TSH fluctuation during pregnancy depending on socioeconomic conditions in district Charsadda Khyber-Pakhtunkhwa Pakistan.

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Abstract

Estimation and interpretation of thyroid function tests in pregnant women is of outmost importance for maternal, fetal and neonatal health. Our objective was to calculate laboratory and geography-specific reference intervals for TSH during first, second and third trimester of pregnancy in the pregnant females in District Charsadda. Fulfillment of extensive questionnaires and estimation thyroid-stimulating hormone (TSH) were performed. The reference population was defined using inclusion criteria regarding different demographic characteristics, socioeconomic conditions and general medical status of women. Reference interval for TSH was 0.2 µ IU/ml-2.02 µ IU/ml for the first trimester 0.2 µ IU/ml-1.84 µ IU/ml for the second trimester 0.56 µ IU/ml-3.09µ IU/ml for third trimester had TSH lower than the upper reference limit. These trimester-specific Population based reference ranges are essential in everyday clinical practice for the correct interpretation of thyroid hormone values and accurate classification of thyroid disorders.

Keywords: Thyroid Stimulating Hormone (TSH), Pregnancy, trimester, Fluctuation, Thyroid Function Test, socioeconomic, hormone.

1. Introduction

Thyroid stimulating hormone (TSH) is a glycoprotein in nature hormone produced by the anterior pituitary gland and is regulated by hypothalamic pituitary axis. It is the primary stimulus in thyroid gland for the production of thyroid hormone. It influences growth effects on thyroid follicular cells to increase the size of thyroid [1]. The thyroid secretes two important hormones, thyroxine (T4) and triiodothyronine (T3), both with the effect of controlling growth, the metabolism and body development, acting in the production of structural proteins, enzymes and other hormones [2]. Pregnancy is a locus of multiplex of fluctuation in most of the hormones of the body in all three trimesters [3]. The thyroid gland and its activity are greatly influenced by pregnancy [4. During pregnancy the thyroid is hyper stimulated, resulting in changes in thyroid hormone concentrations [5. During pregnancy significant alteration happens in the maternal thyroid gland with a subsequent effect on the fetus [6]. An elevated TSH level in the serum indicates hypothyroidism in pregnancy [7]. Hypothyroidism affects 1.5 percent to 4% of pregnant women worldwide, according to many studies. Overt hypothyroidism (OH) affected 0.3 to 0.5 percent of individuals, while the remainder had subclinical hypothyroidism (SCH) [8]. Currently, reference range for TSH with an upper limit of 2.5 mU/l in the first trimester and 3.0 mU/l in the second or third trimester

to diagnose subclinical and overt hypothyroidism [9]. Trimester-specific intervals are especially important during pregnancy when thyroid insufficiency may be associated with adverse obstetric outcome and fetal neuro developmental deficits. Gestational age-specific reference intervals are now available for thyroid function tests [10]. Avoiding maternal (and fetal) hypothyroidism is of major importance because of potential damage to fetal neural development, an increased incidence of miscarriage, and preterm delivery. Maternal hyperthyroidism and its treatment may be accompanied by coincident problems in fetal thyroid function. Autoimmune thyroid disease is associated with both increased rates of miscarriage, for which the appropriate medical response is uncertain at this time, and postpartum thyroiditis [11]. When hypothyroidism comes to overt hypothyroidism, preeclampsia, gestational hypertension, fetal mortality, preterm delivery, spontaneous abortions, repeated abortions, and cretinism have all been linked to overt hypothyroidism during pregnancy [12]. In connection to hypothyroidism in the subclinical stage preeclampsia, prenatal hypertension, fetal mortality, early delivery, spontaneous abortions, and recurrent abortions [13]. Several international researches have sought to discover biological and demographic–socioeconomic parameters linked to prenatal psychological discomfort. Prior research has shown low income levels and unemployment as major contributors [14]. Lack of social and relationship support, prior stressful experiences [15], domestic abuse [16], and unexpected pregnancies [17] are also factors to consider. Prior research in Pakistan sought to uncover characteristics linked to psychological discomfort in women from various cities [105]. Similarly, several previous research on pregnant women in Pakistan found that up to 70% of them experienced prenatal psychological discomfort and emotional disturbances [18]. Premature birth, low birth weight, delayed neurological development, and low IQ are all indications that prenatal psychological strain can contribute to negative birth and child outcomes [19]. The purpose of this study is to investigate the specific TSH level for individual trimester during pregnancy and to investigate the socioeconomic condition for fluctuation of TSH in pregnant women. We also compare the local TSH test value and ATA value of TSH for trimester specific references range at district charsadda.

2. Objectives

Our main objectives are.

- To investigate the TSH (thyroid stimulating hormone) level in each trimester.
- To compare the TSH level with socioeconomic parameters.

3. Materials and Methods

The present study was conducted in District Charsadda, Khyber Pakhtunkhwa Pakistan. The studied area is located about the northeast from city of Peshawar, Charsadda is a large municipality with the population exceeding 50,000 people. The area is located at an altitude of 302 meters (991 ft) with coordinates of 34°.14' N latitude and 71°.74' E longitude. The total area of the district is 996 Km2 with a population size of 1,616,198 which comprises of 49% (approximately) male and 51% female population. The district comprises three Tehsils that is Shabqadar, Tangi and Charsadda[20].

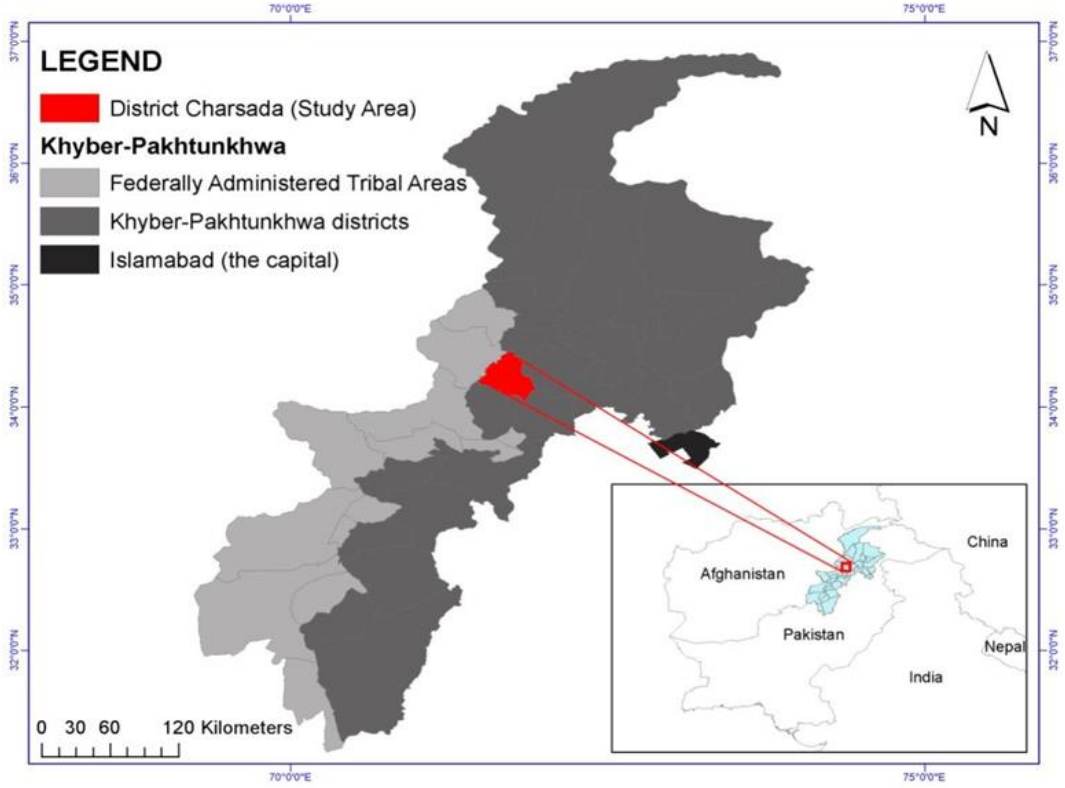

Figure 1: Map of District Charsadda Khyber Pakhtunkhwa [21]

3.1. Field work

In this study the blood samples collected from 60 pregnant females 20 samples for each of trimester by visiting different government and private clinics. Ethical permission was taken from university respective department for hospitals (Alkhidmat Hospital Charsadda) and clinic (Dr. Taleema Ali Clinic) visits proper permission was also taken. 3 ml blood was taken from each female. Once blood is taken it is directly transfer to EDTA tubes for storage. Each tube is properly labeled and then shift to laboratory (Department of Botany/Zoology) where it is store in the refrigerator at 4°C.

3.2. Questionnare based information

A total of 60 questionnaires were filled (20 in each trimester). These pregnant females were interviewed at the time of blood sample collection. The questionnaire mostly covered total of 18 questions to socioeconomic and pregnancy related problems.

3.3. Sample preparation

The collected blood samples (3 ml) were then centrifuged at 1000 rpm for two minutes and serum was collected in separate Eppendorf micro tube. Detection buffers 75 µL was added to 150 µL of serum and mixed well. Already prepared TSH strips were used to analyze the test. Small amount of the mixture was added into the strips for further process. Chroma (II) Immunoassay reader was used for the investigation of TSH level. The loaded strips were inserted in the reader; about 12 minutes were given for screening the samples. TSH level was then noted on the screen for each sample.

4. Results

4.1.Questionnaire based analysis

All the demographic characters, socioeconomic conditions, and pregnancy related problems are listed in the Table 1. Percentages of all the parameters are calculated.

Table 1: Demographic charactristics of women

Socioeconomic conditions of women	Number of Samples 60	Percentage%
Age Groups		
20-29	42	70
30-35	18	30
No Of Children		
Primary	16	27
1 to 3	29	48
<3	15	25
Education		
Uneducated	39	65
High	15	25
Higher secondary	6	10
Monthly Income		
Below 20000	17	28
21000-50000	40	67
Above 50000	3	5
Violence History		
Yes	4	6
No	56	94
Antenatal Care		
Regular	44	73
Irregular	15	25
No	1	2
Nutrition		
Once a week	34	56
Twice a week	18	30
No	8	14
Employment status		
Yes	4	6
No	56	94
Anemia		
Yes	28	47
No	32	53
Pregnancy Induced Hypertension		
Yes	15	25
No	45	75
Gestation Diabetes		
Yes	2	4
No	58	96
Asthma		
Yes	0	0
No	60	100

Body Condition		
Normal	48	80
Underweight	7	12
Obese	5	8
Mode of delivery		
Primary	14	23
Normal	41	68
Operation	5	9
Alive		
Primary	15	25
1 to 3	37	61
<3	4	7
No	4	7
Still Births		
1 to 3	19	32
No	41	68
Miscarriages		
Yes	9	15
No	51	85
Presence of Goiter		
Yes	0	0
No	60	100

A total of 60 pregnant females enrolled in this study 20 of each trimester investigated based on questionnaire mostly covered of socioeconomic conditions and pregnancy related problems

In age group 70% of the pregnant females at the age of 20-29 years while the remaining 30% of females at the age of 30-35%. The second socioeconomic condition is the No of children 27% of the females at their first pregnancy while the 48% of females have children 1 to 3 the remaining 25% of females have children above the 3. The third socioeconomic condition level of education 65% of females are uneducated 25% of female at their level of high education while the remaining 10% of females at their level of higher secondary education. The next socioeconomic condition is Monthly Income 28% of the females have monthly income below the 20000(PKR) while 65% of the females have monthly income 21000-50000 (PKR) only the remaining 5% of females have monthly income above the 50000. The antenatal care was provided regularly to 73% of the total females 25% of females were get care at irregular basis only the 2% of females were not get their antenatal care. The nutrition level of the pregnant females 56% of the females get their nutrition once a week 30% 0f females get their nutrition twice a week while the remaining of 14% females having not a proper nutrition in a week. The employment status of the females only the 6% of

females were employed while the remaining 94 % of females have no employment status.The medical conditions of the pregnant females in the case of anemia 47% of females suffer from anemia while the remaining 53% of females have their normal condition. The 25% of females suffer from hypertension while the remaining 75% of females are normal. Gestation diabetes the 4% of females were diabetic while the remaining 96% of females were normal. Body condition of the pregnant females show 3 different categories 80% of the females were normal 12% of females were at the underweight condition while the remaining 8% of females were at the condition of obese. The fetal outcome was categorized into 3 groups. The 1st is Alive 25% of females were at their first pregnancy 61% of females were 1 to 3 alive babies 7% of females were alive babies above 3 while the remaining 7% of females were no alive babies. 2nd is still births 32% of females have history of still births 1 to 3 time while in 68% of cases it was not reported. 3rd is miscarriages 15% of females were history of miscarriages while the remaining 85% of cases it was not reported all of the percentage of socioeconomic conditions were shown in the: Table 1 and Figure 2.

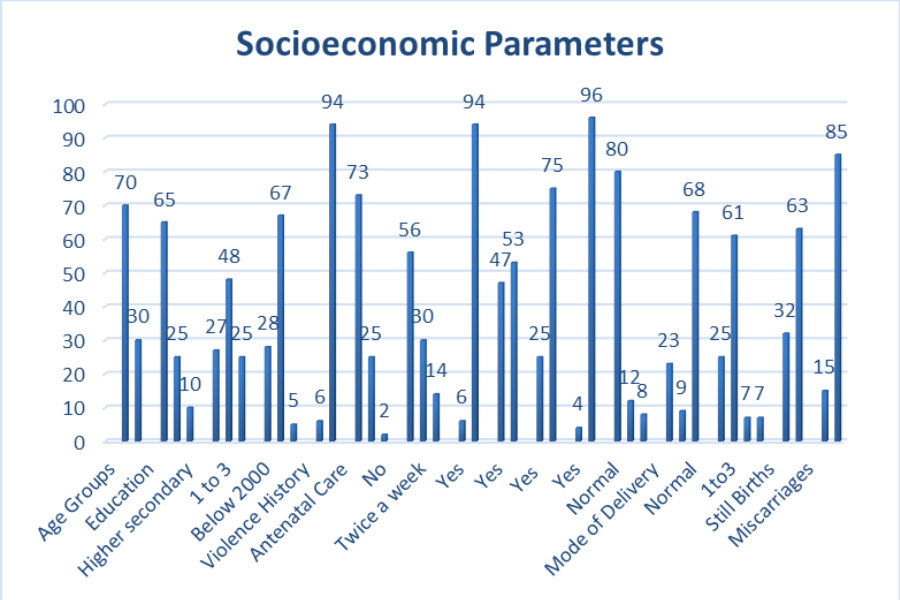

Figure 2: History of socioeconomic conditions of investigated women

Laboratory Analysis

Table 2: TSH value of tested samples and standard (ATA) TSH values

Parameter	Laboratory TSH Test values		Standard TSH (ATA) values	
TSH	**Minimum**	**Maximum**	**Minimum**	**Maximum**
1st Trimester	**0.2 µ IU/ml**	**2.02 µ IU/ml**	**0.6 µ IU/ml**	**3.4 µ IU/ml**
2nd Trimester	**0.2 µ IU/ml**	**1.8 µ IU/ml**	**0.3 µ IU/ml**	**3.6 µ IU/ml**
3rd Trimester	**0.5 µ IU/ml**	**3.09 µ IU/ml**	**0.3 µ IU/ml**	**4.0 µ IU/ml**

All the tested samples have maximum TSH range of 1st trimester is 2.02µ IU/ml and minimum 0.2 µIU/ml the standard maximum range by ATA is 0.6-3.4 µ IU/ml during 1st trimester. The maximum TSH range of 2nd trimester is 1.8 µ IU/ml and minimum 0.2 µ IU/ml the standard maximum range by ATA is 0.3-3.6 µ IU/ml during 2nd trimester. The maximum TSH range of 3rd trimester is 3.09 µ IU/ml and minimum 0.5µ IU/ml Standard maximum range by ATA is 0.3-4.0 µ IU/ml during 3rd trimester as shown in Table: 2 and Figure 3.

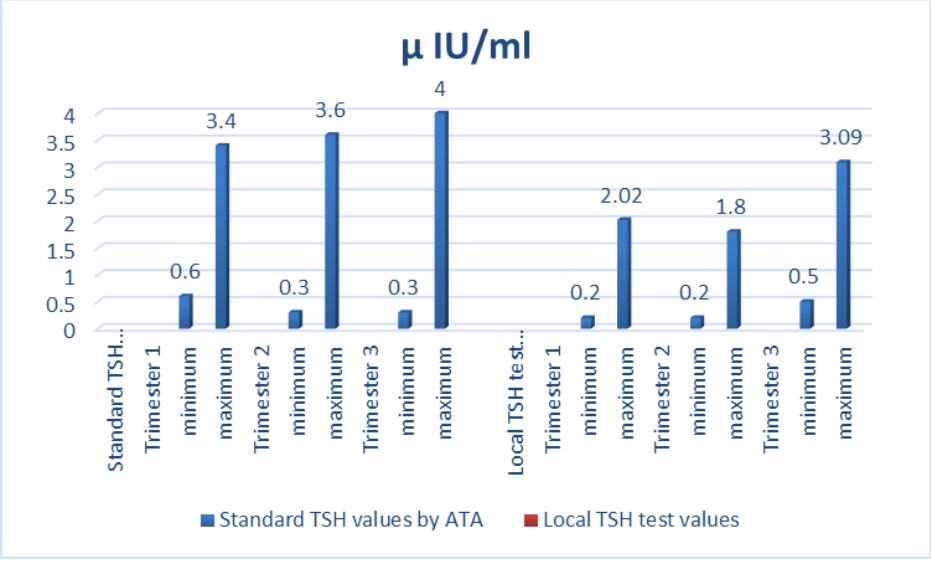

Figure 3: Comparison of tested TSH values with Standard TSH (ATA) values

4.1. Reasons for Fluctuation of TSH from its normal ranges of ATA value

4.1.1. Reasons of minimum TSH level

Minimum level of TSH in the examined women may due to the socioeconomic conditions such as High number of children (8), High age group (35), Low income (20,000 PKR), Poor nutrition, Unawarness (uneducated), and Health problems such as Anemia and Hypertension

4.1.2. Reasons of maximum TSH level

The maximum ranges of the test samples are very low (2.02) for 1st trimester as compare to ATA (3.4). (1.8) for 2nd trimester as compare to ATA (3.6). (3.09) for 3rd trimester as compare to ATA (4.0). The reason behind this fluctuation may Uneducated women (65%), Low income (67%), Low nutrition (56%), Violence history (6%), Irregular antennal care (25%) and Health problems such as anemia (47%) and hypertension (25%).

5. Discussion

Several hormonal changes and metabolic demands occur during pregnancy, resulting in complicated consequences on thyroid function [22]. Changes in the pituitary thyroid axis include an increase in thyroid hormone-binding globulin as well as total T4, T3, and serum thyroglobulin (TG). Furthermore, iodine clearance by the kidneys increases during pregnancy, whereas the moderate thyrotrophic effects of increased hCG may exert negative feedback on TSH production [23], incorrectly implying hyperthyroidism in normal pregnant women in the first trimester [24]. Overt and subclinical hypothyroidism is predicted to affect 0.3-0.5 and 2-3 percent of pregnant women, respectively [25]. Recent research has found that untreated hypothyroidism during pregnancy increases the risk of maternal anemia, preeclampsia, postpartum hemorrhage, placental abruption, and spontaneous abortion, as well as causing low birth weight, prematurity, congenital malformations, and impaired fetal brain development, resulting in a lower intelligence quotient (IQ) in children [26]. Hyperthyroidism, on the other hand, has been reported in around 0.2 percent of pregnant women [27], and can result in preeclampsia, stillbirths, premature delivery, intrauterine growth retardation, and low birth weight [28]. Despite the above, the serial variations in blood thyroid hormone levels suggest a need to better establish "pregnancy-specific" normative

reference ranges for thyroid function tests in order to detect TSH fluctuations early in pregnancy. Our study represents the first study performed in Greece [29]. It provides reference ranges for thyroid hormones during the first and second trimester of pregnancy. International guidelines recommend determining serum TSH as the first-line screening variable for thyroid dysfunction before conception and during pregnancy [30]. According to our results and in agreement with previous studies [31], the derived reference intervals for TSH were different (narrower and lower) from those proposed by the manufacturer. More specifically, our TSH reference intervals was 0.2μIU/ml 1.84μIU/ml for the second trimester, compared to standard ATA is 0.3-3.6 μ IU/ml. Consequently, women with subclinical hyperthyroidism would not have been identified, and normal women would have been misclassified as having subclinical hyperthyroidism if the manufacturer's TSH limits were used. Regarding TSH, our intervals were only slightly different. Many cross-sectional studies have provided trimester-specific free TSH reference levels in pregnant women [32]. However, these stated reference ranges differ between research due to differences in ethnicity, iodine consumption, sample size, evaluation of reference population, and immunometric test utilized. Table 1 summarizes the 2nd trimester-specific reference intervals for TSH from 20 women investigated, as well as their demographic features. Geographic diversity in hormonal levels is caused by ethnic differences as well as differences in iodine dietary factors. Furthermore, different laboratory reagents identify unique circulating TSH isoforms, resulting in variations even for the same sample [33]. Recent consensus guidelines do not advocate universal thyroid function screening during pregnancy in Pakistan but recommend testing high-risk pregnant women with a past or present history of a thyroid disorder, and patients with a first degree relative with thyroid disease or any other clinical indication of a thyroid disorder. However, there is a significant overlap of symptoms between the various groups of thyroid derangements and a strict clinical diagnosis is not possible [34]. There is also a significant subset of the population residing in rural areas without adequate antenatal checkup facilities and the inability to afford access to healthcare facilities, which can exclude many women with potential thyroid dysfunction. Under these circumstances, we feel there could be a need for more vigorous thyroid screening in the area in order to accurately determine the disease burden and effectively manage patients.

6. Conclusion

From this study we first report TSH level fluctuation in District Charsadda (TSH level of 1st trimester 0.2 µ IU/ml-2.02 µ IU/ml). (TSH level of 2nd trimester 0.2 µ IU/ml-1.8 µ IU/ml).(TSH level of 3rd trimester 0.5 µ IU/ml-3.09 µ IU/ml). These studies further investigate the reasons behind the minimum and maximum level of TSH. High number of children's, low income, low nutrition, and unbalance diet, irregular antenatal care, uneducated, health problems such as anemia and hypertension. This study will help generate the importance of reference ranges of thyroid profile in pregnant females. It will also help the gynecologist, pathologist, and patients interpret TSH, results because of reference ranges per trimester. It can further help in decreasing maternal morbidity and mortality levels, along with fetal complications in Pakistan.

7. Future Recommendations

- We recommend that further studies should be conducted by analyzing more samples.
- This study also recommends that FSH, Cortisol, Progesterone should also be included to deeply investigate the reason behind the TSH fluctuation.

8. Conflict of interest

The authors declared that present study was performed in absence of any conflict of interest.

9. Acknowledgment

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References

1 Z. Gluvic, M. Obradovic, A. J. Stewart, M. Essack, S. J. Pitt, V. Samardzic, E. R. Isenovic, "Levothyroxine treatment and the risk of cardiac arrhythmias–focus on the patient submitted to thyroid surgery" Frontiers in endocrinology, 12 (2021).

2 T. R. Mezzomo, J. Nadal, "Effect of nutrients and dietary substances on thyroid function and hypothyroidism" Demetra: Food, Nutrition & Health, 11(2), 427-444 (2016).

3 Soldin OP,Tractenberg RE, Hollowell JG, Jonklaas J, Janicic N, Soldin SJ. Trimester – specific changes in maternal thyroid hormone during gestation. Clin Obstet Gynecol. 2004; 14:1084-90.

4 Baecke M, Spaanderman ME, van der Werf SP. Cognitive function after pre-eclampsia: an explorative study. J Psychosom Obstet Gynecol 2009; 30: 58-64.

5 R. Moreno-Reyes, D. Glinoer, H. Van Oyen, S. Vandevijvere, "High prevalence of thyroid disorders in pregnant women in a mildly iodine-deficient country: a population-based study" The Journal of Clinical Endocrinology & Metabolism, 98(9), 3694-3701 (2013).

6 B. R. Haugen, "Drugs that suppress TSH or cause central hypothyroidism" Best practice & research Clinical endocrinology & metabolism, 23(6), 793-800 (2009).
Saunders TA, Lobel M, Veloso C, Meyer BA. Prenatal maternal stress is associated with delivery analgesia and unplanned cesareans. J Psychosom Obstet Gynecol 2006; 27: 141-6.

7 Azami M, Darvishi Z, Sayehmiri K. Systematic review and meta-analysis of the prevalence of anemia among pregnant Iranian women (2005–2015). Shiraz E Med J. 2016;17(4–5):e38462.

8 T. I. Korevaar, "The upper limit for TSH during pregnancy: why we should stop using fixed limits of 2.5 or 3.0 mU/l" Thyroid research, 11(1), 1-3 (2018).

9 A. Alemu, B. Terefe, M. Abebe, B. Biadgo, "Thyroid hormone dysfunction during pregnancy: A review" International journal of reproductive biomedicine, 14(11), 677 (2016).

10 L. De Groot, M. Abalovich, E. K. Alexander, N. Amino, L. Barbour, R. H. Cobin, S. Sullivan, "Management of thyroid dysfunction during pregnancy and postpartum: An

Endocrine Society clinical practice guideline" The Journal of Clinical Endocrinology & Metabolism, 97(8), 2543-2565 (2012).

11 Medicinalstyrelsen K. Förebyggande åtgärder mot endemisk struma (Preventive measures for endemic goitre) Medicinalväsendet 30 1936.

12 Allan WC, Haddow JE, Palomaki GE, Williams JR, Mitchell ML, Hermos RJ, et al. Maternal thyroid deficiency and pregnancy complications: implications for population screening. J Med Screen 2000;7(3):127-30.

13 Shields BM, Freathy RM, Knight BA, Hill A, Weedon MN, Frayling TM, et al. Phosphodiesterase 8B Gene Polymorphism Is Associated with Subclinical Hypothyroidism in Pregnancy. J Clin Endocrinol Metab 2009;94(11):460812.

14 Ford HB, Schust DJ. Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol 2009;2(2):76-83.

15 issenberg R, van den Boogaard E, van Wely M, van der Post JA, Fliers E, Bisschop PH, et al. Treatment of thyroid disorders before conception and in early pregnancy: a systematic review. Hum Reprod Update 2012.

16 van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, et al. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update 2011;17(5):605-19.

17 Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). Thyroid Therapy for Mild Thyroid Deficiency in Pregnancy (TSH) (https://clinicaltrials.gov/ct2/show/ NCT00388297).

18 Fransson E, Örtenstrand A, Hjelmstedt A. Antenatal depressive symptoms and preterm birth: A prospective study of a Swedish national sample. Birth 2011; 38:10-16. [CrossRef]

19 Leigh B, Milgrom J. Risk factors for antenatal depression, postnatal depression and parenting stress. BMC Psych 2008; 8:24-30. [CrossRef]

20 A. Khan, R. D. Mitchell III, S. Niaz, S. Ayaz, I. Khattak, H. Naeem, M. A. Zaman, "Seroprevalence of Anaplasma spp. among sheep and goats in Charsadda District, Pakistan" Small Ruminant Research, 176, 5-10 (2019).

21 S. U. Baig, S. M. J. Shah, B. N. Khattak, "Land, Income and Land-Use Diversification in Khyber Pakhtunkhwa Province of Northern Pakistan" (2018).

22 M. Abalovich, N. Amino, L. A. Barbour, R. H. Cobin, L. J. De Groot, D. Glinoer, A. Stagnaro-Green, "Management of thyroid dysfunction during pregnancy and postpartum: An Endocrine Society Clinical Practice Guideline" The journal of clinical Endocrinology & Metabolism, 92(8_supplement), s1-s7 (2007).

23 J. M. Hershman, "The role of human chorionic gonadotropin as a thyroid stimulator in normal pregnancy" The Journal of Clinical Endocrinology & Metabolism, 93(9), 3305-3306 (2008).

24 D. Glinoer, "The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology" Endocrine reviews, 18(3), 404-433 (1997).

25 P. Karakosta, L. Chatzi, E. Bagkeris, V. Daraki, D. Alegakis, E. Castanas, M. Kampa, "First-and second-trimester reference intervals for thyroid hormones during pregnancy in "Rhea" mother-child cohort, Crete, Greece" Journal of thyroid research, (2011).

26 M. Ohashi, S. Furukawa, K. Michikata, K. Kai, H. Sameshima, T. Ikenoue, "Riskbased screening for thyroid dysfunction during pregnancy" Journal of pregnancy, (2013).

27 J. G. Hollowell, N. W. Staehling, W. D. Flanders, W. H. Hannon, E. W. Gunter, C. A. Spencer, L. E. Braverman, "Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III)" The Journal of Clinical Endocrinology & Metabolism, 87(2), 489-499 (2002).

28 J. H. Mestman, "Hyperthyroidism in pregnancy" Best Practice & Research Clinical Endocrinology & Metabolism, 18(2), 267-288 (2004).

29 T. Giassa, I. Mamali, E. Gaki, G. Kaltsas, G. Kouraklis, K. B. Markou, T. Karatzas, "Iodine intake and chronic autoimmune thyroiditis: a comparative study between coastal and mainland regions in Greece" Hormones, 17(4), 565-571 B. (2018).

30 B. Vaidya, S. Anthony, M. Bilous, B. Shields, J. Drury, S. Hutchison, R. Bilous, "Detection of thyroid dysfunction in early pregnancy: universal screening or targeted high-risk case finding" The Journal of Clinical Endocrinology & Metabolism, 92(1), 203-207 (2007).

31 D. Glinoer, C. A. Spencer, "Serum TSH determinations in pregnancy: how, when and why" Nature Reviews Endocrinology, 6(9), 526-529 (2010).

32 T. Männistö, H. M. Surcel, A. Ruokonen, M. Vääräsmäki, A. Pouta, A. Bloigu, E. Suvanto, "Early pregnancy reference intervals of thyroid hormone concentrations in a thyroid antibody-negative pregnant population" *Thyroid, 21*(3), 291-298 (2011).

33 R. Silvio, K. J. Swapp, S. L. La'ulu, K. Hansen-Suchy, W. L. Roberts, "Method specific second-trimester reference intervals for thyroid-stimulating hormone and free thyroxine*" Clinical biochemistry*, 42(7-8), 750-753 (2009).

34 Talat, A., Khan, A. A., Nasreen, S., & Wass, J. A. (2019). Thyroid screening during early pregnancy and the need for trimester specific reference ranges: a cross-sectional study in Lahore, Pakistan. *Cureus, 11(9*).