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Trimester-Specific Thyrotropin Hormone Reference Levels in Mosul City, Iraq

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Abstract

Thyroid disorders during pregnancy is widespread and it is linked with adverse obstetric outcomes. Serum thyroid – stimulating hormone (TSH), also known as thyrotropin hormone is optimal indicator for monitoring and evaluating thyroid function. There are significant changes in thyroid physiology during gestation. Thus different TSH levels are recorded in pregnant women in different countries, Therefore. the aim of current study : To determine trimester (Tri) specific levels of TSH in the apparently healthy pregnant women in Mosul city. A cross – sectional study was conducted at Hospitals, Primary Health Centers and Outpatient clinics in Mosul city and collected from December 2020 to April 2021. Total enrolled pregnant women were 200 of age 18 – 32 years, 80 of them were excluded from study for meeting one or more of exclusion criteria . The remaining of 120 women were included in study, 40 women were assigned to each Tri. Thyrotropin and anti – thyroid peroxidase were measured for participants. The study results showed TSH levels increased over the Tri with very significantly higher TSH levels in the third in compared with first Tri. Accordingly the reference levels of TSH 0.33-2.79 mIU/L, 0.43-3.88 mIU/L, 0.57-3.39 mIU/L for the first, second and third Tri respectively. Our conclusion that the upper limit of TSH reference ranges for pregnant women in the first and second Tri was closer to that suggested by the American Thyroid Association(ATA) guidelines 2011 and some other countries.

Keywords: TSH; Trimester specific; Reference levels; Mosul city

1. Introduction

Thyroid – stimulating hormone (TSH) also known as thyrotropin is a glycoprotein hormone. [1] It is consist of two chains alpha and beta chain , human chorionic gonadotropin (HCG) and other glycoprotein hormone have identical alpha subunit as TSH . [2]

During pregnancy there are various physiological changes leading to alter in the maternal thyroid function . These include increased in the thyroxin – binding globulin (TBG), HCG , renal filtration rate , thyroid hormone production and maternal iodine requirements . [3,4]

HCG has direct inducing action on the thyroid gland Via TSH receptor , It reaches its peak near the 10 ~ 12 weeks of the pregnancy , then it declines during the second and third trimester .

The thyrotrophic activity of HCG causes an increase in thyroid hormone levels at the end of the first trimester . This leads to concomitant decrease in both thyrotropin – releasing hormone (TRH) and TSH values due to negative feedback mechanism [5] Which are associated with a decrease in HCG, thereafter trend towards a rise in TSH as pregnancy progresses[6].

Reference levels of TSH gradually increase in the second and third Tri . However , it is still lower than that of non-pregnant women[5].

It is important to be aware of these changes in TSH level during pregnancy It is of importance in medical practice . because using non pregnant level to interpret thyroid function test(TFT) may lead to misdiagnosis . [7]

Therefore It is essential to estimate specific reference levels for TSH in each Tri , in each country and province . Anti – thyroid peroxidase (Anti – TPO) is found in 2% - 17% of randomly selected pregnant women [8] .

Recent researches suggested that pregnant women with positive Anti – TPO may have failed response to HCG [9] .

Elevated Anti – TPO levels are associated with lower (thyroxine) T4 and Higher TSH levels [10] .

Aim of the present study is to determine Tri specific thyrotropin hormone reference intervals in apparently

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healthy pregnant women that live in non-coastal area in Mosul city, Iraq. Mosul city is chiefly and provincial capital with multiethnic, which lies northern Iraq across the Tigris river, in addition Greater Zab river irrigate Mosul, our population's rely on fresh water for drinking, Mosul known to be an area of iodine deficiency.

2. Subjects and Methods

2.1. Subjects :

This study was carried out in Al-khanssa,AL-Btool Maternity Teaching Hospital , Al-Noor Primary Health Center and Out – patient clinics in Mosul city during period from December 2020 to April 2021.

It is a cross–section study . Ethical approval was obtained from committee of Ethics at Nineveh Health Directorate , Mosul , Iraq

Two hundred women were enrolled in this study with age(18-32) years, they were interviewed, (72)of them fulfill one or more of the exclusion criteria and (8)of them with positive Anti –TPO, the remaining 120 pregnant women were included in the present study , 40 pregnant women were assigned to each Tri . For each subject was interviewed and the general information was taken to fill the questionnaire(name, age, weight,height,parity,gravida ,number of abortion, gestational age, medical history concentrating on thyroid disorders , drug history). The gestational age was calculated based on the women's last menstrual period and / or the early report of ultrasound.

The gestational age was classified into the following trimesters: first trimester (1st Tri) with gestational age less than 13 weeks(wks) ; second trimester(2nd Tri) with gestational age range from 13-28 wks and third trimester(3rd Tri)with gestational age ≥28 - 40 wks .

2.1.1 Inclusion criteria:

Subject inclusion criteria are apparently healthy pregnant women with uncomplicated single intrauterine gestations .

2.1.2 Exclusion criteria

Women with the following criteria were excluded from the current study :

1-Any women on the following drugs :

Thyroid drugs , estrogen , medication known to effect thyroid function like amiodarone , lithium , steroids and non-steroidal anti – inflammatory drugs .

2-Twin pregnancy .

3-Positive family or personal history of thyroid disorders.

4-Women with acute or chronic diseases including diabetes mellitus , hypertension , cardiac , renal and hepatic disorders .

5-Women with recurrent miscarriages and infertility .6-The presence of positive Anti-TPO.

7-History or presence of hyperemesis gravid arum .

8-History of polycystic ovarian disease .

2.2.Methods

Blood samples were obtained from all participants in the study :

Five milliliter (ml) of venous blood had been collected in plain tube, incubated at 37 C^0 for 15 minutes (min), centrifugation for 10 min at 3000 rpm. Aspiration of supernatant serum was divided into two aliquot, freezed at -20 C^0 till the time of assay. Each of which used for the following measurements

1.TSH utilizing Access 2(Beckman Coulter) NHANES, USA utilizing electrochemi-luminescence (ECL)technique, with the limit of detection 0.005 mIU/Measuring range (0.01-50.0)mIU/L reference range of the kit used (0.38-5.33)mIU/L . [11]

2.Anti – TPO was measured on the Algeria Orgentic using the enzyme –linked immunosorbent assay" ELISA" .Sensitivity was determined to be 5 IU/ml ,measuring range was 0-3000 IU/ml and cut-off 75 IU/ml.[12]Negative less than 50 IU/ml, Borderline50-75IU/ml, and the Positive was morethan75IU/ml.

2.3.Statistical Analysis:

All data are analyzed in a computer using IBM SPSS for Windows version 26.0(SPSS software,Chicago,ILL). Statistical mean and standard deviation (SD) was obtained .Levels of TSH were not normally distributed(assessed using the Kolmogorov-Smirnov test of normality), to compare difference between three groups were using the Kruskal –Wallis test , Mann-whiney test was used to compare mean difference between two group and the difference is considered statistically significant at $P \leq 0.05$ [13] . The range of 2.5th to 97.5th centile was indicated the reference range for each Tri .

.3.Results :

Two hundred women were enrolled in this study ,72 of them were excluded from study for meeting one or more exclusion criteria, throughout three trimesters the positive Anti-TPO were 3, 3 and 2 for the 1st, 2nd and 3rd Tri respectively. The eight of the positive Anti-TPO pregnant women were excluded from the study, The remaining 120 pregnant women were included in the final study population , 40 women were assigned to each Tri with age range (18 – 32) years , mean ± standard error(SE) of age were 24. 85 ± 0.68 , 25.5 ±0.59, 24.62 ± 0.64year in the 1st , 2nd , 3rd Tri respectively ,

Table(1)

Characteristics	1st Tri N= 40 Mean± SE	2nd Tri N=40 Mean± SE	3rd Tri N=40 Mean± SE
Age, year	24.85±0.68	25.5±0.59	24.62±0.64
Gestational age, wks	7.92±0.44	21.05±0.88	36.95±0.41

General characteristic of each group. Tri=trimester

TSH levels increased with progression of pregnancy from 1.19 ± o.73 , 1.53 ± 0.80 , 1.87 ± 0.77 (mIU / L) for the 1st, 2nd and 3rd Tri,respectively,Table(2)

TSH levels increased over the Tri with a significant difference was observed in TSH levels in 3rd Tri versus the 1st Tri P = 0.001, while just a significant difference was observed in TSH levels between the 2nd and 3rd , 1st and 2nd Tri (p = 0.05) , Fig(1).

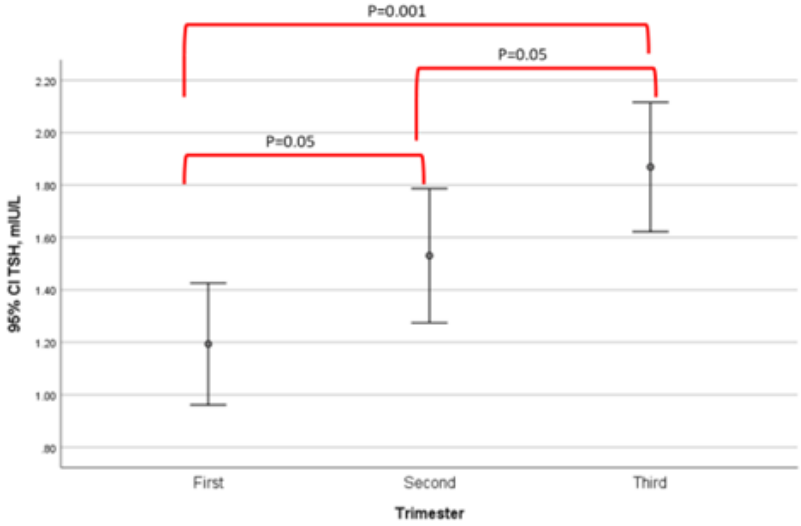

Fig.1.Distrubution of TSH level among different trimester in pregnant women

Table(2) **Trimester specific mean and percentile value of thyroid stimulating hormone**

	Tri	Mean±SD	Percentile 2.5th and 97.5th	5th and 95th
TSH (mIU/L	1st Tri	1.19±0.73	0.33-2.79	0.34-2.70
	2nd Tri	1.53±0.80	0.43-3.88	0.55-3.75
	3rd Tri	1.87±0.77	0.57-3.39	0.72-3.18

The mean ± SD, 2.5th and 97.5th , 5th and 95th percentile for TSH in each Tri, Table(2)

Accordingly,2.5th and 97.5th TSH centile for each Tri of current study was 0.33 – 2.79 (mIU / L) in the 1st Tri , 0.43 – 3.88 (mIU / L) in the 2nd Tri and o.57 – 3.39 (mIU / L) in the 3rd

4.Discussion :

During pregnancy thyroid status influenced by various factors such parity, body mass index [14] , iodine status, geographical area, ethnic and assay methods which consequently may influence the normal levels of thyroid TFTs Thus each population should establish its own reference levels for normal TFT to avoid serious effect on the mother and fetus . [15]

In the present study we observed that the TSH mean value in the 1st Tri (1.19 ± 0.73 mIU / L) is lower than in the 2nd Tri (1.53 ± 0.8 mIU / L) but 3rd Tri (1.87 ± 0.77 mIU / L) has higher mean value , Table(2). The initial decline in TSH levels due to HCG thyrotrophic activity cause arise in the serum concentration of thyroid hormones towards the end of 1st Tri . This is turn contributes to concomitant reduction of TRH and TSH levels due to negative feedback mechanism which associated with a decrease of HCG , thereafter trend towards rise in TSH as pregnancy progress .[5,6]

This upward sloping curve in the level of TSH was also noticed by previous Iraq studies Almomin etal.,2016 [16] and inconsistent with Alhamd

etal.,2013 in wasitte of Iraq [17] . Table (3), this is difference due to inclusion and exclusion criteria or due to different assay methods. Our results were in agreement with results of study done in India with mean±SD 1.11±0.57 1ST Tri,1.58±0.61 2nd Tri and 1.63±0.64 3rd Tri Rani etal.,2018 [11] .

Table(2) show the reference intervals of TSH in the 2nd Tri was wider than those in the 1st Tri and 3rd Tri, a finding that disagreement with Almomin etal.,2016 ,who reported reference interval 0.04-3.77, 0.30-3.21 and 0.60-4.50 mIU/L for the 1st Tri , 2nd Tri and 3rd Tri respectively [16] . Khalil etal.,2018 conducted study in Emirates they reported reference interval 0.094-3.33 , 0.052-4.56 and 0.44-4.75 mIU/L for the 1st Tri, 2nd Tri and 3rd Tri respectively [18] evidently they were in agreement with our results , a similar findings were reported in Egypt by sheriba etal.,2018 who noticed reference intervals 0.87-2.42 mIU/L(µIU/ml) in the 1st Tri ,0.94-2.55 mIU/L in the 2nd Tri and1.44-2.71 mIU/L in the 3rd Tri [19]

Table 3: Comparison of mean of thyroid stimulating hormone between our study and previous Iraq studies

	TSH mIU/L Mean		
	First trimester	Second trimester	Third trimester
Present study	1.19	1.53	1.87
Almomin et al [16]	1.51	1.58	1.87
Alhamd et al [17]	2.38	1.67	1.26

The upper limits of TSH reported in the current study are 2.79 mIU/L for the 1st Tri and 3.39 mIU/L for the 3rd Tri are closer to that suggested by American Thyroid Association (ATA) guidelines 2011, that recommended 0.1 – 2.5 , 0.2 – 3.0 and 0.3 – 3 mIU / L for 1st Tri ,2nd Tri and 3rd Tri respectively [20] .

We compared our study results to those reported in different countries (India[11] , China[21], Australia [22] and Netherlands[8]) using Beckman Access or DxI and 2.5% - 97.5% percentiles of TSH , Table(4) Shows that upper limit of TSH in the 1st Tri in all studies were around 3.0 (mIU / L) except study 2 as well as the lower limit of TSH in the 2nd Tri were around 0.4 except that reported in study 3 .

Although all these five studies were used Beckman as instrument for measuring TSH still this difference can explain different ethnicity and geographical factors.

Table 4

Comparism trimester reference range of thyroid stimulating hormone between different countries using Beckman Access or DxI

country	Year	Refer ence.	Author	Sample size	2.5th and 97.5th centile of TSH mIU/L(µIU/ml)		
					1st trimester	2nd trimester	3rd trimester
Present study 1				120	0.33-2.97	0.43-3.88	0.57-3.39
India 2	2018	11	Rani et al	162	0.08-2.24	0.42-2.84	0.40-3.14
China 3	2015	21	Zhang et al	1521	0.06-3.13	0.07-4.13	0.15-5.02
Australia 4	2013	22	Ekinci et al	129	0.03-3.05	0.42-3.36	0.34-2.83
Netherland s 5	2007	8	Benhadi et al	2475	0.27-2.96	0.38-3.04	

5.Conclusion :

The upper limit of TSH reference ranges for pregnant women in the 1st Tri and 2nd Tri was closer to the upper limit suggested by ATA guidelines 2011 and some other countries. We recommended to establish trimester specific reference level of TSH in each countries and even province to overcome maternal and fetal complications moreover recommend to measure TFTs for women as apart pre – marital examination to overcome an important and invisible cause for female infertility and It is used as control for TFTs during pregnancy for each women .

6.Conflicts of interest

There are no conflicts to declare.

7.Formatting of funding sources

All tests were done on my private account

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9.References

[1]. Arrangoiz, R ., Cordera, F., Caba, D., Muñoz, **M., M oreno, E. and de León , E,**L. Comprehensive Review of Thyroid Embryology, Anatomy, Histology, and Physiology for Surgeons. *Int. J. Otolaryngology and Head & Neck Surgery* , 7**(4)**, 160-188,(2018) . https://doi.org/10.4236/ijohns.2018.74019

[2].Delitala A.P., Capobianco G., Cherchi P.L., Dessole S. and Delitala G., Thyroid function and thyroid disorders during pregnancy: a review and care pathway. *Arch Gynecol Obstet*,299(2),327-338, (2019). [PubMed]

[3] Muller. L., Taylor P.N and Lazarus J.H., Thyroid function in pregnancy. *Ann Thyroid* ,3(27),(2018).

[4]. Khadilkar.S., Thyroid‑Stimulating Hormone Values in Pregnancy: Cutoff Controversy Continues*?.J Obstet Gynecol India*,69,389-394, 2019). https://doi.org/10.1007/s13224-019-01272-

[5].Park,C., Evaluation of Pregnancy and Thyroid Function. *Korean J Clin Lab Sci, 50(1):1-10, (2018). https://doi.org/10.15324/kjcls.2018.50.1.1*

[6]. McNei .A and Stanford,P,E., Reporting Thyroid Function Tests in Pregnancy.*Clin Biochem Rev,36(4),109-126, (2015).*

[7]. Glinoer D.and Spencer CA., Serum TSH determination in pregnancy: how, when and why? ..*Nat Rev Endocrinol*, 6,526-529,(2010).

[8]. Benhadi N., Wiersinga W.M., Reitsma J.B., Vrijkotte T.G., Van der Wal M.F. and Bonsel G.J., Ethnic differences in TSH but not in free T4 concentrations or TPO antibodies during pregnancy. *Clin Endocrinol* (Oxf),66(6),765-770,(2007).

[9]. Korevaar T.I. , Steegers E.A., Pop V.J., Broeren M.A. and Chaker L., Thyroid Autoimmunity Impairs the Thyroidal Response to Human Chorionic Gonadotropin: Two Population-Based Prospective Cohort Studies. *J Clin Endocrinol Metab* ,102,69-77,(2017). [PubMed]

[10]. Pearce E.N., Oken E., Gillman M.W., Lee S.L., Magnani B., Platek D. and Braverman L.E., Association of first-trimester thyroid function test values with thyroperoxidase antibody status, smoking, and multivitamin use. *Endocr Pract* ,14,33-9,(2008).

[11].Rani K.S., Tirupati S . , Sarathi S. and Kumar .K.D.,Trimester –specific reference ranges for thyroid function tests in South Indian women.*Thyroid Res Pract*,15,117-21(2018).

[12].Mouse S.,El-SawyS.S. and Abdelraouf M., Trimester-specific thyroid hormone changes in normal pregnant Egyptian women. *Egypt J Intern Med,31,412-415,(2019).*

[13].Daniel W.W.Biostatistics:A foundation for analysis in health science 10th ed,John Wiley and Sons Inc.,New York,USA,Chapter 13,Non parametric and distribution free statistics,(690-708),(2013).

[14]. .Korevaar T.I., de Rijke Y.B., Chaker L.,Medici M. and Jaddoe V.W., Stimulation of Thyroid Function by Human Chorionic Gonadotropin During Pregnancy: A Risk Factor for Thyroid Disease and a Mechanism for Known Risk Factors. *Thyroid* ,27,440-50,(2017). [Crossref] [PubMed]

[15]. Murillo-Llorente,M,T., , Fajardo-Montañana,C and Pérez-Bermejo,M ., . Reference Values of Thyroid Hormones During the First Trimester of Pregnancy in Valencian Community (Spain) and Their Relationship with Iodine Intake.*Nutrients,12(5),(2020).*

[16].Almomim A.M., Mansour A.A and Sharief M., Trimester-specific reference intervals of thyroid function testing in pregnant women from Basrah,Iraq using electrochemiluminescent immunoassay.*Diseases* ,4,pii:E20(2016).

[17].AlhamadA.K.,Mohammed H.Q. and Al-dhahiryJ.K., Evaluation of thyroid function at different stages of pregnancy in Iraqi women. *AJPS*, 13(2),139-146(2013).

[18].Khalil A.B.,Sali h B.T.,Chinengo O.,Bardies M., Turner A. andWareth L,A.,Trimester specific reference ranges for serum TSH and Free T4 among United Arab Emirates pregnant women.*Pract Lab Med*,12,1-16(2018)

[19].SheribaN.A.,IbrahimN,A.,MohamedN,R. and HegabA.M.,Assessment of normal range of thyroid function tests in healthy Egyptian pregnant women. *Thyroid Res Pract*,15(2),70-74(2018).

[20]. Stagnaro-Green A., Abalovich M., Alexander E., Azizi F., Mestman J and Negro R.., American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid* 21,1081-125,(2011).

[21]. Zhang J., Li W., Chen Q-B., Liu L-Y., Zhang W., and Liu M-Y., Establishment of trimester-specific thyroid stimulating hormone and free

thyroxine reference interval in pregnant Chinese women using the Beckman Coulter UniCel™ DxI 600. *Clin Chem Lab Med* ,53:1409-14(2015).

[22]. Ekinci E.I., Lu Z.X., Sikaris K., Bittar I., Cheong K.Y,and Lam Q.,Longitudinal assessment of thyroid function in pregnancy. *Ann Clin Biochem*,50,595-602, (2013).