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The Significance of Trimester-Specific Thyroid Hormones Reference Intervals in Iraqi Pregnant Women

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Abstract

In Iraq, there is no obvious considerable data regarding trimester-specific reference intervals of thyroid hormones despite documented multiple physiological hormonal changes during pregnancy. Thus, this study aimed to determine trimester-specific reference intervals for serum TSH, FT3, and FT4 and assess the incidence of thyroid function test misinterpretation and misdiagnosis among pregnant women using non-pregnant reference intervals. A thyroid function test was performed for 774 enrolled pregnant women. Reference intervals of TSH, FT3, and FT4 were ascertained in each pregnancy trimester. It was then compared to the adult non-pregnant values, and the incidence of misinterpretation was later calculated. TSH and FT4 reference interval values were lower than non-pregnant reference interval values. The application of non-pregnant women references values in pregnant women caused a serious misinterpretation and misdiagnosis in 66 (8.5%) pregnant women regarding TSH, and 34 (4.4%) pregnant women regarding FT4, while no misdiagnosis was noticed regarding FT3. The trimester-specific reference interval values of TSH, FT3, and FT4 in Iraqi pregnant women showed an obvious variation from non-pregnant reference intervals and the urgent advice to use the trimester-specific reference intervals to avoid misclassification of thyroid dysfunction during pregnancy.

Keywords: gestation, iodine, pregnancy, reference interval, thyroid-stimulating hormone

Introduction

The optimal function of the maternal thyroid gland is critical for both the fetus and mother's health. Thyroid hormones are crucial for the development and growth of fetal skeletal and nervous systems, especially during the first trimester of pregnancy.[1] Diagnosis of maternal thyroid gland diseases during early pregnancy is important for timely medical intervention since these diseases are common, with a prevalence ranging from 2% to 5%.[2,3]

Gestational maternal thyroid gland dysfunctions can be associated with many maternal and adverse fetal outcomes. For example, subclinical hypothyroidism is found in about 2.3% of pregnant women and is characterized by symptomless abnormally high thyroid-stimulating hormone (TSH) and normal free thyroxine (FT4). It can be associated with many obstetric complications like hypertension, repeated abortions, preterm delivery, placental abruption, intra-uterine growth retardation, increased cesarean section, and increased insulin resistance rate.[4,5] In addition to various impairments of cognitive and neurological development and decreased intelligence quotient in the child.[6-10] Regarding thyroid autoantibodies, such as thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab), most available research indicates that the high serum level of these antibodies is associated with the increased rate of recurrent abortions and preterm birth.[11-13] At the same time, the prevalence of gestational subclinical hyperthyroidism is around 1.7% and usually not associated with any significant adverse pregnancy outcomes.[14]

Profound maternal thyroid physiological changes occur during pregnancy. The purpose is to fulfill both the maternal and fetal demanded amount of thyroid hormones, especially during the early stages of pregnancy. It is because the fetus depends on the maternal thyroid hormones for development and maturation (till approximately the 20th week of gestation) when the fetal thyroid tissue starts to secrete a sufficient amount of thyroid hormones.[2,15] Most important physiological changes include increased serum level of thyroxine-binding globulin (TBG), which elevates the serum level of total thyroxine (TT4) and total triiodothyronine (TT3) in pregnant women compared to non-pregnant women. In addition, thyroid gland stimulation with increased serum level of human chorionic gonadotrophin (HCG) is caused by its structural similarity to TSH. It stimulates the thyroid

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gland (agonist effect) to secrete more thyroxine (T4) and triiodothyronine (T3) hormones, especially during the first trimester of pregnancy.[16]

All these changes during pregnancy can affect the maternal thyroid gland homeostasis and complicate the correct interpretation of thyroid function test results.[17-19] These mentioned gestational facts and variations necessitate the determination of thyroid hormones reference interval values for each trimester of gestation. The purpose is to minimize the chance of misinterpreting thyroid function test results and to diagnose and control gestational thyroid gland dysfunctions with its dangerous maternal and adverse fetal outcomes.[20] Therefore, Endocrine Society (ES), European Thyroid Association (ETA), and American Thyroid Association (ATA) guidelines for diagnosis and management of thyroid gland diseases during pregnancy and postpartum 2017 established a presumed geographic and ethnic variation in serum level of TSH throughout the pregnancy. With a decline in its lower value of reference range, trimester-specific reference ranges for TSH should be determined for each different population. Also recommended that each region and center decide on its gestational age-specific reference ranges for thyroid hormones through blood specimens from pregnant women free of any thyroid diseases and negative for thyroid (TPO-Ab, Tg-Ab) autoantibodies.[21-23]

The majority of centers and laboratories in different countries and regions still depend on the thyroid hormone reference ranges in non-pregnant women to evaluate and interpret the thyroid hormone test results during pregnancy. This is because only several studies have been established to define gestational trimester-specific reference intervals for thyroid hormones in different regions worldwide.[24-26] In Iraq, no thyroid hormones trimester-specific reference range has been set for pregnant women because of the limitation of the available data. Therefore, this study aimed to determine thyroid hormones trimester-specific reference ranges by using routine laboratory analysis methods and applying these new reference intervals to get a precise and correct interpretation of thyroid function test results among Iraqi pregnant women.

Method

This study was performed from February 2019 to October 2021 at Al-Noor University College in Mosul City, Iraq. This study was in collaboration with local obstetrical and gynecological centers, where pregnant women were asked to participate through the kind efforts and help from the medical staff in the obstetrical and gynecological clinics during their recurrent visits to the clinics. Few pregnant women declined to participate in this study due to socioeconomic reasons. The study's protocol and design were approved by the internal scientific review board and ethics committee at Al-Noor University College.

This was a cross-sectional study; apparently, healthy pregnant women with single intrauterine uncomplicated gestations were enrolled following prior written approval. A questionnaire was used to get the complete records of all participants in this study. The questionnaire included details of personal, geographic, clinical, chronic disease, medications, and family history, with special attention to the personal or family's record of thyroid gland dysfunctions or medications. Obstetrical and gynecological history was recorded to determine the parity, gravida, abortions, ectopic pregnancy, stillbirth, and preterm labor. Gestational age was determined by using both the date of the last menstrual period and the ultrasound examination. A detailed general and systemic physical examination are done.

Exclusion criteria included personal and/or family 's record of thyroid gland dysfunctions, thyroid treatments, systemic diseases like hypertension, diabetes mellitus, record of abortions, stillbirth, ectopic pregnancy, hyperemesis gravidarum, preterm delivery, evidence of fetal genetic abnormalities (e.g., trisomy), presence of visible or palpable goiter (diffuse or nodular), overt hypothyroidism or hyperthyroidism, and pregnant women positive for thyroid peroxidase-Ab (>35 IU/mL) and/or thyroglobulin-Ab (>40 IU/mL). For each blood specimen, five parameters were measured, including FT4, free triiodothyronine (FT3), TSH, and antithyroid antibodies, including TPO-Ab and Tg-Ab. All were measured using a COBAS e 601 analyzer (Roche diagnostics), a highly-specialized immunoassay analyzer using Electro-chemiluminescence (ECL) technique (Roche Diagnostics/Germany) by using its specified kits.

All data were analyzed using the Statistical Package for the Social Sciences Version 16.0 (SPSS Inc., Chicago, IL, USA). Mean, median, standard deviation, and 2.5th, 50th, and 97.5th percentiles were calculated for thyroid TSH, FT3, and FT4 during the three trimesters of pregnancy in rapprochement with the manufacturer's non-pregnant reference interval. TSH data were normalized using log transformation and summarized as a geometric mean.[27] To compare data between the trimesters, One−Way ANOVA was used. A two−tailed p-value<0.05 was considered statistically significant.

Results

The total participants in this study were 864 pregnant women. After excluding the TPO-Ab and/or Tg-Ab positive pregnant women and the cases of overt hypothyroidism, only 774 pregnant women were enrolled in this study. The primary characteristics of the study population in Table 1 show that 366 women are in the first trimester, 252 women are in the second trimester, and

Table 1. The Primary Characteristics of Study Population

	1st Trimester	2nd Trimester	3rd Trimester	Total
Number (n)	366	252	156	774
Age (year)				
Mean±SD	23.4±2.44	24.6±3.64	26.1±3.89	24.7±3.32
Gestational age (weeks) mean±SD	8.2±1.8	18.6±3.1	32.2±2.4	-
Primigravida	213	136	73	422 (54.5%)
Multigravida	153	116	83	352 (45.5%)

Note: SD = Standard Deviation

Table 2. Thyroid Gland Diseases Incidence in Each Trimester

	1st Trimester	2nd Trimester	3rd Trimester	Total
TPO-Ab	28 (3.2%)	15 (1.7%)	10 (1.2%)	53 (6.1%)
Tg-Ab	18 (2.1%)	10 (1.2%)	3 (0.3%)	31 (3.6%)
TPO-Ab and/or anti Tg-Ab	40 (4.6%)	22 (2.5%)	10 (1.2%)	72 (8.3%)
Overt hypothyroidism	12 (1.4%)	6 (0.7%)	0 (0.0%)	18 (2.1%)
Hyperthyroidism	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Notes: TPO-Ab = Thyroid Peroxidase Antibody, Tg-Ab = Thyroglobulin Antibody.

156 women are in the third trimester.

The mean age of participants in the first, second, and third trimesters was 23.4±2.44 years, 24.6±3.64 years, and 26.1±3.89 years, respectively. While the mean age of all participants was 24.7±3.32 years, the median age was 27.7 years (range = 15 – 40 years). Gestational age during the first, second, and third trimesters was 8.2±1.8 weeks, 18.6±3.1 weeks, and 32.2±2.4 weeks, respectively (Table 1).

Regarding the trimesters' distribution, the first trimester involved 366 pregnant women, with a mean age of 23.4 years and a median gestational age of 8.2 weeks. The second trimester involved 252 pregnant women with a mean age of 24.6 years and a median gestational age of 18.6 weeks. At the same time, the third trimester involved 156 pregnant women, with a mean age of 26.1 years and a median gestational age of 32.2 weeks. Primigravida women were 422 (54.5%), and multigravida women were 352 (45.5%).

Tabel 2 shows that the TPO-Ab and/or Tg-Ab were positive in 72 (8.3%) pregnant women; 53 (6.1%) were TPO-Ab positive, and 31 (3.6%) were Tg-Ab positive. Depending on the reference interval of the laboratory kit (non-pregnant women), overt hypothyroidism was diagnosed in 18 (2.1%) pregnant women, while no case of hyperthyroidism had been diagnosed. As a result, these 90 pregnant women were excluded, and the study population's total number became 774 pregnant women.

Figures 1, 2, and 3 show the trimester-specific reference range of thyroid hormones TSH, FT3, and FT4, determined by the 2.5th, 50th, and 97.5th percentile of these hormones in each pregnancy trimester. The TSH reference interval in the first trimester, second, and third trimester was 0.09 to 2.83 mIU/L, 0.26 to 2.98 mIU/L, and 0.38 to 3.11 mIU/L, respectively, with an obvious increase in both the upper and lower values toward the end of pregnancy. The FT4 reference interval in the first, second, and third trimester was 10.88 to 19.86 pmol/L, 9.82 to 17.44 pmol/L, and 8.92 to 15.22 pmol/L, respectively, with a slight decline in both the upper and lower values toward the end of pregnancy. The FT3 reference range in the first, second, and third was 3.64 to 6.49 pmol/L, 3.32 to 5.66 pmol/L, and 3.11 to 5.39 pmol/L, respectively, with obvious stability in both the upper and lower values toward the end of pregnancy. TSH and FT4 values statistically showed significant differences (p-value<0.05) among different trimesters of pregnancy. These differences were seen between the first, second, and third trimesters, with increasing values toward the third

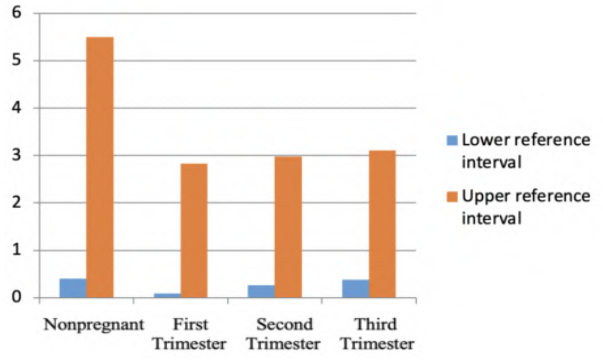

Figure 1. Tthyroid-Stimulating Hormone Reference Intervals

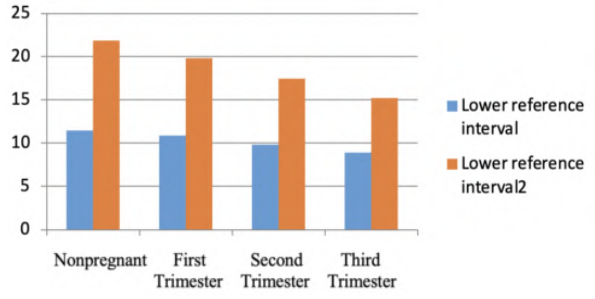

Figure 2. Free Thyroxine (FT4) Reference Intervals

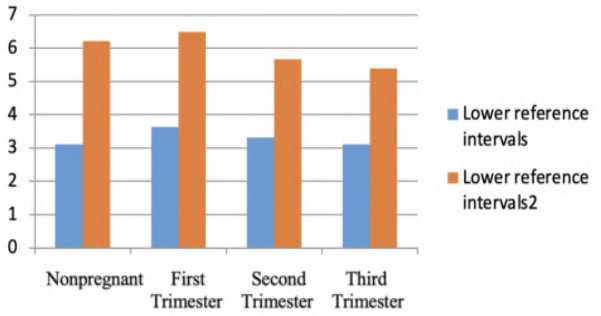

Figure 3. Free Triiodothyronine (FT3) Reference Intervals

Table 3. Hazard of Misidentification When Applying Non-Pregnant Reference Interval Values in Pregnant Women

Hormone	Misclassified / n (%)			
	1st Trimester (n = 366)	2nd Trimester (n = 252)	3rd Trimester (n = 156)	Total (n = 774)
TSH	41 (11.2)	19 (7.5)	6 (3.8)	66 (8.5)
Free T4	14 (3.8)	14 (5.5)	6 (3.8)	34 (4.4)
Free T3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: TSH = Thyroid-Stimulating Hormone, T4 = Thyroxine, T3 = Triiodothyronine.

trimester for TSH values while decreasing values are seen toward the third trimester for FT4 values.

In this study, a lower TSH reference interval occurred in the first trimester (0.09 µIU/mL) and was significantly lower than the adult non-pregnant reference interval (0.4 µIU/ml). The upper TSH reference range occurred during the third trimester (3.11 µIU/mL) and was significantly lower than the adult non-pregnant reference interval (5.5 µIU/mL). For FT4, the reference range gradually declined from the first trimester to the second trimester and then to the third trimester. The lower reference limit of FT4 occurred in the third trimester (8.92 pmol/L), whereas the upper reference interval occurred in the first trimester (19.86 pmol/L).

To discover the possibilities of misdiagnosis and misclassification of different thyroid gland diseases during pregnancy, the new trimester-specific reference intervals of thyroid hormones were obtained by this study. It was then compared to the manufacturer's adult non-pregnant reference interval for TSH, FT4, and FT3, and the results are summarized in Table 3. Regarding TSH, 66 (8.5%) pregnant women obtained misidentification of thyroid gland dysfunction, while 31 (4.0%) of enrolled pregnant women would not be identified despite their elevated TSH. In comparison, 35 (4.5%) of enrolled pregnant women would faulty be identified with a decreased TSH level. The first trimester presented the highest incidence of misidentification of TSH results (11.2%). Regarding FT4, 34 (4.4%) pregnant women obtained misidentification of thyroid gland dysfunction, and 19 (2.4%) pregnant women with elevated results would not be identified. While, 15 (1.9%) of enrolled pregnant women would faulty be identified with decreased FT4 levels. The second trimester presented the highest incidence of misidentification of FT4 results (5.5%). Regarding FT3, no misidentification or misdiagnosis had been noticed.

Discussion

Gestation gives rise to many physiological and hormonal changes in different body systems. The pituitary-thyroid hormonal axis will be affected during pregnancy due to the activation of TSH receptors by the HCG, so the negative feedback effect of TSH will decline due to the partial structural similarity of these two hormones.[28-30] At 10-12 weeks of gestation, the TSH reaches maximum serum level. The TBG levels will increase; nevertheless, serum levels of TT4 and TT3 do not rise more than 50%.[7] So, assessing thyroid gland function is more precise by measuring TSH and FT4.

The status of iodine nutrition was not determined because of the Iraqi community's daily consumption of iodized salt for many years. Therefore, all enrolled pregnant women were assumed to have sufficient amounts of iodine. In this study, the prevalence of thyroid gland autoimmune diseases in pregnant women has been determined, and 8.3% of enrolled pregnant women were positive for TPO-Ab and/or Tg-Ab. In contrast, it was 10.0–12 % in Vanderpump's study,[31] and 14.8% in Godines-

Enriquez, *et al.*, study.[15]

During this study, it was determined that with trimester-specific reference values for TSH, FT4, and FT3 in enrolled pregnant women, there were significant differences between trimesters for both TSH and FT4 values (p-value<0.05). During the first trimester, TSH level significantly declined compared to the non-pregnant controls (p-value<0.05). Also, a significant rising trend in TSH levels was noticed from the first to the third trimester (p-value<0.05). A significant decreasing trend was observed regarding FT4 levels from the first to the third trimester (p-value<0.05).

The thyroid hormone values obtained in pregnant women were interpreted by applying both the obtained gestational age-specific reference intervals for thyroid hormones in pregnant women and the reference intervals for those in non-pregnant women. There was an obvious risk of misdiagnosis and misclassification of different thyroid diseases (Table 3), such as hypothyroidism and hyperthyroidism, if the thyroid hormone function tests were interpreted using the non-pregnant reference intervals, which leads to disease underestimation or overestimation with the subsequent wrong treatment. The interpretation of TSH values was particularly important because TSH is the cornerstone in analyzing the results of thyroid hormone. The cause of its low lower limit value (0.09 mIU/mL) during the first trimester was the mimic effect of HCG. Then TSH would increase gradually toward the end of the third trimester due to fading up of the HCG effect, but this process is still poorly understood.[32] This process was responsible for the highest incidence of misidentification of TSH results (11.2%) during the first trimester if non-pregnant intervals references were used. This dangerous misinterpretation of TSH test values would occur, especially in pregnant women with pre-conception hypothyroidism, because they need regular monitoring throughout pregnancy and careful adjustment of treatment dose.[33]

The TSH results of this study were in line with two studies in Iraq,[34,15] a study in Egypt,[36] and another study in the United Arab Emirate.[37] At the same time, there was partial incompatibility with the result of another Iraqi study.[38] A study by Yang, *et al.*, showed that the TSH reference interval has a relatively lower cutoff value compared to previous different studies.[39] This difference may be due to the maternal iodine status of pregnant women enrolled in this study,[40] laboratory methods used, or ethical factors.

Regarding FT4 results, there was a significant difference compared to non-pregnant women's values, so this study agreed with a previous study by Padmakumar, *et al.*,[41] and indicated the necessity of determining the trimester-specific reference values to interpret the results of thyroid hormone assays in pregnant women. In this study, FT4 significantly declined with the progress of pregnancy from the first to the third trimester. The TSH significantly increased with the progress of pregnancy from the first to the third trimester, while FT3 showed no differences. It was difficult to compare different studies due to various causes and study variations, such as the size of the enrolled study sample, assay methods used, inclusion and exclusion criteria, iodine status, diverse centile range, and ethical and geographical factors.

The results of this study come in partial agreement with the Mehran, *et al.*, study.[42] Although no significant difference was found in TSH values among the pregnancy trimesters by Azizi, *et al.*, study,[43] another study conducted in India by Sekhri *et al.*,[44] stated that there was a decline in TSH value during the first trimester, followed by an increase during the second and third trimester. In contrast, FT4 values showed a gradual decline throughout the pregnancy trimesters but were significantly lower during the second and third trimesters. While FT3 values started declining during the second trimester and remained stable during the third trimester.[44]

Another study used liquid chromatography-tandem mass spectrometry and immunoassay techniques; FT4 showed significant differences between these techniques during the first trimester, while no significant difference was noticed regarding TSH.[45] A study from China showed a considerable decline in TSH values during the first trimester compared to non-pregnancy values, while there was an increase in the second trimester, and throughout pregnancy, there was a gradual decrease in FT4 and FT3.[46] Another study done by Kurioka, *et al.*, in Japan indicated significant changes in TSH and FT4 during the first trimester and showed a substantial decrease of FT4 during the progress of pregnancy.[47]

Lastly, the US national academy of clinical biochemistry recommended that the interpretation of serum thyroid hormone test values must be done using the trimester-specific reference interval values of thyroid hormones.[48] In summary, this study and most available data from previous studies confirmed the differences in the thyroid hormones reference interval values between pregnant and non-pregnant women and demonstrated the necessity to establish the thyroid hormones trimester-specific reference intervals to avoid serious misdiagnosis of thyroid gland diseases during pregnancy.

Limitation

Interindividual variation cannot be excluded because thyroid hormones could not be measured consecutively in each enrolled pregnant woman. The urinary iodine concentration was not measured to assess the iodine status because of the assumption that all participating pregnant women ingested sufficient iodine quantity through the daily consumption of iodized salt for many years, and

the ultrasound examination of the thyroid gland was not done. Poor methodology standardization is a significant limiting factor.

Conclusion

This study shows a significant difference in thyroid hormone reference intervals in pregnant women compared to nonpregnant. Using the thyroid hormone values of nonpregnant women to interpret thyroid hormone values in pregnant women can cause many misinterpret test results. Hence, it is very important to achieve the trimester-specific reference interval values to get a correct and proper diagnosis of thyroid diseases and dysfunction, such as missing many cases of hypothyroidism which can cause dangerous maternal and fetal complications (such as fetal mental retardation, which can be prevented by thyroxine) or giving unnecessary thyroid treatment to normal pregnant women misdiagnosed as hypothyroidism cases.

For Iraqi pregnant women, this study recommends using the thyroid hormones reference intervals determined for each trimester of pregnancy to interpret the thyroid function test (TFT) values. This study suggests doing more national studies to obtain national gestational age-specific reference interval values of different thyroid hormones in Iraq. It can be done by using larger study samples, with particular attention to the assay methodology, iodine status, statistical method, and inclusion and exclusion criteria of the enrolled pregnant women.

Abbreviations

TSH: Thyroid-Stimulating Hormone; FT4: Free Thyroxine; TPO-Ab: Thyroid Peroxidase Antibody; Tg-Ab: Thyroglobulin Antibody; TBG: Thyroxine-Binding Globulin; TT4: Total Thyroxin; TT3: Total Triiodothyronine; HCG: Human Chorionic Gonadotrophin; T4: Thyroxine; T3: Triiodothyronine; ES: Endocrine Society; ETA: European Thyroid Association; and ATA: American Thyroid Association; FT3: Free Triiodothyronine; ECL: Electro-Chemi-Luminescence; SPSS: Statistical Package for Social Sciences and Problem Solutions; µIU/mL: Micro International Unit/Milliliter; pmol/L: Picomol/Liter; SD: Standard Deviation; TFT: Thyroid Function Test.

Ethics Approval and Consent to Participate

Not applicable.

Competing Interest

The authors declare that there are no significant competing financial, professional, or personal interests that might have affected the performance or presentation of the work described in this manuscript.

Availability of Data and Materials

Materials and data of this study are available from the corresponding author for non-commercial goals and upon a reasonable request.

Authors' Contribution

HMA designed and developed this research and directed the overall work. MAA, KMS, and MY were responsible for samples and data collection. All authors shared data analysis and interpretation. Last, they reviewed and approved the final version of the manuscript.

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