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ORIGINAL ARTICLE

Determination of trimester specific reference intervals for thyroid hormones during pregnancy in Malaysian women

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Abstract

Objective: To establish trimester specific reference intervals for thyroid hormones during pregnancy in Malaysian women. *Study design*: Cross-sectional observational study performed in the Obstetric Clinic, University Malaya Medical Centre. A single blood sample from 626 women at various trimesters of pregnancy was analyzed for thyroid function. TSH, FT4, TT4, FT3 and TT3 values at each trimester of pregnancy were calculated. *Results*: From the TSH, FT4, TT4, FT3 and TT3 results, reference values based on mean $\pm$2SD was calculated for the hormones at each trimester of pregnancy. *Conclusion*: We calculated clinically relevant trimester-specific reference values for thyroid function tests through pregnancy to facilitate improved management of thyroid disease in pregnancy in our local population.

Keywords: trimester-specific, reference values, pregnancy, thyroid function

INTRODUCTION

Laboratory reference intervals for thyroid function tests have traditionally been derived from non-pregnant subjects who are free from thyroid disease. Their validity in pregnant women is debatable as pregnancy produces profound physiological changes in the mother, which in turn complicate the interpretation of maternal thyroid function tests. Pregnancy is associated with significant, but reversible changes in thyroid function studies, which are among the most profound seen as a result of a normal physiological state.[1-6] Proper maternal thyroid function during pregnancy is important for the health of both the mother and developing child. Furthermore, human choronic gonadotropin (hCG) can stimulate the thyroid gland during the first trimester because of its structural similarity to thyrotrophin (TSH).[7] Maternal thyroid hormones are critical to foetal neurodevelopment and change significantly during pregnancy.[8-10] Therefore, thyroid status is frequently assessed during pregnancy, both to evaluate suspected thyroid abnormalities, and to monitor the status of pre-existing thyroid disease. However, the usual clinical and laboratory assessment can be potentially misleading. The findings associated with the hypermetabolic state of normal pregnancy can overlap with the clinical signs and symptoms of thyroid disease. Both normal pregnancy, and pregnancy complicated by conditions such as hyperemesis gravidarum (HG), can be associated with thyroid function study changes that are strongly suggestive of hyperthyroidism, in the absence of primary thyroid disease.[5,11,12] Therefore, a local reference range for thyroid hormones in pregnant women is needed.[13-18] The availability of gestational age-dependent reference intervals for thyroid hormones will allow an accurate interpretation of thyroid hormone results in complicated pregnancies, which may have abnormal thyroid function, such as pre-eclampsia and HG.[13,15,18] For this reason, it is advisable for laboratories to establish their own reference values based on the populations they serve.

MATERIALS AND METHODS

Six hundred and twenty-six women attending the Obstetric Clinic, University Malaya Medical Centre, a 1000-bedded teaching hospital were

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recruited. Women with known thyroid disease, or taking medications other than nutritional supplements (e.g., prenatal vitamins) that might alter thyroid function were excluded from the data collection. All subjects were consuming iodide salt. Therefore, none of the subjects had iodide deficiency problem.

The study was approved by the University Malaya Medical Centre Ethical Review Committee on research involving human subjects, and all women gave informed written consent for the study. All women attending routine venepuncture were informed about the study and were invited to be involved. The sample of women recruited was therefore representative of the attending population.

Between 3-5 ml of venous blood was collected from each subject. The blood was collected into plain containers and allowed to clot. Each sample was centrifuged at 1000 rpm for 10 min to achieve separation. The serum obtained was stored at -20^{0}C until analysis was performed. Thyroid function tests were carried out by measuring the serum levels of thyroid stimulating hormone (3rd generation TSH), free and total thyroxine (FT_4, TT_4), free and total triiodothyronine ((FT_3, $TT_{3)}$ thyroid peroxidase and thyroglobulin antibodies (TPO-Ab, Tg-Ab) using the Abbott AxSYM immunoassay platform.

Data was entered into a data base and analyzed using SPSS for Windows v.15 (SPSS Inc, Chicago, IL, USA). Skewness of all the thyroid hormone parameters and the Kolmogorov-Smirnov tests were used to assess normality of the data. Where indicated, data was log transformed before analysis. After obtaining as near possible to Gaussian distributions, the value for reference intervals were calculated as representing 95% confidence intervals. A one way ANOVA analysis was performed to explore the impact of pregnancy trimester on the thyroid hormone parameter means. A *p*-value of <0.05 was considered to be significant.

RESULTS

Of the 626 antenatal mothers included into the whole study group, 69 were 20–24.9 years of age, 278 were 25.0–29.9 years of age, 187 were 30.0–34.9 years of age, 77 were 35.0–39.9 years of age and 15 were 40.0–45.0 years of age (data not shown). Six hundred and twenty six women each gave a single blood sample. Their mean age was 29.5 years (range 20-45 years). The distribution of the women based on their 1st:2nd:3rd trimesters were 130:229:267 respectively.

Table 1 shows the mean ± SD of the measured thyroid function tests in pregnant women. We found that the mean TSH levels decreased during the first trimester and then increased significantly ($p<0.05$) in the second and third trimester. Comparatively, the increase in the mean TSH levels from the second to the third trimester was not statistically significant ($p>0.05$).

Mean FT4 levels were increased in first trimester, and then declined significantly ($p<0.05$) during the second and the third trimesters. This finding was echoed in comparing the significant decline of the mean FT4 level from the second to the third trimester of pregnancy.

Mean TT4 levels was the only thyroid hormone parameter that displayed no significance in the ANOVA analysis in its decrease from the first to the second trimester ($p>0.05$) and the third trimester respectively.

Mean FT3 values (Table 2) showed declining levels form the first trimester that were significant in the second and third trimesters ($p<0.05$). However, the decrease in the mean FT3 levels from the second to the third trimester was not statistically significant ($p>0.05$). In the first trimester, the mean TT3 values (Table 2) showed declining levels which then increased significantly during the second trimester the third trimester. The mean TT3 values remain unaltered with no statistical significance for the second to the third trimester.

DISCUSSION

In the current setting, most clinicians will use non-pregnant reference intervals to manage thyroid disease in pregnancy. This study showed that mean TSH was seen to rise progressively through the three trimesters of pregnancy (Table 1). A similar finding is echoed in a longitudinal study of thyroid function in pregnancy by Price *et al*[18] where the TSH concentrations showed an upward trend that became significant during the third trimester. Their finding about TSH was closely similar to our study. Khandakar *et al* performed a case-control study to find out alteration in serum thyroid hormones levels in normal pregnant women as compared to nonpregnant women in Dhaka City.[8] For this purpose, they randomly selected 35 pregnant women during third trimester and 21 non-pregnant women of childbearing age as control. Their finding about TSH was closely similar to our study.

Erem *et al* investigated maternal thyroid function in 29 pregnant women with goitre and

TABLE 1. Reference intervals for thyroid hormones established in pregnant women

Subjects	Mean ± SD				
	TSH mIU/L	FT4 pmol/L	TT4 nmol/L	FT3 pmol/L	TT3 nmol/L
First Trimester N= 130	1.04 ± 0.08	13.86 ± 5.9	143.56 ± 38.26	3.3 ± 1.04	1.18 ± 0.38
Second Trimester N= 229	1.82 ± 0.07	9.35 ± 2.07	140.89 ± 26.99	2.83 ± 0.41	1.29 ± 0.24
Third Trimester N= 267	1.92 ± 0.06	8.40 ± 1.30	138.03 ± 22.79	2.73 ± 0.43	1.29 ± 0.30
Overall N= 626	1.69 ± 0.04	9.89 ± 3.74	140.23 ± 28.17	2.89 ± 0.64	1.27 ± 0.30

Key: N =Number of subjects. SD = Standard deviation

51 pregnant women without goitre in the eastern black sea region of Turkey, which is an endemic goitre area.[17] They found that all TT4, FT4, TT3, FT3, and TBG levels increase during pregnancy. Erem *et al*. also showed that serum TSH levels declined in pregnant women without goitre compared with nonpregnant women without goitre. In our study, serum levels of TSH and TT3 in pregnant women showed changes closely similar to Erem *et al.*[17] But in contrast to them we saw declining in serum levels of FT4, TT4 and FT3 in advancing pregnancy. In our study, free T4 and FT3 strongly declined during the third trimester. Khandakar *et al*[8] also found overall mean of each thyroid function test showed significantly increasing TT4 and declining FT4 and FT3. Changes in albumin and free fatty acid concentrations affect the binding of T4 and T3 to carrier proteins, lowering the blood levels of FT4 and FT3 as pregnancy progresses.[12, 13]

Panesar *et al*[13] carried out a prospective study with 343 healthy pregnant women (5-41 weeks) and 63 non-pregnant controls to establish gestation-related reference intervals for thyroid hormones in pregnant Chinese women. They found that FT3 decreased during pregnancy, whereas FT4 initially increased, peaking between 9-13 weeks and then decreased, the decline becoming significant by week 21, and TSH changes was similar to FT4. We also found declining FT3 over the pregnancy. FT4 changes during pregnancy in our study were nearly similar to their study. In contrast to Panesar *et al*., we did find any significant change in TSH in each trimester.

Kumar *et al*[16] measured serum levels of T3, T4, and TSH in 124 pregnant women that were apparently normal, healthy young primigravidas with no known metabolic disorders and normal carbohydrate gestational intolerance test. They found that mean TT3 increased during the second trimester and then declined in the third trimester compared to the first trimester. This is in contrast to our study where the TT3 level remained the same. Kumar *et al*. also showed mean TT4 level rose in the second trimester and then decreased during the third trimester which was mirrored in our work. Kumar *et al*. also saw mean TSH levels rising progressively through the trimesters of pregnancy.

CONCLUSION

In conclusion, thyroid hormone reference intervals vary depending on the trimester of pregnancy. We have established trimester-specific reference intervals for thyroid function tests on the Abboott Axysm in an iodine replete, multi-ethnic pregnant population of Malaysian women. It is hoped that this data will encourage

TABLE 2. Multiple comparison of changes in thyroid hormone concentration during pregnancy means.

Hormone	Trimester	Trimester	* Significance of mean difference	95% Confidence Interval	
				Lower Bound	Upper Bound
TSH	First	second	significant	-1.03	-0.51
		third	significant	-1.12	-0.62
	Second	first	significant	0.51	1.03
		third	non significant	-0.31	0.11
	Third	first	significant	0.62	1.12
		second	non significant	-0.11	0.31
FT4	First	second	significant	3.69	5.33
		third	significant	4.63	6.23
	Second	first	significant	-5.33	-3.69
		third	significant	0.25	1.59
	Third	first	significant	-6.23	-4.64
		second	significant	-1.59	-0.25
TT4	First	second	non significant	-4.75	10.08
		third	non significant	-1.69	12.75
	Second	first	non significant	-10.08	4.75
		third	non significant	-3.21	8.95
	Third	first	non significant	-12.75	1.69
		second	non significant	-8.95	3.21
FT3	First	second	significant	0.32	0.64
		third	significant	0.42	0.73
	Second	first	significant	-0.64	-0.32
		third	non significant	-0.03	0.23
	Third	first	significant	-0.73	-0.42
		second	non significant	-0.23	0.03
TT3	First	second	significant	-0.19	-0.04
		third	significant	-0.18	-0.03
	Second	first	significant	0.04	1.93
		third	non significant	-0.05	0.08
	Third	first	significant	0.03	1.18
		second	non significant	-0.08	0.05

**The mean difference is significant at the .05 level*

the appreciation of the changes that occur in thyroid function during pregnancy, and allow clinicians to use trimester-specific reference intervals to identify mothers who may require medical management to minimize the risk of abnormal neurodevelopment to their children.

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