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Trimester-specific reference intervals of thyroid hormones for normal pregnant women at a tertiary care hospital in Mumbai, Maharashtra

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Abstract: *Background:* Maternal thyroid dysfunction has been associated with adverse pregnancy outcomes. *Objectives:* The present study was conducted to determine trimester-specific reference intervals (TRIs) for serum thyroid stimulating hormone (TSH), total circulating thyroxine (TT4), total circulating triiodothyronine (TT3), free triiodothyronine (FT3) and free thyroxine (FT4) on healthy pregnant women in Mumbai region. *Materials and Methods:* A case-control study designed with 50 normal pregnant women that randomly selected from the first (20 samples), the second (13 samples), and the third (17 samples) trimesters and 50 randomly selected non-pregnant healthy controls. Thyroid function tests were estimated by chemiluminescent immunoassay method. *Results:* TRIs of TSH, TT3, TT4, FT3 and FT4 for first trimester pregnancies were 0.45-3.11µIU/mL, 139-222 ng/dL, 9.24-19.2µg/dL, 1.87-5.40 pg/mL, and 1.06-1.66 ng/dL respectively. TRIs for second trimester pregnancies were 0.63-3.88 µIU/mL, 150-231 ng/dL, 8.75-18.0 µg/dL, 2.40-3.58 pg/mL, and 0.79-1.41 ng/dL. TRIs for third trimester pregnancies were 0.75-3.92 µIU/mL, 162-252 ng/dL, 7.91-14.3 µg/dL, 2.10-2.98 pg/mL, and 0.72-1.34 ng/dL. TRIs for TSH, TT3, TT4, FT4 and FT3 were different from non-pregnant normal reference intervals. *Conclusions:* The reference intervals of thyroid function tests in pregnant women differ among trimesters.

Keywords: Trimester-specific reference intervals, Thyroid hormones, Normal pregnancy.

Introduction

The state of pregnancy represents a functional challenge for the thyroid gland [1]. Proper maternal thyroid function during pregnancy is important for the health of both the mother and developing child [2]. Thyroid disorders are amongst the most common endocrine diseases in India [3]. The frequency and pattern of thyroid disorders depends on sex, age, ethnic and geographical factors and especially on iodine intake [4]. A high iodine intake is associated with lower prevalence of goiter and higher prevalence of hypothyroidism. Low intake is associated with a higher prevalence of hyperthyroidism [5]. Hypothyroidism is more common in older women and 10 times more common in women than men [6]. The prevalence of hyperthyroidism is also reported as more common in women than men [7].

During pregnancy, maternal thyroid function is transformed by three independent but interrelated factors, (1) an increase in human chorionic gonadotrophin (hCG) concentrations that stimulate the thyroid gland, (2) significant increases in urinary iodide excretion, resulting in a fall in plasma iodine concentrations, and (3) an increase in thyroxine-binding globulin (TBG) during the first trimester, resulting in increased binding of thyroxine. In the aggregate, these factors may be responsible for the increased thyroid demand, or thyroid "strain" observed during pregnancy [8-10].

Therefore, non-pregnant reference intervals should not be used in pregnancy as they can mislead on the diagnosis and treatment of thyroid disorders during pregnancy.

Establishment of reference intervals in each trimester of pregnancy is thus of great importance, and these values should be used to diagnose thyroid disorders during pregnancy. There is no normative statistics documented for thyroid hormones on healthy pregnant women in Mumbai region. The aim of this study was to establish trimester-specific reference range for thyroid hormone during pregnancy.

Material and Methods

This was a case-control study undertaken in Department of Biochemistry, Grant Government Medical College and Sir J.J. Group of Government Hospitals, Mumbai. All participants completed a medical history form and provided informed consent. The Institutional Ethical Committee at the Grant Medical College and Sir J.J. Group of Government Hospitals, Mumbai, India, approved this study.

Study population: A case-control study designed with 50 normal pregnant women that randomly selected from the first (20 samples), the second (13 samples), and the third (17 samples) trimesters and 50 randomly selected non-pregnant healthy female controls. From October 2007 to June 2010, healthy pregnant women who visited Sir J.J. Hospital for routine antenatal checkups were consecutively and prospectively enrolled. Exclusion criteria included women with a visible or palpable diffuse or nodular goiter, a history of thyroid disease or medication usage, positive for anti-thyroid peroxidase antibody, anti-thyroglobulin antibody.

Methods: Qualified midwives informed participants about the rationale of the study to obtain written consent to allow for laboratory measurements. Obstetric history was taken using a standard questionnaire, and physical examination was performed. Gestational age was calculated from the first day of the last normal menstrual period, and gestational age 1-12, 13 to 27 and 28-40 weeks comprised the first, second, and third trimesters of pregnancy.

Blood sample collection: Venous blood samples collected in plain test tube with aseptic precautions at the time of admission before starting any treatment. After 2 hours of collection, sample was centrifuged at 3000 rpm for 5 minutes. Serum was separated and collected in polythene tube with cork. Serum was immediately stored at -20^{o}C until assayed. The sera with no sign of hemolysis used for the analysis of TSH, TT3, TT4, FT3 and FT4. We used fully automated enzyme amplified chemiluminescent immunoassay based Immulite 1000 analyzer.

Laboratory Measurements: Measurement of concentration of TSH, TT3, TT4, FT3 and FT4 by using commercial kits manufactured from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA. Daily quality controls were performed and all measurements were controlled.

Statistical Analysis: Numerical variables were reported in terms of mean and standard deviation. Data were analyzed using the MS Excel. Statistical Package for the Social Sciences (SPSS) Inc., Chicago, USA software was used for statistical analysis. To assess the difference between categorical variables Chi□square test was used. Independent sample *t*`test was used to compare the means of two separate sets of independent samples.

For comparison of means of more than two samples-analysis of variance test was used. $P<0.05$ and $P<0.001$ indicated statistically significant and highly significant difference respectively. Mean represented the central tendency of quantitative data and standard deviation represented the discrete tendency.

Percentile values for TSH, TT3, TT4, FT3 and FT4 were presented with 2.5th, 25th, 50th, 95th, and 97.5th percentiles. Reference ranges for TSH and fT4 in each trimester were defined as the range between 2.5th and 97.5th percentiles according to National Academy of Clinical Biochemistry recommendations [11].

Results

A total of 100 women were recruited in the study. Of these, 50 were enrolled in the non-pregnant control group, with an average age 25.4 ± 4.96 years; 20 in the 1st trimester group, 25.7 ± 4.16 years; 13 in 2nd trimester group, 26.2 ± 4.34years; and 17 in the 3rd trimester group, 26.4 ± 4.48 years (Table 1).

Table-1: Baseline demographic data of each group			
Group	**N**	**Age (Years)**	**Gestational age (Weeks)**
Non-pregnant controls	50	25.4 ± 4.96	0.00 ± 0.00
Pregnant subjects			
1st trimester (1–12 weeks)	20	25.7 ± 4.16	8.12 ± 3.09
2nd trimester (13–27 weeks)	13	26.2 ± 4.34	25.2 ± 2.67
3rd trimester (28–40 weeks)	17	26.4 ± 4.48	32.5 ± 2.94

To establish a trimester-specific reference range of thyroid function, 20, 13, and 17 eligible pregnant women in 3 trimesters after screened in each trimester group, respectively, meanwhile 50 eligible women in non-pregnant control group. The trimester-specific reference range of thyroid function is listed in Table 2. The TSH reference range in non-pregnant control group, 1st trimester, 2nd trimester, and 3rd trimester was 2.56-3.95, 0.45-3.11, 0.63-3.88 and 0.75-3.92 µIU/mL, respectively; the lower limit and upper limit increased along with the trimester, but always lower than lower limit of non-pregnant control group.

The TT3 reference range in non-pregnant control group, 1st trimester, 2nd trimester, and 3rd trimester was 110-152, 139-222, 150-231 and 162-252 ng/dL, the upper limit and lower limit also increased along with the trimester, but always higher than non-pregnant control group. The TT4 reference range in non-pregnant control group, 1st trimester, 2nd trimester, and 3rd trimester was 7.23-10.8, 9.24-19.2, 8.75-18.0 and 7.91-14.3 µg/dL, the upper limit and lower limit also declined along with the trimester, but always higher than non-pregnant control group.

The FT3 reference range in non-pregnant control group, 1st trimester, 2nd trimester, and 3rd trimester was 3.56-3.98, 1.87-5.40, 2.40-3.58 and 2.10-2.98 pg/mL, the lower limit increased in 1st and 2nd trimester but declined in 3rd trimester but always lower than non-pregnant control group while upper limit declined with the trimester. In addition, The FT4 reference range in non-pregnant control group, 1st trimester, 2nd trimester, and 3rd trimester was 0.95-1.83, 1.06-1.66, 0.79-1.41 and 0.72-1.34 ng/dL, respectively, the upper limit and lower limit both declined along with the trimester.

Table-2: Established of trimester-specific reference range of thyroid function in each groups						
Group	**N**	**TSH (µIU/mL)**	**TT3 (ng/dL)**	**TT4 (µg/dL)**	**FT3 (pg/mL)**	**FT4 (ng/dL)**
Laboratory range	--	0.40-4.00	81.0-178	4.50-12.5	1.50-4.10	0.80-1.90
Non-pregnant controls	50	2.56-3.95	110-152	7.23-10.8	3.56-3.98	0.95-1.83
Pregnant subjects						
1st trimester (1–12 weeks)	20	0.45-3.11	139-222	9.24-19.2	1.87-5.40	1.06-1.66
2nd trimester (13–27 weeks)	13	0.63-3.88	150-231	8.75-18.0	2.40-3.58	0.79-1.41
3rd trimester (28–40 weeks)	17	0.75-3.92	162-252	7.91-14.3	2.10-2.98	0.72-1.34

The mean ± standard deviation, median, 2.5th, 5th, 95th, 97.5th, percentiles for TSH, TT3, TT4, FT3 and FT4 were determined in each trimester of pregnancy. The reference intervals for each trimester are shown in Table 3. Analysis of these parameters between each trimester showed significant variation. The limits of the reference intervals were calculated as percentile 2.5 – percentile 97.5. Mean TSH value significantly increased from trimester 1st to 2nd ($P < 0.05$) as compared to trimester 2nd to 3rd ($P < 0.001$). TT3 significantly increased with the progression of gestational period ($P < 0.001$). TT4, FT3 and FT4 significantly decreased with the progression of gestational period ($P < 0.05$).

Table-3: Trimester-specific percentile values of thyroid hormones							
Thyroid hormones	**Trimester**	**Mean ± SD**	**Percentile values**				
			2.5th	**25th**	**Median**	**95th**	**97.5th**
TSH (µIU/mL)	Non-pregnant	3.29 ± 0.54	2.56	2.97	3.24	3.93	3.95
	1st trimester	1.73 ± 0.84	0.45	1.55	1.62	3.10	3.11
	2nd trimester	1.89 ± 1.04	0.63	1.26	1.59	3.54	3.88
	3rd trimester	2.34 ± 0.98	0.75	1.59	2.45	3.46	3.92
TT3 (ng/dL)	Non-pregnant	125 ± 17.1	110	112	126	149	152
	1st trimester	166 ± 26.9	139	146	157	215	222
	2nd trimester	204 ± 26.7	150	196	208	230	231
	3rd trimester	210 ± 30.9	162	198	211	251	252
TT4 (µg/dL)	Non-pregnant	7.30 ± 1.55	7.23	5.97	6.94	10.2	10.8
	1st trimester	14.6 ± 3.02	9.24	13.2	15.1	18.4	19.2
	2nd trimester	12.8 ± 3.24	8.75	10.7	11.9	17.7	18.0
	3rd trimester	9.14 ± 2.26	7.91	8.49	8.73	10.8	14.3
FT3 (pg/mL)	Non-pregnant	3.42 ± 0.55	3.56	2.87	3.20	3.91	3.98
	1st trimester	3.39 ± 0.97	1.87	2.76	3.23	5.14	5.40
	2nd trimester	3.15 ±0.36	2.40	2.98	3.30	3.48	3.58
	3rd trimester	2.44 ± 0.27	2.10	2.21	2.44	2.84	2.98
FT4 (ng/dL)	Non-pregnant	1.44 ± 0.28	0.95	1.18	1.45	1.79	1.83
	1st trimester	1.34 ± 0.18	1.06	1.23	1.34	1.65	1.66
	2nd trimester	0.99 ± 0.21	0.79	0.82	0.89	1.32	1.41
	3rd trimester	0.88 ± 0.18	0.72	0.78	0.81	1.29	1.34

Discussion

Gestational thyroid dysfunction is common and associated with maternal and child morbidity and mortality. During pregnancy, profound changes in thyroid physiology occur, resulting in different thyroid stimulating hormone (TSH) and free thyroxine (FT4) reference intervals compared to the non-pregnant state. Therefore, international guidelines recommend calculating trimester- and assay-specific reference intervals per center [12].

Due to a lot of physiological alterations during pregnancy, understanding of thyroid function tests needs trimester-specific reference intervals (TRIs) for a particular population. There is no normative statistics documented for thyroid hormones on healthy pregnant women in Mumbai region. This study showed that mean TSH and TT3 levels were seen to rise progressively through the three trimesters of pregnancy, but was significant compared with non-pregnant women. Whereas concentrations of TT4, FT3 and FT4 strongly declined significantly in the first, second and third trimester but mean levels of TT4 was higher than controls while mean FT3 and FT4 levels were reduced as compared with non-pregnant women.

In our study, the upper limit, lower limit, and mean values of TSH in the 3 trimesters rose gradually; the upper limit of the 3rd trimester approached the non-pregnant control group, which were in agreement with the previous studies [13-16]. Mehran L et al studied 215 cases for measurement of thyroid function tests by immunoassay method of which 152 iodine-sufficient pregnant.

Their study showed that significant difference was observed in average values of TSH among trimesters. This finding was closely like to our study. The percentiles and average values of both TT3 and TT4 increased

markedly from first trimester to third trimester. In our study TT3 and TT4 showed increasing in both the second and the third trimester [17].

Zhang D et al performed a prospective, observational study to find out the trimester-specific reference range of thyroid function in normal pregnant women as compared to non pregnant women in Nanjing. Their study showed that trimester-specific reference range of serum TSH and TT3 significantly increased with the progression of gestational period and TT4, FT3 and FT4 significantly decreased with the progression of gestational period. This finding was closely like to our study [18]. Sun R et al established the reference intervals for pregnant women in Zhejiang province, China. A total of 9038 cases were recruited in their study. Their median and percentile values of TSH, TT3, TT4, FT3 and FT4 did not closely matched but near to our results [19].

Finally, numerous limitations that existed in the present study had to be acknowledged. First, the sample size for each group was relatively small. Second, maternal blood iodine level was not considered in our study. Third, fasting condition was not essential for the women enrolled in our study, and that factor might influence our results [20].

Conclusion

TSH and TT3 levels showed an increasing trend while TT4, FT3 and FT4 concentrations reveals reduced manner from the first trimester to the third throughout gestation. The reference intervals of thyroid function tests in pregnant women differ among trimesters. TRIs may help in the diagnosis and appropriate management of thyroid dysfunction during pregnancy which will prevent both maternal and fetal complications.

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