https://doi.org/10.31351/vol33iss2pp79-85

Trimester-Specific Free Thyroid Hormones Levels among Pregnant Women in Nineveh Governorate, Iraq

Marrwa M.Naif [*,1], Hazim A.Allawi[2] and Sura Khairialdeen Mohialdeen[2]

[1]Ministry of Health and Environments, Nineveh Health Directorate, Nineveh, Iraq
[2]Department of Biochemistry, College of Medicine, University of Mosul, Nineveh, Iraq.
[*]**Corresponding author**
Received 13/12/2022, Accepted 29/5/2023, Published 27/6/2024

Abstract

There are numerous physiological changes during pregnancy that lead to alteration in the thyroid function and consequently lead to a change in the thyroid hormones levels. Therefore, the aim of the current study is to establish trimester –specific reference serum levels (TSRSLs) for free triiodothyronine (FT3) and free thyroxine (FT4) in apparently healthy pregnants in Nineveh governorate, Iraq.

Accordingly, a cross-sectional study was carried out from December 2020 to April 2021 in Al-Batool Teaching hospital, Al-khanssa Maternity Teaching Hospital, Al-Noor Primary Health Center (PHC) and Out-patient clinics in Nineveh governorate. One hundred and fifty pregnants aged (16-42) years were enrolled in this study. Participants were grouped according to the trimester of pregnancy into three groups involving 50 participants each . FT4 and FT3 were measured for each participant. The result of this study showed the TSRSLs for FT4 in the first, second and third trimesters were (0.70-1.29, 0.49-1.31 and 0.58-1.28 ng/dl), respectively; while ,for the FT3 they were (2.63-3.96, 2.04-3.3 and 2.11-3.40 pg/ml), respectively. Serum FT3 level was significantly higher in the first trimester compared to the second and third trimesters. While serum FT4 level was significantly lower in the second trimester compared to first and third trimesters. We concluded TSRSLs of FT4 and FT3 in pregnant women in Nineveh governorate is comparable to that recorded elsewhere.
Keywords: Free thyroid hormone , Reference levels, Pregnant women , Nineveh governorate

مستويات هورمونات الغدة الدرقية الحرة والمحددة حسب ثلوث الحمل بين النساء الحوامل في محافظة نينوى ، العراق

مروى موفق نايف [*،١] حازم علاوي [٢] وسرى خيرالدين محي الدين[٣]

[١] وزارة الصحة والبيئة ، دائرة صحة نينوى ، نينوى،العراق
[٢]فرع الكيمياء الحياتية، كلية الطب، جامعة الموصل، نينوى،العراق.

الخلاصة

هناك العديد من التغيرات الفسيولوجية في وظائف الغدة الدرقية تحدث خلال الحمل والتي تؤدي الى اختلاف في وظائف الغدة الدرقية وبالتالي تؤدي الى اختلاف في مستويات هورمونات الغدة الدرقية, لذلك فان هذه الدراسة تهدف الى تحديد مستويات مصل الدم المرجعية محددة حسب ثلوث الحمل لثلاثي يودوثيرونين والثايروكسين الحرين في النساء الحوامل الاصحاء ظاهريا في محافظة نينوى , العراق. وفقا لذلك اجريت دراسة مقطعية للفترة من كانون الاول ٢٠٢٠ الى نيسان ٢٠٢١ في مستشفى البتول التعليمي, مستشفى الخنساء التعليمي للولادة, مركز النور للرعاية الصحية الاولية والعيادات الخارجية للمرضى في محافظة نينوى.

مئة وخمسون امرأة حامل تراوحت اعمارهم ما بين ١٦ – ٤٢ سنة قد شاركوا في هذه الدراسة. تم تجميع المشاركات وفقا لثلوث الحمل في ثلاث مجموعات تضم كل مجموعة ٥٠ مشاركة .وقد تم قياس مستويات هورموني الثيروكسين وثلاثي يودوثيرونين الحرين في مصل الدم لكل مشاركة. اظهرت نتيجة هذه الدراسة ان مستويات مصل الدم المرجعية محددة حسب ثلوث الحمل للثايروكسين الحر في الثلث الاول والثاني والثالث من الحمل كانت (0.58-1.28, 0.49-1.31, 0.70-1.29 نانوغرام /دسيليتر)، على التوالي؛ بينما كانت بالنسبة لثلاثي يودوثيرونين الحر (2.11-3.40, 2.04-3.3, 2.63-3.96 بيكوغرام / مل) على التوالي.وكان مستوى ثلاثي يودوثيرونين الحر في مصل النساء الحوامل اعلى وبمغزى احصائي في ثلث الحمل الاول مقارنة بالثلثين الثاني والثالث؛ بينما كان مستوى الثايروكسين الحر في مصل النساء الحوامل اقل وبمغزى احصائي في ثلث الحمل الثاني مقارنة بالثلثين الاول والثالث .خلصت الدراسة الى ان مستويات مصل الدم المرجعية محددة حسب ثلوث الحمل للثايروكسين ولثلاثي يودوثايرونين الحرين في النساء الحوامل في محافظة نينوى هي مقاربة لما سجل في اماكن اخرى .
الكلمات المفتاحية: هورمونات الغدة الدرقية الحرة، المستويات المرجعية،النساء الحوامل، محافظة نينوى.

Introduction

The thyroid gland is the first endocrine gland that starts to develop in the day 20 to 24 of gestation [1].

Total thyroid hormones include free form and protein bounded form, these are the forms in blood; free triiodothyronine (FT3) and free thyroxine (FT4) are free fractions of thyroid hormones [2].

Only 0.3% and 0.03% of T3 and T4, respectively are circulating in free form, which is regarded as the active hormone fraction. Thyroid receptors have more affinity for T3 than T4; thus, T4 is relatively inactive [1].

The most important point is that the chemical structure of human chorionic gonadotropin hormone (hCG) is identical to that of thyroid – stimulating hormone (TSH), and has a direct inducing action on the thyroid gland via binding TSH receptor which reaches its peak at week 10 -12 of gestation, thereafter declines to a plateau after week 12 of gestation[3,4].

The hCG thyrotrophic activity causes an increase in the serum concentration of total triiodothyronine and total thyroxine .In addition, to a transient increase in FT4 at the end of first trimester .This in turn contributes to the concomitant reduction in both TRH and TSH levels due to negative feedback mechanism . Then hCG level declines with a decrease FT4 in the second and third trimesters and an increase of TSH level as pregnancy progress[5].Total thyroid hormones are present at nanomolar concentration, while the free fraction of thyroid hormone (FT4 and FT3) are present at picomolar concentration[6]. States of thyroxine-binding globulin (excess or deficiency) are commonly seen in clinical practice in association with a variety of pathophysiologic condition especially the high thyroxine-binding globulin state of pregnancy. This lead to the measurement of free hormone concentrations better than total hormone measurements [7]. Since free hormone levels reflect the actual physiological effects of thyroid hormones rather than total hormone levels [8].

The aim of the current study is to establish trimester specific reference serum levels (TSRSLs) for FT3 and FT4 in apparently healthy pregnant females in Nineveh governorate –Iraq.

Subjects and Methods

Subjects

This study is a cross- sectional study. It was carried out in Al-Batool Teaching hospital, Al-khanssa Maternity Teaching Hospital, Al-Noor Primary Health Center (PHC) and Out- patient clinics in Nineveh governorate from December 2020 to April 2021 after obtaining ethical approval from the Committee of Ethics at Nineveh Health Director, Mosul, Iraq .Each pregnant women was interviewed and the general information was taken to fill the questionnaire; regional area and full obstetric history including last menstrual period (LMP), parity, gravida ,number of abortion and history of infertility, were collected. Thyroid clinical examination was performed ; and thyroid peroxidase antibody (anti-TPO) was estimated.

The calculation of gestational age was based on women's last menstrual period (LMP) and/or report of ultrasonography examination.

According to the gestational age the subjects were grouped based on the trimester of pregnancy: first trimester with gestational age (less than 13 wks.), second trimester with gestational age (13-27wks.) and third trimester with gestational age equal or more than 28 wks. [9,10]. One hundred and fifty pregnants aged (16-42) years were enrolled in this study. Participants were grouped according to the trimester of pregnancy into three groups involving 50 participants each.

Inclusion criteria

Participants included in the study were apparently healthy pregnant females with uncomplicated single intrauterine gestations.

Exclusion criteria

Pregnants with the following criteria were excluded from the study:

1-Medications that may affect serum free thyroid hormones levels or measurements such as estrogen, thyroid drugs, lithium, amiodarone, non-steroidal anti - inflammatory drugs and steroids.

2-Positive personal or family history of thyroid disorders.

3-Diabetes mellitus, hypertension, cardiac, renal and hepatic disorders.

4-History of recurrent miscarriages, infertility, fetal malformation and other pregnancy complications.

5-Positive Anti-TPO.

6-Presence or history of polycystic ovarian syndrome or hyperemesis gravidarum.

Methods

Five ml of venous blood were taken from all participants in this study, it collected in plain tube then incubated for 15 min at 37°C, centrifugation for 10 min at 3000 rotation per minute to obtain serum to be measured for Anti-TPO as well as FT3and FT4.

FT3and FT4 were performed by electrochemiluminescence technique using Beckman Access 2 Immunoassay System, NHANES, USA with the FT3 reference range (2.5-3.9 pg/ml)and FT4 (0.61-1.12 ng /dl) [11].While Anti-TPO level was measured utilizing the enzyme linked- immunosorbent assay by Algeria ORGENTIC [11].

Statistical analysis

The statistics were evaluated by computerized statistical package for the social sciences SPSS program for windows version 26.0 (SPSS software, Chicago, ILL). Statistical mean,

standard deviation (SD), median and range were obtained Shapiro-Wilk test was used for testing normality.

Data of FT3 were normally distributed ; to compare the mean difference of FT3 among the three trimesters, ANOVA test was used. While independent student t-test was used to compare the means of two trimesters. Data of FT4 were not normally distributed ; to compare the mean difference of FT4 among three trimesters, Kruskal-Wallis H test was used. While Mann-Whiney test was used to compare the means of two trimesters. The reference levels of FT3 and FT4 for each trimester were defined as 2.5th and 97.5th percentile. The difference is considered statistically significant at $p< 0.05$.

Results

Table 1. shows the mean ±SD and range(min-max) of chronological age and gestational age. Table 2 shows the mean ± SD, median and range for FT3 were calculated for each trimester. Table 3 shows the mean ± SD, median and range for FT4 were calculated for each trim. Table (4) and (5) show the range of the reference for FT3 and FT4 in three trims (the central 95% range between the 2.5th and 97.5th percentiles). Table 4. shows the value of TSRSLs for FT3 at first trim was higher than that of second and third trim. Table (5) shows the value of TSRSLs for FT4 at first trim was higher than that of second trim and third trim.

Table1. Chronological and gestational age of participants.

Parameters		1st trimester no. =50	2nd trimester no. =50	3rd trimester no. =50	*P-value
Age (yr.)	Mean± SD	27.00±6.54	27.14±5.45	25.78±6.16	NS(0.4)
	Min - Max	16-42	17-40	16-42	
Gestational age(wks.)	Mean± SD	8.12±2.73	20.70±4.305	36.66±3.00	<0.001
	Min-Max	2-12	13-27	28-40	

*Kruskal-Wallis H test that was used to compare difference between three trims (1st= first, 2nd = second and 3rd = third trimester). Significant difference between groups(trims) exists at $p< 0.05$, NS: not significant

Table 2. General descriptive of FT3 concentration for each trimester.

FT3 (pg/ml)	1st trim no.= 50	2nd trim no .=50	3rd trim no. =50	*p-value
Mean ±SD	3.29±0.36	2.71±0.30	2.63±0.26	<0.0001
Median	3.28	2.67	2.60	
Range(Min-Max)	(2.62-3.99)	(2.04-3.32)	(2.08-3.51)	
Pair Difference	1st vs. 2nd	1ST vs. 3rd	2nd vs. 3rd	
****P-value**	<0.0001	<0.0001	NS(0.2)	

*ANOVA test(One Away Analysis of Variance) that was used to compare mean difference between three trims(1st ,2nd and 3rd trims).** Two independent sample t-test that used to compare mean difference between two trims(1st vs. 2nd , 1st vs. 3rd and 2nd vs. 3rd).Significant difference between groups(trims) exists at $p< 0.05$, NS: not significant. SD = standard deviation, 1st = first, 2nd = second , 3rd = third , trim= trimester, no.= number

Table 3. General descriptive of FT4 concentration for each trimester

FT4 (ng/dl)	1st trim no.= 50	2nd trim no.= 50	3rd trim no. = 50	*P-value
Mean± SD	0.94±0.15	0.83±0.21	0.92±0.14	0.002
Median	0.92	0.80	0.94	
Range(Min-Max)	(0.70-1.30)	(0.49-1.31)	(0.56-1.32)	
Pair difference	1st vs. 2nd	1st vs. 3rd	2nd vs. 3rd	
****P-value**	0.001	NS(0.5)	0.007	

*Kruskal- Wallis H test that was used to compare mean difference between three trims (1st ,2nd and 3rd trims)
** Mann-whiney U test used to compare mean difference between two trim(1st vs. 2nd , 1ST vs. 3rd and 2nd vs. 3rd).Significant difference between groups(trims) exists at $p< 0.05$, NS: not significant SD = standard deviation, 1st = first, 2nd = second , 3rd = third , trim= trimester, no.= number.

Table 4. Trimester –specific reference serum level of FT3

Parameter	Trim	2.5th and 97.5th
FT3(pg/ml)	1st	2.63-3.96
	2nd	2.04-3.3
	3rd	2.11-3.40

Table 5. Trimester- specific reference serum level of FT4

Parameter	Trim	2.5th and 97.5th
FT4(ng/dl)	1st	0.70-1.29
	2nd	0.49-1.31
	3rd	0.58-1.28

Discussion

The reference ranges for different parameters of thyroid function tests during pregnancy must be well established for each population of each country and governorate,
because different factors may explain the disparity in the results for TSRSLs between different countries, some were due to maternal factors such as age, body mass index, racial origin, iodine status, gestational age and parity. Other factors were related to the way of assessment of the serum thyroid hormones levels. Moreover, the disparity were related to difference in sample size, geographical area, inclusion and exclusion criteria. Accordingly, wide a range of TSRSLs of thyroid hormones levels were obtained that may delay in diagnosis and management of thyroid disorders [12,13].

TSH is an optimum indicator for evaluating and monitoring of thyroid functions. During pregnancy assessment of only serum TSH and total thyroid hormones are not enough due to alteration in protein binding and the effect of hCG[14.15]. For this reason, in the current study the TSRSLs of FT3 and FT4 in pregnant women in Nineveh governorate were measured to be used for comparison with that of other populations. This study represented the first study performed in Nineveh governorate. In 2019 Iraq was classified with insufficient iodine intake and this classification based on national representative data [16].

Iodine is a main microelement use for thyroid hormones synthesis [17]. Maternal thyroid hormones are important in the first half of gestation till maturation of fetal thyroid gland. Moreover, thyroid hormones is necessary for fetal growth mainly for the development of neurological system [18].

In the current study, as shown in Table (6 and 7),the mean value of FT3 and FT4 in third trimester were lower than that of the first trim of pregnancy. This decline for FT3 and FT4 might be due to estrogen induced stimulation of TBG sialyation [5].

Different TSRSLs of FT3 and FT4 had been reported in different areas using different assay methods; (Table 6), or same method to that used in the current study; (Table 7).

Concerning TSRSLs of FT3 for studies in Table (6); study no. 5 recorded the only first trim reference range which was lower than that reported in the present study. Furthermore, the upper limit of FT3 for three trims of studies no. 2 and 4 was higher than that of the current study..

The percentile 5th and 95th of FT3 for current study was (2.69-3.9, 2.13-3.26, 2.23-3.07 pg/ml) for the first ,second and third trimester respectively, in comparison with the current study with study no. 3 which calculated TSRSLs by 5th and 95th percentile as shown in Table (6) the upper limit of FT3 for first and third trimester of study no. 3 was lower than that of present study, while the lower limit of FT3 for first and second trimester was higher than that of present study.

As shown in Table(6).The lower and upper limit of FT4 for first trim as well as the lower limit for second and third trims of the present study were lower than all studies (2,3,4and 5)

The upper limit of TSRSLs of FT4 for third trim of the current study was higher than that of other studies (3 and 4) except study no. 2 (17.23 pmol/L).Different assay methods and other factors such as sample size, geographical area, inclusion and exclusion criteria. may explain the difference in the results.

Table (7) showed the results of different studies for FT3 and FT4 parameters using same assay method Access 2(Beckman Coulter).

The high value of TSRSLs for FT4 that recorded in first trim was noticed due to the effect of thyrotrophic activity of hCG [5].

Trimester specific reference serum levels of FT4 for different studies (6,7 and 8) showed the value of TSRSLs of FT4 in pregnant women at first trim was higher than that at the second and third trim. Nearly, similar findings were reported in the current study mainly when compared to the TSRSLs of FT4 for first trim with that of the third trim in regard to the lower and upper limit of TSRSLs of FT4.The TSRSLs of the present study (9.01- 16.6 pmol/L) for FT4 for the first trim appear higher than that reported by study no. 7 (9.0-15.1 pmol/L).Studies no.(6 and 8) reported TSRSLs of FT4 (0.84-1.43 and 0.68-1.44 ng/dl), respectively, which appear higher that than reported by the current study (0.70-1.29 ng/dl).The TSRSLs of FT3 was reported by only two studies no. 7and 8. Table (7). Nearly similar TSRSLs of the three trims for the current study to that reported by study no. 7.

Study no.8 reported lower values of TSRSLs for FT3 than that reported in the present study. Moreover, the present study and study no.8

had the same trend of decrease of the lower limit for FT3 TSRRs in the second trim then increase in third trim.

Table 6. Trimester –specific serum reference levels of thyroid hormones in different populations measured by different assay method

no.	Country, sample size	Author, year percentile reference no.	Thyroid test	1st Trim	2nd Trim	3rd Trim	Iodine Status	Exclusion criteria	Methods /Instruments
1	Nineveh ,Iraq Current Study (150)	2020 2.5th and 97.5th	FT4 ng/dl	0.70 -1.29	0.49 - 1.31	0.58 – 1.28	……..	a, b, c, d, e, f, g ,h ,i , j	ECL Beckman Coulter
			FT4 pmol/L	9.01-16.6	6.31-16.85	7.46-16.47			
			FT3 pg/ml	2.63-3.96	2.04-3.3	2.11-3.4			
			FT3 pmol/L	4.03-6.08	3.13-5.06	3.24-5.22			
2	Poland (170)	Kostecka-Matyja *et al.*, 2017, 2.5th and 97.5th 19	FT4 pmol/L	11.99 - 21.89	10.46-16.67	8.96 -17.23	Proper iodine prophylaxis	a ,b, d, g, j	ELC/Elecsys analyzer ,Roche Diagnostics
			FT3 pmol/L	3.63 -6.55	3.29 – 5.45	3.1 – 5.37			
3	Egypt (360)	Sheriba *et al.,2018* 5th and 95th 20	FT4 ng/dl	0.89 -1.4	0.85 – 1.18	0.81 – 1.08	…….	a, b, c, d, g, h, j, k,	ELISA
			FT3 pg/ml	2.74 -3.5	2.66 – 3.27	2.22 – 2.9			
4	Ankara Turkey (1258)	Bulur *et al.,2019* 2.5th and 97.5th 8	FT4 ng/dl	0.72 -1.79	0.71 - 1.26	0.65 -1.12	…… .	a, b, c, d, e, f, g, , i	Chemilumine scence LIAISON
			FT3 pg/ml	2.45 -4.03	2.37 - 3.85	2.31 -3.77			
5	Guangzhou/ China (130)	Huijia Lin *et al.*, 2021 2.5th and 97.5th 21					……..	a, b, c, d, e, g, j, k	ECL/Abbott 1200 analyzer
			FT4 pmol/l	10.95 - 16.79	…….	…….			
			FT3 pmol/l	3.19 -5.91					

Similar unit of thyroid test for 1st ,2nd ,3rd **a-**Any thyroid drug**. b-** Any medication affecting TFTs. **c**-Twin pregnancy. **d-** Personal or family history of thyroid disease. **e-**Women with recurrent miscarriage , fetal abnormalities and other pregnancy complication **. f-** Women with infertility. g-Women with acute or chronic illness. **h**-Hyperemesis. **i** -polycystic ovarian. **j-** Positive Anti-TPO **k** -Positive Anti-thyroglobulin.

FT4: pmol/L= ng/dl*12.87 FT3:pmol/L=pg/ml *1.536

Table 7. Trimester –specific serum reference levels of thyroid hormones in different populations measured by Access (Beckman coulter).

no.	Country ,Sample size,	Author, year percentile reference no.	Thyroid test	1st Trim	2nd Trim	3rd Trim	Iodine Status	Exclusion criteria
١	Nineveh ,Iraq Current Study (150)	2020 2.5thand 97.5th	FT4 ng/dl	0.70 -1.29	0.49 - 1.31	0.58 – 1.28	……..	a, b, c, d, e, f, g, h, i, j .
			FT4 pmol/L	9.01-16.6	6.31-16.85	7.46-16.47		
			FT3 pg/ml	2.63-3.96	2.04-3.3	2.11-3.4		
			FT3 pmol/L	4.03-6.08	3.13-5.06	3.24-5.22		
6	Korea (417)	Kim HJ,etal., 2017 2.5th and 97.5th 22	FT4 ng/dl	0.84 -1.43	0.68 -1.21	0.67-1.13	More than adequate UIC:427.3µg/	a, b, c, d, j, k
7	China Zhejiang province (9038)	Rulin et al et al.,2018 2.5th and 97.5th 23	FT4 pmol/l	9.0-15.1	6.82-11.31	6.71 - 11.44	Sufficient	a, b, c,d,e, f,g,h,i,j, k
			FT3 pmol/l	3.8-5.99	3.51-5.28	3.31-5.17-		
8	South India (162)	Rani et al., 2019 2.5th and 97.5th 24	FT4 ng /dl	0.68 -1.44	0.59 -1.21	0.53-1.15	Sufficient	a , b, c, d, e ,g, h, j, l
			FT3 pg/ml	2.08 -3.48	1.81 -3.81	1.86 -3.38		

Similar unit of thyroid test for 1st ,2nd ,3rd . **a-**Any thyroid drug**. b-** Any medication affecting TFTs. **c**-Twin pregnancy. **d-** Personal or family history of thyroid disease. **e-**Women with recurrent miscarriage , fetal abnormalities and other pregnancy complication **. f-** Women with infertility. g-Women with acute or chronic illness. **h**-Hyperemesis. **i** -polycystic ovarian. **j-** Positive Anti-TPO **k** -Positive Anti-thyroglobulin .l-Any women with UIC <150 µg/L. FT4: pmol/L= ng/dl*12.87 FT3:pmol/L=pg/ml *1.536.

Conclusions

The TSRSLs for FT3 and FT4 have some similarity with that recorded elsewhere and we concluded a decrease in FT4 level across three trims.

Acknowledgement

We would like to thank the College of Medicine, University of Mosul for providing the necessary requirements in completing this work.

Ethics Statements

It was approved by the Ethical Committee at Nineveh Health Directorate, Mosul, Iraq before the start of the study.

Conflict of Interest

There are no conflicts to declare.

Funding

All tests were done on my private account.

Authors' Contributions

Marrwa M.Naif designed the study, investigation, methodology, data analysis and interpretation, software, preparation of the original manuscript, writing review and editing. Hazim A.Allawi supervised and revised the original manuscript, project administration. Approval, agreement and rearrangement for all parts of the study were given by Sura Khairialdeen Mohialdeen and Hazim A.Allawi .

References

1. Arrangoiz R, Cordera F, Caba D, Muñoz M, Moreno E, de León EL. Comprehensive review of thyroid embryology, anatomy, histology, and physiology for surgeons. International Journal of Otolaryngology and Head & Neck Surgery.201 8 ; 7:160 -188.
2. Brent GA. Mechanism of thyroid hormone action.J Clin Invest. 2012 Sep;122(9):3035-43.
3. Moleti M, Trimarchi F, Vermiglio F. Thyroid physiology in pregnancy. Endocr Pract. 2014;20:589-96.
4. Muller I, Taylor PN, Lazarus JH. Thyroid function in pregnancy. Ann Thyroid. 2018; 3:27.

5. Park C. Evaluation of pregnancy and thyroid function. Korean J Clin Lab Sci. 2018 ;50(1):1-10.

6. Soldin OP. Thyroid function testing in pregnancy and thyroid disease: trimester-specific reference intervals. Ther Drug Monit. 2006 Feb;28(1):8-11.

7. Spencer CA. Assay of thyroid hormones and related substances. In: Feingold KR, Anawalt B, Boyce A, et al. Endotext 2017. South Dartmouth (MA): MDText.com, Inc.; 2000.

8. Bulur O, Atak Z, Ertugrul DT, Beyan E, Gunakan E, Karakaya S, et al.Trimester-specific reference intervals of thyroid function tests in Turkish pregnants. Gynecological Endocrinology 2020 May 3;36(5):413-6

9. . Zhang D, Cai K, Wang G, Xu S, Mao X, Zheng A,et al. Trimester-specific reference ranges for thyroid hormones in pregnant women. Medicine. 2019 Jan;98(4).

10. Sheng Y, Huang D, Liu S, Guo X, Chen J, Shao Y, et al. Reference intervals of thyroid hormones and correlation of BMI with thyroid function in healthy Zhuang Ethnic pregnan women. BioMed Research International 2018 Nov 14; 2018.

11. Rifai N. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics 8 E; South Asia Edition; e-Book. Elsevier India; 2019 Jul 16

12. Murillo-Llorente MT, Fajardo-Montañana C, Pérez-Bermejo M. Reference values of thyroid hormones during the first trimester of regnancy in Valencian Community (Spain) and their relationship with iodine intake.Nutrients. 2020;12(5): 1433.

13. Amouzegar A, Khazan M, Hedayati M,Azizi F. An assessment of the iodine status and the correlation between iodine nutrition and thyroid function during pregnancy in an iodine sufficient area. Eur. J. Clin. Nutr. 2014; 68: 397–400.

14. Medici M, Korevaar TI, Visser WE, Visser TJ,Peeters RP. Thyroid function in pregnancy: what is normal? Clin Chem 2015 May 1;61(5):704-13.

15. Surks MI, Boucai L. Age- and race-based serum thyrotropin reference limits. J Clin Endocrinol Metab. 2010;95(2):496–502.

16. Iodine Global Network.[Internet]. Annual report. 2019;p11.[cited 3 Oct.2021].Available from http://www.ign.org/index.cfm

17. Portulano C, Paroder-Belenitsky M, Carrasco N.The Na+/I− Symporter (NIS):

18. Mechanism and medical impact. Endocrine Reviews. 2014 ;35:106 -149.

19. Korevaar TI, Medici M, Visser TJ,P eeters RP. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. Nat Rev Endocrinol 2017;13 (10):610

20. Kostecka-Matyja M, Fedorowicz A, Bar-Andziak E, Bednarczuk T, Buziak-BerezaM, Dumnicka P, et al. Reference values for tsh and free thyroid hormones in healthy pregnant women in Poland: A Prospective, Multicenter Study.Eur Thyroid J. 2017 Apr;6(2):82-88.

21. 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)

22. Lin H, Ye M, Zhou Z, Yuan L, Lash GE, Zhang G, et al. Reference values and the effect of clinical parameters on thyroid hormone levels during early pregnancy. Biosci Rep. 2021 Jan 29;41(1):BSR20202296.

23. Kim HJ, Cho YY, Kim SW, Kim TH, Jang HW, Lee SY,et al. Reference intervals of thyroid hormones during pregnancy in Korea, an iodine-replete area. Korean J Intern Med. 2018 May;33(3):552-560

24. Sun R, Xia J. The reference intervals of thyroid hormones for pregnant women in zhejiang province. Lab Med. 2017 Dec 22;49(1)

25. Rani K.S., Tirupati S., Sarathi S. Kumar.K.D.,Trimester –specific reference ranges for thyroid function tests in South Indian women.Thyroid Res Pract 2018;15;117.