.9)	0.6-5.0(2.1)	0.1-2.5
2nd Trimester	0.16-3.3(1.0)	0.44-5.78(2.4)	0.2-3.0

TSH: Thyroid stimulating hormone

ATA: American Thyroid Association.

trimester and 196(51.04 %) females were in their second trimester. There were 109(57.97%) primigravida in the first trimester and 137(69.9%) in the second trimester. Mean age of subjects presenting in the first and second trimester was  $25.37 \pm 3.78$  years and  $26.54 \pm 4.65$  years respectively (Table-1). Mean, median, confidence interval, 5th, 50th, 95th percentiles for TSH, FT4, and TT3 were computed in the first and second trimesters (Table-2).

By using cut-off values of TSH for non-pregnant females on our study subjects, sub-clinical hyperthyroidism (SCH) was found in 26(13.82%) females in first trimester, while in the second trimester, SCH was found in 4(2.04%) females (Table-3). By using 5th and 95th percentile, trimester-specific values derived from our reference population, SCH cases were reduced to 2(1.06%) and 1(0.51%) case in the first and second trimester subjects respectively. Applying non-pregnant reference values on our subjects, only 1(0.5%) case of sub-clinical hypothyroidism was found in the second trimester. But after applying the trimester-specific reference interval, 6(3.19%) cases of sub-clinical hypothyroidism were reported in the first trimester and 8(4.08%) in the second trimester.

Following the non-pregnant values, it could be deduced that 30(15.95%) cases among subjects in the first trimester and 10(5.10%) in the second trimester, making a

total of 40 (10.41%), were misdiagnosed or would have been over-treated.

## Discussion

During pregnancy, mother's thyroid gland starts its augmented functioning just after conception, manifesting in the form of raised T4 and T3 concentrations along with increased TBGs, peaking in the 7th week and stabilising by the 16th week of gestation. Afterwards it remains high throughout pregnancy. Negative feedback system causes TSH concentration to remain low throughout pregnancy.<sup>14</sup> For accurate assessment of thyroid function, it is necessary to measure all key players i.e. TSH, T3, T4, anti-thyroid peroxidase (TPO) antibodies, and thyroglobulin antibodies. But, due to financial burden, measuring most crucial marker like TSH gives directions to thyroid dysfunctioning.<sup>15</sup> FT4 was done instead of total T4 (TT4) because thyroid hormone binding proteins are frequently present in abnormal concentrations in physiological states, such as pregnancy, or in association with drug therapies and abnormalities in binding proteins that affect hormone binding. For this reason, artefactual changes in TT4 concentration in euthyroid individuals may be common, while free hormone measurements better reflect true thyroid status. Accurate serum FT4 measurements are needed to differentiate overt hypothyroidism from SCH and for the diagnosis of euthyroid hypothyroxinaemia, the controversial diagnostic group that some claim is associated with adverse pregnancy outcomes.<sup>16</sup> When combined with elevated TSH levels, FT4 or TT4 determinations are equally sensitive to identify women with SCH.<sup>17</sup> Results of the current study are consistent with other studies reporting that hyper-functioning of thyroid gland during pregnancy demands the need for trimester-specific reference intervals of thyroid markers.<sup>2-4,18-20</sup>

In the current study reference interval for TSH is substantially close to that recommended by ATA guidelines (Table-4) for those laboratories that do not have a defined reference intervals for thyroid markers in their population.<sup>7</sup> However, in a study<sup>18</sup> results were consistent with normal adult population. Mean TSH levels in our study increased from first to second trimester. These results are consistent with results of other studies.<sup>9,21</sup> Similarly, TSH reference value for the first trimester in this study (0.05-2.8 uIU/ml) was limited as compared to 0.01-4.05 uIU/ml reported in a study conducted in the United States (US).<sup>19</sup> In another US study<sup>22</sup> the same automated immunoassay

analyser (ADVIA CENTAUR analyser) was used and reference interval was found to be 0.04-3.6 uIU/ml. These variations in the reference intervals for first trimester demands the need for recruiting reference values specific for each study population as these are very strong evidences that thyroid functions change with ethnic group.<sup>10,22,23</sup> TSH reference value for second trimester (0.16-3.3uIU/L) was also consistent with those mentioned in ADA guidelines and reported in a research<sup>24</sup> for Asian population (0.06-3.64uIU/ml). Variable results were reported in different ethnic groups like in Korean (0.01-4.26uIU/ml),<sup>8</sup> American (0.39-4.18uIU/ml)<sup>25</sup> and Chinese (0.10-4.34uIU/ml)<sup>20</sup> population. All these findings again emphasise the fact that ethnicity and race has a very strong impact on thyroid profile.<sup>24</sup> Furthermore, our study also found that mean TSH values increased as pregnancy progressed, with value of second trimester being more than first trimester's. Such findings are consistent with other studies.<sup>6,21</sup> FT4 and TT3 values were raised from first to second trimester as reported in other studies.<sup>6,26</sup> In another study<sup>27</sup> on Iranian women, TSH, TT3 and TT4 were raised while FT4 declined from first to second trimester.

One study<sup>18</sup> suggested that Asian pregnant women are more prone to developing gestational thyrotoxicosis, which is a crucial finding also corroborated in our study (8 cases, 2.08% of overt hyperthyroidism). Our study population also included SCH cases with 3.19% subjects in their first trimester and 4.08% in their second trimester. Likewise a research<sup>2</sup> reported a prevalence of 4% cases of SCH.

Although the current study has proved its importance by determining a reference interval for pregnant women which has not been established in the region, but some limitations to our study include its single-centre nature, failure to perform thyroid ultrasonography, lack of anti-TPO and anti-thyroglobulin assays, not confirming iodine-replete population by urinary iodine and lack of pre- and post-pregnancy data of the study population due to lack of follow-up facilities.

## Conclusion

Misclassification of pregnant women with thyroid disorders can only be avoided with proper reference intervals established in pregnancy rather than following the non-pregnant values. The trimester-specific reference intervals determined in the current study are recommended to be followed in all laboratories in Rawalpindi using same methodology as used here for the screening of thyroid dysfunction in pregnant females.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** None.

## References

1. Springer D, Bartos V, Zima T. Reference intervals for thyroid markers in early pregnancy determined by 7 different analytical systems. *Scand J Clin Lab Invest* 2014; 74: 95-101.
2. Mankar J, Sahasrabudhe A, Pitale S. Trimester specific ranges for thyroid hormones in normal pregnancy. *Thyroid Res Pract* 2016; 13: 106-9.
3. Xing J, Yuan E, Li J, Zhang Y, Meng X, Zhang X, et al. Trimester-and assay-specific thyroid reference intervals for pregnant women in china. *Int J Endocrinol* 2016; 2016: 3754213
4. Medici M, Korevaar TI, Visser WE, Visser TJ, Peeters RP. Thyroid function in pregnancy: what is normal? *Clin Chem* 2015; 61: 704-13.
5. Stricker R, Echenard M, Eberhart R, Chevailler MC, Perez V, Quinn FA, et al. Evaluation of maternal thyroid function during pregnancy: the importance of using gestational age-specific reference intervals. *Eur J Endocrinol* 2007; 157: 509-14.
6. 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 Endocrinol Metab* 2016; 20: 167-70.
7. 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.
8. Moon HW, Chung HJ, Park CM, Hur M, Yun YM. Establishment of trimester-specific reference intervals for thyroid hormones in Korean pregnant women. *Ann Lab Med* 2015; 35: 198-204.
9. Sekhri T, Juhi JA, Wilfred R, Kanwar RS, Sethi J, Bhadra K, et al. Trimester specific reference intervals for thyroid function tests in normal Indian pregnant women. *Indian J Endocrinol Metab* 2016; 20: 101-7.
10. Gilbert RM, Hadlow NC, Walsh JP, Fletcher SJ, Brown SJ, Stuckey BG, et al. Assessment of thyroid function during pregnancy: first-trimester (weeks 9-13) reference intervals derived from Western Australian women. *Med J Aust* 2008; 189: 250-3.
11. Labadzhyan A, Brent GA, Hershman JM, Leung AM. Thyrotoxicosis of pregnancy. *J Clin Transl Endocrinol* 2014; 1: 140-4.
12. Larsson A, Palm M, Hansson LO, Axelsson O. Reference values for clinical chemistry tests during normal pregnancy. *BJOG* 2008; 115: 874-81.
13. Demir I, Orbak Z, Karakelleoglu C. Relationship between the level of trace elements and growth in school children group of 6 to 12 ages with goitre. *Endocrine Abstracts* 2017; 51: P001
14. Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, et al. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* 2017; 27: 315-89.
15. Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Thyroid* 2012; 22: 1200-35.
16. McNeil AR, Stanford PE. Reporting Thyroid Function Tests in

- Pregnancy. *Clin Biochem Rev* 2015; 36 :109-26.
17. Wilson KL, Casey BM, McIntire DD, Cunningham FG. Is total thyroxine better than free thyroxine during pregnancy? *Am J Obstet Gynecol* 2014; 211: 132. e1- e6.
  18. 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.
  19. Dashe JS, Casey BM, Wells CE, McIntire DD, Byrd EW, Leveno KJ, et al. Thyroid-stimulating hormone in singleton and twin pregnancy: importance of gestational age-specific reference ranges. *Obstet Gynecol* 2005; 106: 753-7.
  20. Li C, Shan Z, Mao J, Wang W, Xie X, Zhou W, et al. Assessment of thyroid function during first-trimester pregnancy: what is the rational upper limit of serum TSH during the first trimester in Chinese pregnant women? *J Clin Endocrinol Metabol* 2013; 99: 73-9.
  21. Mehran L, Amouzegar A, Delshad H, Askari S, Hedayati M, Amirshakari G, et al. Trimester-specific reference ranges for thyroid hormones in Iranian pregnant women. *J Thyroid Res* 2013; 2013: 651517.
  22. Pearce EN, Oken E, Gillman MW, Lee SL, Magnani B, Platek D, et al. Association of first-trimester thyroid function test values with thyroperoxidase antibody status, smoking, and multivitamin use. *Endocr Pract* 2008; 14: 33-9.
  23. 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.
  24. La'ulu SL, Roberts WL. Second-trimester reference intervals for thyroid tests: the role of ethnicity. *Clin Chem* 2007; 53: 1658-64.
  25. Haddow JE, Knight GJ, Palomaki GE, McClain MR, Pulkkinen AJ. The reference range and within-person variability of thyroid stimulating hormone during the first and second trimesters of pregnancy. *J Med Screen* 2004; 11: 170-4.
  26. Kumar A, Gupta N, Nath T, Sharma JB, Sharma S. Thyroid function tests in pregnancy. *Indian J Med Sci* 2003; 57: 252-8.
  27. Zarghami N, Rohbani-Noubar M, Khosrowbeygi A. Thyroid hormones status during pregnancy in normal Iranian women. *Indian J Clin Biochem* 2005; 20: 182-5.
-