Trimester-specific thyroid hormone reference data in Sri Lankan women

Kamani Dhanushka Liyanarachchi[1], Dimuthu Tharanga Muthukuda[2], Udari Apsara Liyanage[3], Renuka Jayatissa[4], Lakma Tharindi Subasinghe[5], Ariyavidana Hareendra Pradeep[5], Eranga Colombage[2], Lalana Devi Ranasinghe[1], Nandun Prabuddha Liyanarachchi[5], Samantha Ranasinghe[4], Sarath Lekamwasam[6], Noel P Somasundaram[1]

Abstract

Introduction: Regional differences in thyroid hormones are noted, especially during pregnancy.

Objectives: Establish reference values for thyroid function tests for Sri Lankan pregnant women and to determine their comparability with regional data; and determine the prevalence of 2. Thyroid Peroxidase (TPO) antibody positivity and 3. Iodine deficiency among pregnant women with uncomplicated clinical history.

Methods: A cross-sectional study conducted in antenatal clinics of a tertiary care maternity center recruited a minimum of 56 women in each trimester in a multistep approach to derive an "ideal-reference population"; participants with clinically manifested thyroid disease, followed by subjects with sonographically abnormal thyroids and finally those at high risk for thyroid disease as shown by positive TPO levels and urine iodine deficiency were excluded in sequence. Thyroid hormones were measured by chemiluminescence in the ideal reference population. Reference ranges were derived using median and 5th and 95th centiles.

Results: Final sample included 369 women. TSH reference ranges of the first (n=64), second (n=188) and third (n=117) trimesters were 0.014-2.77mIU/L, 0.31-3.2 mIU/L and 0.34-3.4 mIU/L, respectively. TPO antibody level showed a weak but significant correlation with TSH (r=0.10, p=0.021) in the final sample. No significant association was found between urine iodine and thyroid function tests.

Conclusions: TSH reference ranges observed in this study are concordant with the Caucasian reference values more than the regional values. Discrepancies in study methodology, defining and selection of reference population and methods employed in measuring thyroid hormones in different studies may have accounted for these differences.

Introduction

An accurate interpretation of thyroid functions in pregnancy is crucial as maternal thyroid diseases are associated with a multitude of adverse pregnancy outcome [1]. Though fetal thyroid follicular cells are detected by the 10th week of gestation (POG) [2], fetus largely depends on the maternal supply of thyroid hormones until 18-20 weeks. Maternal thyroid hormones regulate early fetal brain development and maternal hypothyroidism, even when subclinical, may cause irreversible adverse fetal cognitive outcomes [3,4].

Thyroid disease is prevalent among South Asians making the topic more pertinent in this region. In a multicenter study in India, 13.1% of women in the first trimester were found to have hypothyroidism based on a TSH cutoff of 4.5 mIU/L [5]. Among pregnant women in Nepal, 13.3% had TSH values above 10mIU/L and 31% TSH values between 6-10 mIU/L [6]. In contrast, Western data show a low prevalence of overt hypothyroidism (0.3%) and subclinical hypothyroidism (SCH) (2.5%), using TSH cut-off of 6 mIU/L [7]. Data is limited in Sri Lanka, and a 3.4% prevalence of SCH, based on third trimester TSH cut-off of 5.2 mIU/L has been reported in the Jaffna District [8].

Ceylon Medical Journal 2022; **67:** 37-44

DOI: http://doi.org/10.4038/cmj.v67i2.9629

[1]*Endocrinology and Diabetes Unit, National Hospital, Sri Lanka,* [2]*Sri Jayewardenepura, General Hospital, Colombo, Sri Lanka,* [3]*Faculty of Medicine, University of Colombo, Sri Lanka,* [4]*Medical Research Institute, Sri Lanka,* [5]*Radiology Unit, National Hospital, Sri Lanka,* [6]*Faculty of Medicine, University of Ruhuna, Sri Lanka.*

Correspondence: UAL, *e-mail: <udari8@gmail.com>.* *Received 07 February 2020 and revised version 30 April 2022 accepted 05 June 2022*

Due to the alterations in physiology, thyroid functions change in a trimester specific manner during pregnancy [15]. Furthermore, thyroid functions during pregnancy show a wide ethnic difference. In general, Indian reference values are significantly different from Western reference values [18]. Even though this could partly be due to differences in the study methodology, a genuine ethnic or geographical variation cannot be ruled out. Due to the scarcity of local data many countries including Sri Lanka tend to use Caucasian reference values which may not be acceptable.

The main aim of our study was to determine trimester-specific thyroid function reference values for Sri Lankan pregnant women and to assess whether they were distinctly different from the regional and Western reference values. We also aimed to determine the prevalence of Thyroid Peroxidase (TPO) antibody positivity and iodine deficiency among pregnant women with uncomplicated medical and obstetric history.

We established an "ideal -reference population" without the presence or risk for thyroid disease from which normative data for thyroid functions, urine iodine and thyroid gland dimensions were derived.

Subjects and methods

Study setting and ethics approval

A descriptive cross-sectional study was conducted at the De Zoysa Maternity Hospital for Women (DMH), a tertiary maternity referral centre in Colombo, Sri Lanka. Approval for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Colombo, the Ethics and Scientific Committee of the Medical Research Institute (MRI), Colombo and the Institutional Ethics Committee at DMH. Participants were recruited following informed written consent.

Study participants

Consecutive pregnant women aged 18-40 years attending antenatal clinics were recruited over a period of two months. According to the calculations of sample size, a minimum of 56 subjects in each trimester were needed. Trimesters of pregnancy were defined as, first trimester-POG ≤12 weeks, second trimester POG 13-27 weeks and the third trimester ≥28 weeks.

Participants were evaluated in three stages at the study setting and they progressed from Stage 1 to 3 having fulfilled the inclusion and exclusion criteria specific to the particular stage.

In the first stage a focused history was obtained using an interviewer administered pre-designed questionnaire followed by clinical examination of the thyroid gland. Women with obstetric complications in the current pregnancy (e.g. hyperemesis gravidarum, threatened abortions, gestational diabetes, hypertension), multiple pregnancies, past obstetric history of any pregnancy losses history of thyroidal illness, thyroid surgery, body irradiation and chronic illness were excluded. Further, those who consumed anti thyroid drugs, thyroxin, or drugs that were known to interfere with thyroid function, and family history of thyroid illnesses among first degree relatives were also excluded from the study.

Subjects with clinically overt hypothyroidism, hyperthyroidism or thyroid nodularity were excluded in Stage 1. Those with clinically diffuse goiter were not excluded.

In the second stage of the study, subjects underwent ultrasonography of the thyroid using a portable ultrasound scanner (7.5 MHz linear array probe, Sonoscape-S8) by three radiologists experienced in ultrasonography of small parts. Those with thyroid nodules, nodularity, hypoechogenicity or heterogeneous echo pattern were excluded due to possible association with thyroiditis and iodine deficiency. Inter-rate agreement between the radiologists was established. In the third stage, a venous blood sample was collected for the assays of Free T4, Free T3, TSH and Thyroid Peroxidase (TPO) antibodies and a spot urine sample was collected for the measurement of urine iodine content.

After exclusion of subjects with high TPO antibodies and low urine iodine excretion [9], trimester specific reference ranges of thyroid function tests were calculated.

Biochemical analyses

Chemiluminometric immune analysis using Siemens ADVIA Centaur® XP system was used for the analysis of blood samples. Performance of the machine with regards to the thyroid function tests among non-pregnant women, provided by the manufacturer is summarized in Table 1. Quality control was assessed with commercially available kits using 2 levels (low and high) for each test.

Urinary iodine was measured by modified microplate method using Sandell-Kolthoff reaction [10]. In-house internal quality control analysis and external quality control were performed in collaboration with the Center of Disease Control (CDC) Laboratory in Atlanta under the EQUIP programme.

Statistical analysis

Data were subdivided according to the trimester. For each trimester TSH, FT3, FT4 and urine iodine were expressed as mean, SD, median, and 5^{th} and 95^{th} percentiles. The 5^{th} to 95^{th} centiles were used as the reference range.

Since measurements in the first and the second trimesters were not normally distributed, Mann-Whitney U and Spearman correlation were used to evaluate associations.

Table 1. Analytical sensitivity and measurement precision of the machine for the thyroid function tests

	Reference range	*Analytical sensitivity*	*Total precision value %*
TSH	0.55-4.78 miu/L	0.008-150 miu/L	4.82
Free T4	0.89-1.76 ng/dL	0.1-12 ng/dL	4.06
Free T3	2.3-4.2 pg/mL	0.2-20 pg/mL	3.2
TPO antibodies	<60 U/mL	15-1300 U/mL	5.35

Results

Developing the reference population

Steps used and the number of participants assessed in each stage are demonstrated in Figure 1.

Figure 1. **Progression of participants at different stages.**

Five hundred and sixty participants were excluded at the end of the first stage. This included 88 with obstetric complications in the current pregnancy, 86 with history of thyroid related illness, surgery or drugs, 128 with first degree family history of thyroid disease, 50 with history of hyperemesis gravidarum, 68 with previous pregnancy loss 20 with multiple pregnancy and 50 with known chronic illnesses. Seventy were excluded based on clinical assessment suggestive of overt hyper or hypothyroidism.

Of the 840 participants in the second stage, 146 were excluded following the detection of nodularity (143; 17%), heterogeneous echo pattern (30; 3.6%) and hypoechogenicity (8; 1%) by ultrasonography (11-14). Isolated cases of hyperechogenicity (5; 0.6%), increased vascularity (11; 1.3%) and diffuse gland enlargement (9; 1.1%) without other ultrasonic abnormalities, however, were not excluded, to make allowance for the physiological changes of thyroid gland in pregnancy.

Three hundred and twenty one participants were excluded following lab testing. Of the 694 subjects who underwent blood and urine analyses, 103 (16%) had positive TPO antibodies while 208 (31.1%) had low urine iodine excretion. One person (0.3%) had an elevated urine iodine value (Figure 2).

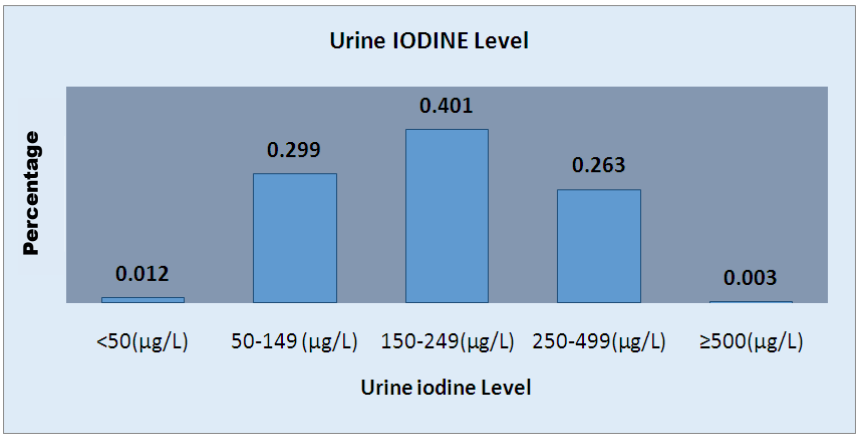

Figure 2. **Distribution of urine iodine among 692 participants in the 3rd Stage.**

Due to technical issues 13 TPO results and 28 urine iodine results were unavailable and they too were excluded.

The reference ranges were derived from a final sample of 369 which included 64 participants in the first trimester, 188 in the second trimester and 117 in the third.

Associations of thyroid functions with urine iodine and TPO status

Six hundred and sixty eight participants in the third stage were used for the assessment. POG showed no significant associations with TPO antibody levels P=0.36) or urine iodine level (P=0.22). We found no association between TPO antibody and urine iodine levels (P=0.65).

Further, no significant associations were found between urine iodine and thyroid function tests. TPO antibody level, however, showed a weak but significant correlation with TSH (r=0.10, P=0.021) while correlation with both free T4 (P=0.62) and free T3 (P=0.51) were not significant.

Reference ranges for each trimester

The mean thyroid hormone level and the reference range of each trimester are shown in Table 2.

Table 2. Thyroid hormone reference ranges and mean values for each trimester

	TSH (miu/L)					*Free T4 (ng/dL)*					*Free T3 (pg/mL)*				
	Mean	*Median*	*SD*	*5th*	*95th*	*Mean*	*Median*	*SD*	*5th*	*95th*	*Mean*	*Median*	*SD*	*5th*	*95th*
First trimester	1.17	1.06	0.77	0.014	2.77	1.34	1.33	0.26	0.98	1.91	3.3	3.24	0.52	2.7	4.55
Second trimester	1.49	1.34	0.87	0.31	3.2	1.15	1.15	0.18	0.84	1.43	2.95	2.93	0.29	2.52	3.53
Third trimester	1.67	1.58	0.833	0.34	3.4	1.13	1.14	0.20	0.79	1.43	2.82	2.78	0.28	2.26	3.33

With the advancement of the pregnancy, TSH increased whereas both Free T4 and Free T3. Free T4 of the first trimester was significantly lower when compared with free T4 of the second and third trimesters ($p<0.001$ for both). Free T4 between the second and third trimesters, however, was not significantly different (p=0.25).

The thyroid gland dimensions progressively increase with the trimester. The 5th , 95th percentiles and the mean values for in the three trimesters are illustrated in (Figure 3).

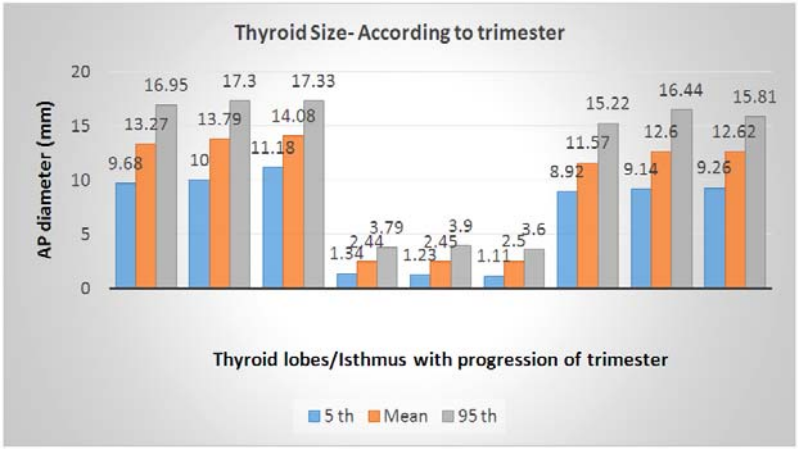

Figure 3. **Dimensions of the thyroid gland measured ultrasonically among 820 subjects.**

Significant difference was seen in the right lobe and the left lobe between the first and the third trimesters (P-0.025 and P-0.002 respectively). No significant changes in size was noted in the thyroid isthmus.

Discussion

We studied the trends of thyroid hormones through the gestation among a group of Sri Lankan women and our results are consistent with previous studies in this area. We observed that TSH increased while FT4 and FT3 reduced with advancing POG. Despite normal TPO levels and urine iodine, there was a significant number of participants with low free FT4 and FT3 in advanced pregnancy.

Based on the data from the USA and Europe, the Endocrine Society and the American Thyroid Association (ATA) have recommended the use of TSH upper limit of 2.5mu/mL in the first trimester and 3mu/L in the second and the third trimesters [15] [16]. Studies in other ethnic groups, however, have shown relatively higher TSH cut-offs leading to the revision of ATA guidelines in 2017 to recommend using local/regional reference values during pregnancy [17]. Apart from the ethnic differences, factors such as assay method, recruitment criteria, iodine status of the population would also contribute to the variation of the thyroid hormones seen in different studies.

Due to the absence of local reference values in Sri Lanka, Caucasian cut-offs are being widely used. It is prudent to think that regional South Asian cut-offs are more appropriate for the Sri Lankan population due to similarities between these populations. Data from South Asia are predominantly from India (Table 3) and they reveal a wide variation of TSH cut-off values in the first trimester ranging from 1.82 mIU/L to 6.65 mIU/L.

The reference ranges of TSH we observed are similar to the Caucasian values. Price *et al* made similar observations where there was no difference in thyroid functions in pregnancy between Asian and Caucasian women [22]. Use of kit manufacture's cutoffs or Indian cutoffs would lead to underestimation of subclinical hypothyroidism particularly in the first trimester, since our cut-off values are lower. Use of the manufacture's cut-offs would have caused 17%, 5.9% and 5.1% of participants in the first, second and the third trimesters, respectively, be classified as having subclinical hyperthyroidism.

Table 3. Studies on reference ranges of thyroid functions in South Asia

Study	*Country*	*Initial sample size and design*	*Exclusion criteria used*	*Centiles used*	*TSH assay used; laboratory reference ranges*	*TSH reference derived mIU/L*		
						1st trimester	**2nd trimester**	**3rd trimester**
Marwaha et al. 2008 [18]	India	Initial 541 Final 331 cross-sectional study	• Clinical criteria • Thyroid Autoantibodies • USS criteria-nodularity, hypoechogenicity • *Urine iodine not done – taken as sufficient*	5th, 95th	Electrochemilu minescence (ECL) 0.27-4.2miu/ml	*n*=107 0.6-5	*n*=137 0.43-5.78	*n*=87 0.74-5.7
Jebasingh et al. 2016 [19]	India-Manipur	Initial ~600 Final 375 cross-sectional study	• Clinical criteria • Thyroid Autoantibodies not done • USS criteria-not done • *Urine iodine not done – taken as sufficient*	5th, 95th	Chemilu minescence assay 0.35-5.5.miu/ml	*n*=109 0.21-1.82	*n*=148 0.71-1.71	*n*=118 0.7-1.93
Sekhri et al. 2016 [20]	India-Delhi	Initial ~600 Final 86 Cohort study	• Clinical criteria • Thyroid Autoantibodies • USS criteria-not done • *Urine iodine not done*	2.5th, 97.5th	ECL 0.27-4.2 µiu/ml	0.09-6.65	0.51-6.66	0.91-4.86
Rajput et al. 2016 [21]	India-	Initial ~1430 Final cross-sectional study	• Clinical criteria • Thyroid Autoantibodies • USS criteria-not done • *Urine iodine not done*	2.5th, 97.5th	ECL 0.35-5.5 miu/L	*n*=301 0.37-3.69	*n*=308 0.54-4.47	*n*=372 0.7-4.64

TSH was undetectably low (≤0.01miu/L) in 3 out of 64 (4.6%) in the first trimester and 1 out of 186 in the second trimester (0.54%). No woman in the third trimester had undetectable TSH. Similar to most other studies, we found that the TSH significantly increased with the progression of gestation [23]. This could be explained by the HCG effect, which is most marked in the early pregnancy.

We observed that in the studies of Marwaha *et al* Sekhri *et al* the TSH reference upper limits are comparable and even slightly higher than manufactures non-pregnant cut-offs in spite of the possible HCG effect in pregnancy. It is difficult to explain, but inclusion of participants with low urine iodine deficiency maybe a plausible explanation.

We followed rigorous exclusion criteria to exclude possible confounders and followed the same clinical criteria used in the establishment of adult non-pregnant reference ranges in the National Health and Nutrition Examination Survey (NHANES III) [24] supplemented by USS criteria. Euthyroid pregnant women with elevated TPO antibodies are known to progress to hypothyroidism [25] and ATA guidelines recommend excluding those with elevated TPO when developing reference ranges [17].

We also used urine iodine deficiency as an exclusion criteria, as iodine deficiency influences thyroid hormone status and recent studies reveal that a significant number of women in Sri Lanka are iodine deficient during pregnancy with the percentage of iodine deficiency exceeding 60% [8] [26].

We did not exclude participants with diffusely enlarged goiters, as the thyroid gland enlarges during pregnancy from 10%, to up to 40% in areas with iodine deficiency [17]. We found a statistically significant increase in the size of the thyroid lobes (Right lobe 6.1% and left lobe 9.07%) in the reference population in the latter trimesters despite normal iodine status suggesting it to be a physiological change.

In addition to changes in TSH, we found that the Free T3 level significantly reduced with the progression of pregnancy. FreeT4 showed a significant reduction from first to second trimester and non-significant reduction from second to third trimester. In the study population more participants were found to have lower levels of free thyroid hormones than manufacturer's cutoffs with the progression of pregnancy (Table 4). Reduction of free thyroid hormones was shown by Panesar *et al* on evaluating Chinese pregnant women, where they found a 25% reduction of the free thyroid hormone levels with advanced POG [27]. The underlying cause for the decrease in FreeT4 is thought to be due to the activity of placental type 3 Deiodinase (Figure 6).

We found a positive correlation between TPO Ab level and TSH suggesting autoimmunity as a possible determinant of hypothyroidism in pregnancy. Furthermore, studies by Casey *et al* and Korevaar *et al* reveled that, approximately one-third of pregnant women with subclinical hypothyroidism were TPO Ab positive [28][29]. Excluding women with auto immunity, significantly reduced TSH upper-limit when deriving reference ranges in the study by Lambert *et al*. (4.15 mIU/l to 3.37 mIU/l in the first trimester and from 3.77 mIU/l to 3.35 mIU/l in the second trimester [30].

Table 4. Percentages of Isolated low FT4 or FT3 in the reference population

	First trimester	*Second trimester*	*Third trimester*
Free T4	1.56%	7%	8.55%
Free T3	non?	1.23%	4.5%

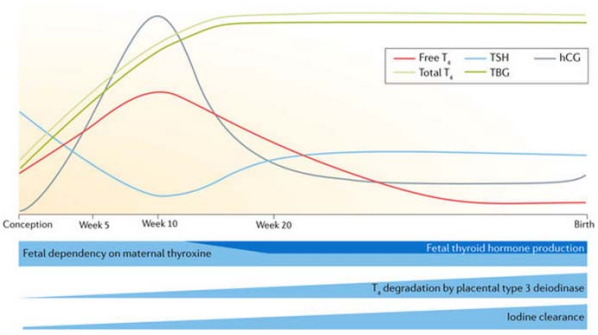

Figure 4. **Showing increased degradation of T4 by type 3 deiodinase in pregnancy.**

(From korevaar *et al*. Nature Reviews Endocrinology 2017, published with permission)

In addition, a recent study by korevaar *et al* revealed that thyroidal response to hCG stimulation is severely impaired in women with thyroid autoimmunity, resulting in lower free F4 levels [31]. This was not evident in our study, possibly due to selection bias.

Limitations and strengths

Limitation would be small sample size. Strength would be the rigid selection criteria used to the ideal ref population.

Conclusion

These data can be used as Sri Lankan reference data allowing clinicians to make more rational interpretation of TFTs in pregnant women. The TSH reference ranges observed in this study are concordant with the Caucasian reference values more than the regional values.

Acknowledgements

Special acknowledgement to Mrs. M.M. Gunatillaka, Head of the Department of Chemical Pathology at the National Hospital of Sri Lanka and Ms T.W. Ediriweera and other staff of the Radio-Immune Lab, National Hospital of Sri Lanka and to the Director, Deputy Director and Matron of the De Soysa Hospital for Women and all staff of the Radiology Unit, Antenatal Clinic and Ward Eight of the De Soysa Hospital for Women, Sri Lanka.

Author contributions

KDL, DTM, NPS designed the study, DTM, KDL, NPS, EC, LDR wrote the proposal, KD, EC, LDR acquired clinical data. NPS, KDL obtained funding for the study from Medical Research Institute. UAL, LTS and AHP performed and interpreted ultrasound scans of the thyroid, RJ, SR performed and interpretedurine iodine estimation. KDL,SL analyzed data, KDL, SL, NPS interpreted data, KDL, DTM, UAL, SL, NPS, NPL drafted the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

None.

Ethical standards

Ethical clearance for the study was obtained from Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka (Reference No: EC-14-144) and Ethics Review Committee of Medical Research Institute (Project number: 21/2014). Institutional ethics clearance was obtained from Ethics Review Committee of the De Soysa Maternity Hospital, Colombo (Project number: 2014/08). Informed written consent was obtained from study participants prior to enrolling in the study

Funding

Funding for conducting the research was provided by the Research Fund, Medical Research Institute (Project number: 21/2014).

References

1. Mannisto T, *et al*. Thyroid diseases and adverse pregnancy outcomes in a contemporary US cohort. *J Clin Endocrinol Metab* 2013; **98**(7): 2725-33.
2. Burrow GN, Fisher DA, Larsen PR. Maternal and fetal thyroid function. *N Engl J Med* 1994; **331**(16): 1072-8.
3. Haddow JE, *et al*. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *N Engl J Med* 1999; **341**(8): 549-55.
4. Su PY, *et al*. Maternal thyroid function in the first twenty weeks of pregnancy and subsequent fetal and infant development: a prospective population-based cohort study in China. *J Clin Endocrinol Metab* 2011; **96**(10): 3234-41.
5. Dhanwal DK, *et al*. Prevalence of hypothyroidism in pregnancy: An epidemiological study from 11 cities in 9 states of India. *Indian J Endocrinol Metab* 2016: **20**(3): 387.
6. Upadhyaya TL, AKC, Paudel S. Prevalence and complications of Hypothyroidism during pregnancy in Western Nepal. *Nepal J Med Sci* 2014; **3**(1): 48-50.
7. Klein R Z, *et al*. Prevalence of thyroid deficiency in pregnant women. *Clin Endocrinol* 1991; **35**(1): 41-46.
8. Yoganathan T, Hettiarachchi M, Arasaratnam V, Liyanage C. Maternal iodine status and the thyroid function of pregnant mothers and their neonates in Jaffna District of Sri Lanka. *Indian J Endocrinol Metab* 2015; **19**(6): 817-23.
9. World Health Organization. Urinary iodine concentrations for determining iodine status in populations (2013).
10. Ohashi T, Yamaki M, Panday CS, Kamarkar MG, Irie M. Simple microplate method for determination of urinary iodine. *Clin Chem* 2000; **46**(4): 529-36.
11. Pedersen OM, Aardal NP, Larssen TB, Varhaug JE, Myking O, Vik-Mo H. The value of ultrasonography in predicting autoimmune thyroid disease. *Thyroid* 2000; **10**(3): 251-59.
12. Marcocci C, Vitti P, Catani F, Catalano F, Concetti R, Pinchera A. Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. *J Clin Endocrinol Metab* 1991; **7**(1): 209-13.
13. Yamashiro Ilka, *et al*. Ultrasound findings in thyroiditis. *Radiol Bras* 2007; **40**(2): 75-9.
14. Aghini-Lombardi, Fabrizio, *et al*. The Spectrum of Thyroid Disorders in an Iodine-Deficient Community: The Pescopagano Survey 1. *J Clin Endocrinol Metab* 1999; **84**(2): 561-6.
15. Stagnaro-Green A, Abalovich M, Alexander E, *et al*. Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and Postpartum. *Thyroid* 2011; **21**(10): 1081-125. doi:10.1089/thy.2011.0087.
16. De Groot, Leslie, *et al*. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2012; **97**(8): 2543-65.
17. Alexander EK, *et al*. 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. *Thyroid* 2017; **27**(3): 315-89.
18. Marwaha RK, *et al*. Establishment of reference range for thyroid hormones in normal pregnant Indian women. *BJOG* 2008; **115**(5): 602-6.
19. Jebasingh FK, Salam R, Meetei TL, Singh PT, Singh NN, Prasad L. Reference intervals in evaluation of maternal thyroid function of Manipuri women. *Indian J Endocrinol Metab* 2016; **20**(2): 167-70. doi:10.4103/2230-8210.176354.
20. Sekhri T, Juhi JA, Wilfred R, *et al*. Trimester specific reference intervals for thyroid function tests in normal Indian pregnant women. *Indian J Endocrinol Metab* 2016; **20**(1): 101-7. doi:10.4103/2230-8210.172239.

21. Rajput R, Singh B, Goel V, Verma A, Seth S, Nanda S. Trimester-specific reference interval for thyroid hormones during pregnancy at a Tertiary Care Hospital in Haryana, India. *Indian J Endocrinol Metab* 2016; **20**: 810-5.

22. Price A, Owen O, Cresswell J, Catch I, Rutter S, Barik S, *et al*. Comparison of thyroid function in pregnant and non-pregnant Asian and Western Caucasian women. *Clin Chim Acta* 2001; **308**: 91-8.

23. Kurioka H, Takahashi K, Miyazaki K. Maternal thyroid function during pregnancy and puerperal period. *Endocr J* 2005; **52**: 587-91.

24. Hollowell, Joseph G, *et al*. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab* 2002; **87**(2): 489-99.

25. Glinoer DANIEL, *et al*. Risk of subclinical hypothyroidism in pregnant women with asymptomatic autoimmune thyroid disorders. *J Clin Endocrinol Metab* 1994; **79**(1): 197-204.

26. Jayatisa R, Gunathilaka MM, Ranbanda JM, Peiris P, Jayasingha J, Ekanayaka P. "Iodine status of pregnant women in Sri Lanka". *Sri Lanka J Diabetes Endocrinol Metab* 2013; **3**(1): 4-7. DOI: http://doi.org/10.4038/sjdem.v3i1.5469

27. Panesar NS, Li CY, Rogers MS. "Reference intervals for thyroid hormones in pregnant Chinese women". *Ann Clin Biochem* 2001; **38**(4): 329-32.

28. Casey BM, *et al*. Subclinical hyperthyroidism and pregnancy outcomes. *Obstet Gynecol* 2006; **107**: 337-41.

29. Korevaar T, *et al*. Stimulation of thyroid function by hCG during pregnancy: a risk factor for thyroid disease and a mechanism for known risk factors. *Thyroid* 2017; **27**: 440-50.

30. Lambert-Messerlian G, *et al*. First- and second-trimester thyroid hormone reference data in pregnant women: a FaSTER (First- and Second-Trimester Evaluation of Risk for aneuploidy) Research Consortium study. *Am J Obstet Gynecol* 2008; **199**: 62e1-62e6.

31 Korevaar TI, *et al*. Thyroid autoimmunity impairs the thyroidal response to human chorionic gonadotropin: two population-based prospective cohort studies. *J Clin Endocrinol Metab* 2017; **102**: 69-77.