

ORIGINAL ARTICLE

TRIMESTER SPECIFIC REFERENCE RANGES OF SERUM TSH, FT3 AND FT4 DURING LAST SEMESTER OF PREGNANCY

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Background: Pregnancy is a stress for thyroid gland. After decades of research it is recommended that normal reference ranges of thyroid hormones test during different phases of pregnancy are necessary. The objective of this study was to establish serum TSH, FT3 and FT4 levels during last trimester of pregnancy. **Methods:** This was a cross-sectional analytical study. Simple convenient sampling technique was applied and sample size was calculated using classical sample size calculation formula of Cochran. Serum TSH, FT3 and FT4 were estimated by ELIZA method. **Results:** Maternal group showed serum TSH levels of 1.79 ± 0.85 mIU/L. Maternal serum FT3 levels were 1.711 ± 2.089 pmol/L. Mean serum FT4 of Maternal group were 1.31 ± 1.07 ng/dL, (Range: 0.78–5.20 ng/dl). **Conclusion:** Our study population trimester specific ranges of TSH, FT4 and FT3 were lower than latest international levels according to Guidelines of American Thyroid Association of 2017. These values may be used to compare thyroid dysfunction in pregnancy.

Keywords: Thyroid hormones, TSH, T3, T4, Pregnancy, Pakistan, Reference value, trimester
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INTRODUCTION

Thyroid hormones are essential for foetal development during prenatal and postnatal period. During pregnancy, increased synthesis of thyroid hormones is needed to meet foetal requirements, which leads to high requirement of iodine.¹ Pregnancy is a stress for thyroid gland; it affects 3% women during pregnancy and 10% women of child bearing age. About 18% of Australian women have thyroid antibody positive test during first trimester of pregnancy.²

Establishment of normal reference ranges of thyroid hormones during different phases of pregnancy is essential. Non-pregnant reference values should not be used for interpretation of laboratory results of pregnant women.³ Trimester specific references of thyroid functions tests are not considered in the laboratories. Intensive research has been conducted worldwide to develop reference ranges of thyroid hormones during each trimester of pregnancy.⁴

During pregnancy, thyroid adapts through changes in thyroid economy and to meet the increased metabolic demands of the body. Thyroid function test of healthy pregnant women are different from those of non-pregnant women. Pregnancy specific, and ideally trimester specific, reference intervals for thyroid function are needed to be practiced.⁵

The Endocrine Society and American Thyroid Association have recommended population based, trimester-specific reference intervals and assay specific values to assess thyroid function during pregnancy.^{6,7} The reference intervals for thyroid

function tests should be based on 2.5th and 97.5th percentile of respective iodine deficient population. The studied population should comprise of healthy, selected women having singleton pregnancy and without any complication. Women with no history of thyroid disease, TPO-Abs-negative women, and women who are not taking anti-thyroid drugs are usually deemed healthy.⁸

In case of non-availability of population-based thyroid function tests reference levels, trimester specific TSH reference intervals should be practiced as recommended by the international guidelines of the Endocrine Society, irrespective of laboratory method. TSH reference interval for first trimester is 0.1–2.5 mU/L, for 2nd trimester 0.2–3.0 mU/L, and for 3rd trimester it is 0.3–3.0 mU/L respectively.⁹

The TSH cut-off values may be misleading to over diagnosis and treatment of thyroid disease in pregnant women because the recommended TSH cut-off value of 2.5–3 mU/L is less than most population-based studies showing higher reference values of TSH.¹⁰ Currently researchers are not using trimester specific reference levels. Männistö *et al*⁷ used population based calculated reference intervals of Northern Finland Birth Cohort (NFBC 1986 population) in their research. NFBC reference intervals for TSH were 0.07–3.1 mIU/L during first trimester and 0.1–3.5 mIU/l during second trimester respectively. Population-based free T4 reference intervals were found to be 11.4–22.4 pmol/L in first trimester and 11.1–18.9 pmol/l in second trimester respectively.¹¹

FT4 immuno-assays in the market are method specific, for normal pregnancy related reference intervals. It is necessary that gestation specific reference intervals should be derived in appropriate reference population, otherwise it may lead to misinterpretation of thyroid status. Tandem mass spectrometry is a gold standard methodology for standardization of thyroid hormones values.¹²

Many researchers have reported that serum TSH reference ranges are lower throughout the pregnancy as compared with age matched non-pregnant women. During first trimester of pregnancy, serum TSH level decreases continuously. Both lower as well as upper normal limits of serum TSH level are lower about 0.1–0.2 mU/L and 1.0 mU/L respectively compared with non-pregnant women.^{13,14}

Serum TSH level is the best indicator of thyroid function during 2nd and 3rd trimester. Reliable trimester specific, population-based reference intervals for TSH are available, based on adequate sample size of singleton pregnancies in iodine sufficient and antibody free population.^{15,16} This study was designed to estimate baseline reference values of serum TSH, FT3 and FT4 levels in normal singleton pregnancy from a tertiary care hospital.

MATERIAL AND METHODS

This was a cross-sectional, hospital-based study. This study was conducted at the Department of Physiology, Institute of Molecular Biology & Biotechnology (IMBB), University of Lahore in collaboration with Departments of Gynaecology and Obstetrics, DHQ Teaching Hospital, Dera Ghazi Khan during Jan–Mar 2020. The study protocol was approved by Ethical Review Committee of Ghazi Khan Medical College, Dera Ghazi Khan and Board of Advanced Studies and Research, University of Lahore. Written informed consent was obtained from subjects. Simple convenient sampling technique was applied.

The study participants were thirty (n=30). Study participants were pregnant mothers with singleton pregnancy attending antenatal clinic during last trimester of pregnancy. All subjects were examined for any symptoms and signs of hypothyroidism and hyperthyroidism. Thirty (30) subjects fulfilling the selection criteria were included in the study. Sample size was calculated using classical sample size formula. Subjects having goitre were evaluated on clinical examination for hypothyroidism, hyperthyroidism excluded from the study. Subjects were selected during last trimester of pregnancy. A pre-tested structured questionnaire was used as data collection tool for interview and Clinical examination method by two-member research team of medical specialists. Family history of goitre and

thyroid surgery and anti-thyroid medication was taken. Age, educational status, socioeconomic status, area of residence, duration of stay and gestational age was also recoded. Blood samples of the subjects were taken aseptically through venepuncture. Serum TSH levels, FT3 and FT4 levels were estimated using Chemi-illumination micro particle assay (C MIA) ARCHITECT fully automated ELIZA. Data was analysed using SPSS-18 for basic statistics. Mean±SD and percentile analysis for TSH, FT3 and FT4 was done.

RESULTS

A maternal group of thirty normal healthy, euthyroid pregnant women (n=30) was selected. Mean age of pregnant women of maternal group was 25.77±5.10 years. Mode of delivery in 16 (53.3%) women was Spontaneous Vaginal Delivery and 14 (46.7%) had to undergo caesarean section for delivery.

Mean serum TSH level was 1.79±0.85 mIU/l (Range: 0.63–3.84 mIU/l), serum FT3 levels was 1.711±2.089 pmol/l (Range: 0.770–12.09 pmol/l). Serum FT4 of maternal group was 1.31±1.07 ng/dl (Range: 0.78–5.2 ng/dl) (Table-1).

Serum TSH levels of maternal group on 5th, 50th, and 95th percentiles were 0.669, 1.790, and 3.304 mIU/l respectively. Serum FT3 levels of maternal group 5th, 50th, and 95th percentiles were 0.842, 1.120, and 3.574 pmol/l respectively. Serum FT4 levels of maternal group were 0.785, 0.990, and 5.09 ng/dl respectively (Table-2).

Table-1: Comparison of group parameters of Maternal Group (n=30)

Parameter	Mean±SD	SEM	Range
TSH	1.785±0.847	0.155	0.630–3.480
FT3	1.711±2.089	0.381	0.770–12.090
FT4	1.314±1.065	0.194	0.780–5.200

Table-2: Percentile of serum FT3, FT4, and TSH of study group (n=30)

Variables	Percentile						
	5 th	10 th	25 th	50 th	75 th	90 th	95 th
Serum FT3	0.842	0.900	0.900	1.120	1.615	3.574	7.519
Serum FT4	0.785	0.842	0.900	0.990	1.068	2.144	5.090
Serum TSH	6	0.708	1.047	1.790	2.367	3.122	3.304

n=number of participants, 50th Percentile=Median

DISCUSSION

This study was performed to establish reference levels for maternal TSH, FT3 and FT4 during last trimester of pregnancy. It was imperative to establish reference levels from study area as no reference levels of maternal serum TSH, FT3 and sFT4 were available for Pakistani pregnant women, for comparison of maternal TSH, FT3 and FT4 levels of

normal pregnant ladies. Many countries have not yet established trimester specific reference ranges of thyroid hormones among normal pregnant women. Rare data is available among South Asian Region countries (SARC) on trimester specific reference ranges of thyroid hormones for pregnant women, which is required for early diagnosis and management of thyroid disorders.¹⁷

Pakistan is also having no trimester specific reference ranges of serum T3, TSH, FT3 and FT4 among healthy pregnant women which are taken as standard for diagnosis and treatment of thyroid disorders during pregnancy. It has been recommended that every tertiary care hospital should establish its own trimester specific reference ranges of thyroid hormones levels based on population of that area.¹⁸ Our study population was also from a tertiary care hospital which had no trimester specific reference ranges for serum TSH, T3, T4, FT3, and FT4 for comparison. These reference ranges are also not available on national level. So our study established reference ranges from Maternal Control Group of study for compared with other countries reference ranges and recommended levels of TSH, FT3, and FT4 by American Thyroid Association.¹⁹

Elhaj *et al* reported trimester specific reference ranges of serum TSH, Serum FT3 and FT4 after a longitudinal study on pregnant Sudanese women. Their study was hospital based with sample size of sixty-three pregnant women (n=63). Our study was also hospital based and sample size thirty pregnant women (n=30) during third trimester. Our study design and sample size were in accordance with Elhaj *et al*. Our results for serum TSH and FT3 levels were significantly higher ($p<0.00$) than Elhaj *et al*.¹⁸

In 2017, Alexander *et al*¹⁹ updated the guidelines of American Thyroid Association (ATA) first published in 2011 for diagnosis and management of thyroid disease during pregnancy and post-partum period. Maternal serum FT3 concentration during 3rd trimester was decreased and was considered more precise and qualitative test. ATA recommended upper TSH level reference limit of 2.5 mU/l in first trimester, 4.0 mU/l during 2nd and 3rd trimesters, in case of non-availability of population based trimester specific reference ranges (5th–98th percentile). FT4 levels between 2.5–97.5 percentile were recommended as 7.4–12.2 ng/dl. Serum FT3 levels were recommended as 4.1–4.4 pg/ml during first and 4.0–4.2 pg/ml during 2nd trimester respectively.

Serum TSH and FT3 levels in our study were lower as compared to standards of ATA¹⁹. This could be because our study population, geographical distribution and dietary habits were different.

Yang *et al*²⁰ reported thyroid functions reference ranges during pregnancy in large Chinese Population and compared with guidelines of American Thyroid Association. Their study was hospital-based, and of longer duration of three years (2013–2016). Their study sample size was 46,262. Our study was also hospital-based with lesser duration and sample size of thirty (30) pregnant women in last trimester of gestation. Our results for serum TSH and FT4 during third trimester of pregnancy are in agreement with the Chinese women.

CONCLUSION

Trimester specific reference ranges of TSH, FT4 and FT3 of our study population were lower than international recommended levels of 2017 Guidelines of American Thyroid Association. These values may be used to compare thyroid disease in pregnancy in our location.

RECOMMENDATIONS

More studies should be conducted in different regions of Pakistan to establish normal range of trimester specific values for thyroid function tests during pregnancy.

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