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ORIGINAL ARTICLE

Reference Intervals of Thyroid Function Tests in First Trimester Vietnamese Pregnant Women

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SUMMARY

Background: **Although TSH suppression by elevated β-hCG is essentially seen during first trimester, differences in TSH reference ranges between various countries have been reported. Physiologic changes during pregnancy may also influence FT4 assays. This study aims to establish method-specific reference intervals (RIs) of TSH, FT4, and FT3 in Vietnamese, first trimester pregnant women.**

Methods: **This cross-sectional study was conducted at My Duc Hospital, Ho Chi Minh, Vietnam. Women with singleton pregnancies in the first trimester and conceived naturally were included. Those with a history of thyroid disease, positive thyroid-specific autoantibodies, diffuse goiter or one thyroid nodule > 10 mm in size or ≥ 2 nodules detected by ultrasound, and taking medications affecting thyroid function were excluded. Serum TSH, FT4, and FT3 were measured by chemiluminescent detection technology on the Access 2 Immunoassay System (Beckman Coulter, Inc., USA). Intra- and interassay coefficients of variations (CV) were 3.6% and 4.4% for TSH, 5.4% and 6.1% for FT4, 6.6%, and 6.0% for FT3, respectively. The 2.5th and 97.5th percentiles were used to determine RIs.**

Results: **Between August 1, 2017, to December 1, 2018, there were 876 pregnant women who fulfilled inclusion and exclusion criteria. They had a mean age of 30.1 years, an average BMI of 21.3 kg/m^2, and 77.3% of them were primigravida. The RIs for TSH, FT4 and FT3 were 0.17 - 2.35 mIU/L, 0.67 - 1.11 ng/dL and 2.82 - 3.90 pg/mL, respectively.**

Conclusions: **Established RIs for TSH, FT4, and FT3 in Vietnamese women would help to reduce the misdiagnosis of gestational thyroid disorders.**

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INTRODUCTION

Hyperthyroidism affects 0.2% of pregnancies while overt and subclinical hypothyroidism account for 0.1 - 0.3% and 2 - 5% of pregnancies, respectively [1]. Moreover, autoimmune thyroid diseases are seen in 5 - 20% of pregnant women, which certainly put them at risk for developing thyroid disorders throughout pregnancy [2].

Maternal thyroid dysfunction could lead to devastating effects on both mothers and their offspring. In fact, severe maternal hyperthyroidism is associated with increased risk of heart failure, preeclampsia, preterm birth, fetal growth restriction, and fetal death [1]. Meanwhile, untreated overt hypothyroidism could lead to miscarriage, stillbirth, perinatal mortality, and significantly reduced intelligence quotient of offspring in their early childhood. Subclinical hypothyroidism is also associated with pregnancy loss, gestational hypertension, preterm birth, and low birth weight as well as postpartum hemorrhage [3].

It is favorable that adverse outcomes as described above are highly avoidable if thyroid disorders are detected and intervened properly. A recent meta-analysis showed that levothyroxine supplementation significantly reduces rates of pregnancy loss and preterm birth in subclinical hypothyroidism and/or autoimmune thyroid diseases [4]. However, detection of thyroid diseases during pregnancy could be challenging because reference intervals (RIs) of thyroid function tests (TFTs), e.g., TSH and FT4, are clearly different from non-pregnant normal ranges. TSH, for example, is largely suppressed by β-hCG during the first trimester while FT4 values measured by most of the commercial immunoassays are greatly influenced by gestational changes of thyroxine-binding globulin (TBG) and albumin [5,6].

Early recommendations of the American Thyroid Association (ATA) in 2011 considered 2.5 mIU/L as the upper limit of normal TSH during first trimester [7]. However, later and larger-scale pregnancy studies in China, South Korea, India, and the Netherlands revealed that there are significant diversities of RIs for TSH in different demographic and ethnic populations. Hence, the 2017 guidelines of the ATA no longer provided the upper limit of TSH but the panel rather recommended a trimester-specific reference range for TSH based on studies of the local community. In addition, inter-assay variations in measurement of FT4 during pregnancy are even greater than TSH [8]. Accordingly, establishment of trimester-specific and method-specific RIs of TSH, FT4, and T3 for local population are urgently required. Such RIs are not only beneficial to maternal thyroid screening and diagnosis but also helpful for treatment, e.g., low-dose levothyroxine therapy is currently recommended for women with positive TPO antibody and TSH level higher than the pregnancy-specific reference range [8].

There is a lack of normal values of TFTs for Vietnamese pregnant women. Therefore, we conducted this cross-sectional study to define normal ranges for TSH and thyroid hormones during first trimester of women with singleton pregnancy.

MATERIALS AND METHODS

This cross-sectional study was conducted at My Duc Hospital, Ho Chi Minh City, Vietnam. The study was approved by the Institutional Ethics Committee of My Duc Hospital (03/2019/MĐ-HĐĐĐ). All women with singleton pregnancies, conceived naturally, and at seven to twelve weeks' gestational age were identified from the hospital database and screened for eligibility. Women with a history of thyroid diseases, positive thyroid-specific autoantibodies, diffuse goiter or one thyroid nodule > 10 mm in size or ≥ 2 nodules detected by ultrasound or taking medications that may interfere with TSH and/or FT4 measurements were excluded.

It was the standard protocol that all pregnant women attending our prenatal care program be screened for thyroid functions during the first trimester. Fasting blood samples were drawn from the median cubital vein. Collected blood samples were immediately transferred to the laboratory. After centrifugation at 4,000 rpm for 15 minutes, supernatants were collected and analyzed. Serum TSH, FT4, and FT3 were measured by chemiluminescent detection technology on the Access 2 Immunoassay System (Beckman Coulter, Inc., USA). Intra- and interassay coefficients of variations (CV) were 3.6% and 4.4% for TSH, 5.4% and 6.1% for FT4, 6.6% and 6.0% for FT3, respectively. Internal quality controls at three different levels (low, middle, and high) were run daily, at the same operating conditions with samples from patients. Z-scores from external quality assessment fell within -1 to +1.

The sample size was calculated using reference intervals for clinical and lab medicine function. We estimated a two-sided 95% confidence interval of upper and lower limits of TSH during the first trimester. We assumed the width of 95% reference interval was 8%. The total sample size required was 876 pregnant women [9, 10].

Baseline characteristics were presented using descriptive statistics. Mean (standard deviation) or median (interquartile range) were provided depending on the skewness of variables. Categorical variables were displayed as number and percentage. The upper and lower limits of TFT references were defined as 2.5th and 97.5th percentiles (95% reference limit). All analyses performed using the R statistical program version 3.5.0 (The R Foundation for Statistical Computing, 2018).

RESULTS

Between August 1, 2017, and December 1, 2018, our database revealed 7,152 women attending the Prenatal Care Unit of the hospital, of which, 876 women fulfilled the criteria of this study. Mean age of participants was 30.1 years, mean body mass index was 21.3 kg/m^2. Two-thirds of women were primigravida (Table 1). Mean gestational age of participants was 11.1 weeks. All TFTs were not normally distributed. TSH and FT4 distributions were skewed left, while FT3 was skewed right (Figure 1). Medians, upper, and lower limits of TSH, FT4, and FT3 were presented in Table 2. First trimester TSH, FT4, and FT3 ranged from 0.17 - 2.35

Table 1. Patients' baseline characteristics.

	Pregnant women (n = 876)
Mean age (years)	30.1 ± 4.2
Gravida	
Multigravida	199 (22.7)
Nulligravida	677 (77.3)
Body mass index (kg/m^2)	21.3 ± 2.6
Mean gestational age (weeks)	11.1 ± 1.0
Systolic blood pressure † (mmHg)	104.2 ± 7.9
Diastolic blood pressure † (mmHg)	64.1 ± 6.2

† - Measured at the first examination. Values are mean ± standard deviation or number of patients (%).

Table 2. Results of thyroids function tests.

Thyroid function tests	TSH (mIU/L)	FT4 (ng/dL)	FT3 (pg/mL)
Median	0.99	0.86	3.36
Upper limit (95% CI ‡)	2.35 (2.22 - 2.41)	1.11 (1.1 - 1.12)	3.90 (3.89 - 3.9)
Lower limit (95% CI ‡)	0.17 (0.13 - 0.19)	0.67 (0.64 - 0.68)	2.82 (2.77 - 2.85)

‡ - Confidence interval.

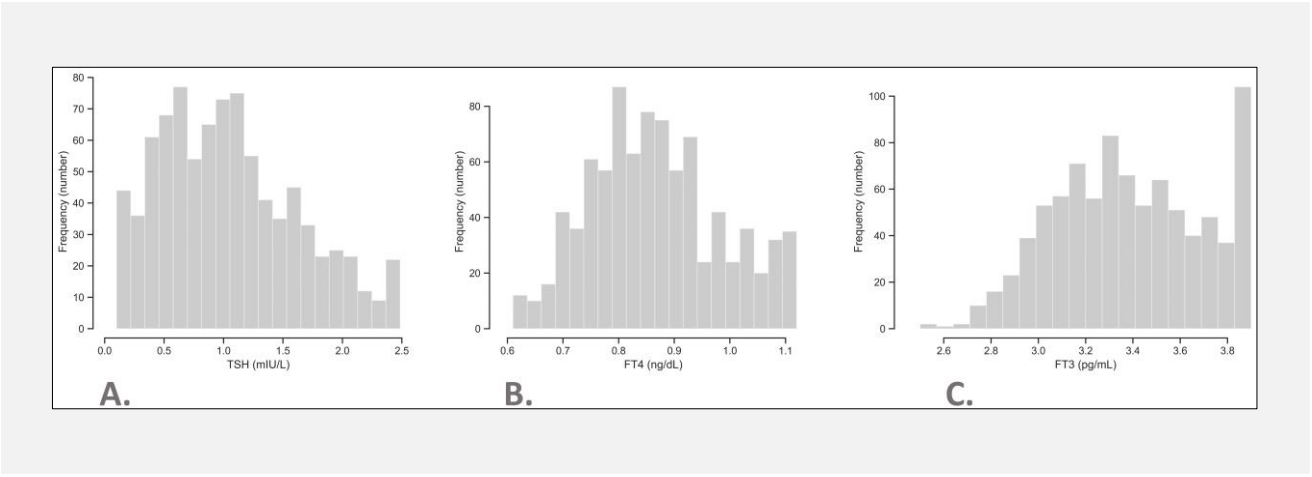

Figure 1. Frequency of TSH, FT4, and FT3 values during the first trimester.

All TFTs were not normally distributed. TSH and FT4 distributions were skewed left (A and B), while FT3 was skewed right (C). Therefore, 2.5th and 97.5th percentiles were used to determine upper and lower limits of TFTs.
Abbreviations: TFTs - thyroid function tests, TSH - thyroid stimulating hormone, FT4 - free thyroxine, FT3 - free triiodothyronine.

mIU/L, 0.67 - 1.11 ng/dL, and 2.82 - 3.90 pg/mL, respectively (Table 2).

DISCUSSION

Newer ATA guidelines in 2017 highlight the importance of local population-based studies to establish RIs for TFTs. When RI of TSH is not available, ATA recommended 2.5 mIU/L as the general cutoff for upper limit (2011) but it was later changed to 4.0 mIU/L (2017). In our study, first trimester RI of TSH was 0.17 - 2.35 mIU/L, which is more consistent with 2011 recommendation and lower than other Asian populations. This could be partially explained by the fact that those studies include a wider spectrum of gestational age, from very early to late in the first trimester. On the contrary, the gestational age of participants in our study were around 12 weeks when β-hCG increases to the highest, and TSH reaches its lowest level. It should be noted that in a cohort of 262 Vietnamese non-pregnant women, aged 18 - 40 years, with no history of thyroid diseases, negative TPO and Tg antibodies, and normal thyroid ultrasound scanning, the 2.5th - 97.5th percentiles of TSH, FT4, and FT3 were 0.57 - 4.44 mIU/L, 0.70 - 1.11 ng/dL, and 2.68 - 3.88 pg/mL, respectively (unpublished data). The first trimester RI of TSH is significantly lower than the RI of TSH in non-pregnant women ($p < 0.05$). Therefore, if first trimester RI of TSH is not available, cases of gestational hypothyroidism are likely to be misdiagnosed. Moreover, the currently reported RI is narrower than first trimester RI provided by the manufacturer (0.05 - 3.7 mIU/L) [11]. From a physiological point of view, FT4 and FT3 during the first trimester should be higher than non-pregnant condition. However, these figures were not significantly different from those in non-pregnant women (0.67 - 1.11 vs. 0.70 - 1.11 ng/dL and 2.82 - 3.90 vs. 2.68 - 3.88 pg/mL, respectively). That could be attributed to limitations of commercial immunoassays. In fact, Lee et al. demonstrated that immunoassays may not reflect true kinetics of gestational FT4 [12]. While FT4 index shows FT4 increases during first trimester and returns to non-pregnant interval during second and third trimesters, FT4 immunoassays show first trimester FT4 falls within non-pregnant interval and declines afterward. In their study, the median of FT4 yielded by one of the two tested immunoassays is significantly lower than non-pregnant median, and that could lead to false diagnosis of hypothyroxinemia if non-pregnant interval is used as the RI. This, again, highlights the importance of establishment of gestational RI for FT4 and FT3 for commonly used assays at local practice.

Strengths of our study include the large sample size, which is larger than the recommendation for this type of study [8,13]. The precision and accuracy of the tests used in the study were maintained via routine internal and external quality controls. There are also some limitations that need to be considered when interpreting our results. The value of maternal iodine was not available. However, in this study, we performed ultrasound to exclude thyroid goiter in return. In addition, the study was conducted at a single hospital, which may not be representative for the whole country. For example, compared to a recent nutrient study which was comprised of 1,944 pregnant women from three metropolitan cities in Vietnam and did not exclude any thyroid disorders, our pregnant group was older (30.1 ± 4.2 vs. 27.6 ± 5.3, $p < 0.05$), higher BMI (21.3 ± 2.6 vs. 20.2 ± 2.5, $p < 0.05$), and more primigravida (77.3% vs. 39.5%) [14].

In summary, we reported first trimester RIs of TFTs in Vietnamese women with singleton pregnancies and conceived naturally. Establishment of these RIs are essential to reduce the misdiagnosis of gestational thyroid disorders.

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Declaration of Interest:
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