Determination of Thyroid Dysfunction and Reference Intervals during the Third Trimester of Pregnancy in Port Harcourt, Nigeria

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Abstract

Background: *Prevalence of thyroid disorders in pregnancy varies with reference intervals of thyroid function tests among various populations globally, considering differences in population-specific characteristics and geographical locations.*

Objective: *To determine the prevalence of thyroid disorders in pregnant women in the third trimester in an iodine-sufficient city using internationally-recommended, assay-specific and laboratory-derived reference limits and to determine third trimester-specific reference intervals for thyroid function tests.*

Subjects and Methods: *Serum thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) were analysed in 178 pregnant women. Thyroid disorders were defined according to three criteria: the American Thyroid Association (ATA) third trimester-reference ranges, assay-specific non-pregnant reference intervals and laboratory-derived third trimester-reference intervals for thyroid function tests.*

Results: *Using the ATA criteria, overall prevalence of thyroid disorders was 18.0%: subclinical hypothyroidism (12.4%), overt hypothyroidism (3.4%), overt hyperthyroidism (1.1%) and isolated hypothyroxinemia (1.1%). Using the assay-specific reference intervals, overall prevalence of thyroid disorders was 11.7%: subclinical hypothyroidism (7.3%), overt hypothyroidism (2.2%), overt hyperthyroidism (1.1%) and isolated hypothyroxinemia (1.1%). Using the laboratory-derived reference intervals, overall prevalence of thyroid disorders was 13.4%: subclinical hypothyroidism (8.4%), overt hypothyroidism (2.8%), overt hyperthyroidism (1.1%) and isolated hypothyroxinemia (1.1%). Laboratory-derived reference intervals for TSH, FT4 and FT3 were 0.40 – 3.70 mIU/L, 8.89 – 18.85 pmol/L and 2.88 – 6.05 pmol/L respectively.*

Conclusion: *Laboratory-derived lower and upper reference intervals for TSH, FT4 and FT3 were observed to be lower than the assay-specific reference intervals and higher than the ATA third trimester TSH reference intervals.*

Key Words: *prevalence, thyroid disorders, reference intervals, pregnant women, third trimester*

INTRODUCTION

Pregnancy poses a challenge to the thyroid gland because of the associated hormonal changes and increased metabolic demands.[1,2] Plasma levels of thyrotropin (TSH), free thyroxine (FT4) and free triiodothyronine (FT3) tend to be lower during pregnancy than in the non-pregnant state Longitudinal studies have proven that FT4 levels in healthy pregnant women with adequate iodine supply are lower by about 10 - 15% at delivery compared to non-pregnant female subjects, and this reduction is amplified in women with limited thyroid reserve.[1,2]

As pregnancy progresses, thyroid function tests are altered as the thyroid gland adapts to these changes.[1,2] Serum free thyroid hormone levels decrease with increasing gestation and factors that may contribute to this decrease include increased thyroxine metabolism in the second and third trimesters due to increased placental types II and III deiodinases, transplacental transfer of thyroid hormones to the developing fetus, as well as increased renal clearance of iodide due to increased maternal glomerular filtration rate.[2-4] In a bid to compensate for these losses and maintain adequate plasma thyroid hormone levels there is an increase in the maternal thyroid volume with a concomitant increase in iodine requirement.[1-3] The World Health Organization (WHO) recommends a daily iodine intake of 250 µg in pregnancy.[1]

Compelling evidence indicate that TSH levels increase with increasing gestation and TSH reference range is higher during the second and third trimesters than in the first trimester.[1,2] However, the serum human chorionic gonadotropin (hCG) level is markedly increased particularly in the first half of pregnancy and as a result of its intrinsic thyrotropic activity and suppression of serum TSH release, the reference intervals for TSH tend to be lower throughout pregnancy compared to the non-pregnant state.[1,2,4] Both the lower and upper reference limits of serum TSH are decreased by about 0.1 – 0.2 mIU/L and 1.0 mIU/L respectively compared with the TSH reference intervals of non-pregnant women.[1] As a result of these changes in thyroid hormones and TSH during pregnancy, various assay-specific and internationally recommended trimester-specific reference ranges (TSR) have been developed. Use of TSH reference ranges of 0.1 – 2.5 mIU/L in the first trimester, 0.2 – 3.0 mIU/L in the second trimester and 0.3 – 3.0 mIU/L in the third trimester recommended by the American Thyroid Association (ATA) in 2011 for the definition of thyroid disorders have been widely adopted.[1] More recent TSH upper reference limit of 3.5 mIU/L in the third trimester have been proposed by the joint American Association of Clinical Endocrinologists (AACE) and the ATA task force [5], and also by the European Thyroid Association (ETA).[6]

Population-based studies have been advocated to determine pregnancy-specific reference intervals, keeping in view different population characteristics.[1,4] Some centers have been able to estimate their own trimester- and assay-specific reference limits.[4,7] However for those that have not been able to do so, internationally-derived TSR are recommended for use.[1]

The purpose of this study is to screen for thyroid disorders in pregnant women in the

third trimester in an iodine-sufficient city in Nigeria[8] using both internationally-derived TSR and laboratory reference limits, and to determine third trimester-specific reference intervals for thyroid function tests. To the best of the authors' knowledge, this type of study has not been done before in this institution.

METHODOLOGY

This study was conducted in a tertiary health care facility in Port Harcourt, a major seaport city in southern Nigeria. Approval was obtained from the Ethical Committee of the hospital and an informed consent was also obtained from every participant. This is an initial report of a large prospective cross-sectional study which screened for thyroid dysfunction among pregnant women in the third trimester as a preliminary step before eventually screening for congenital hypothyroidism among their newborn babies. Apparently healthy consenting women with singleton pregnancies who were scheduled for delivery, both vaginally and through elective caesarian section, were consecutively included in this study. Some information was abstracted from their medical records. The women were also interviewed with the use of questionnaires about their socio-demographic characteristics, reproductive history, family and personal history of thyroid disease or other associated medical conditions like diabetes and hypertension, the use of thyroid medications and the use of iodized salt. All women with thyromegally, known history or evidence of thyroid disorders or associated medical conditions and those on antithyroid drugs, levothyroxine or any medication that could affect thyroid function were excluded from this study.

Participants were physically examined and blood specimens were collected from them. Serum TSH, FT4 and FT3 were analysed by the Vitros ECiQ immunodiagnostic autoanalyser which uses an immunometric assay for TSH and competitive immunoassay technique for FT4 and FT3. All thyroid function tests were performed using reagents provided by Vitros immunodiagnostics according to the instructions on the package insert. The universal 2011 American Thyroid Association (ATA) third trimester TSH reference interval used was 0.3 – 3.0 mIU/L. The assay-specific non-pregnant reference intervals provided by the manufacturer were: 0.47 – 4.68 mIU/L for TSH, 10.06 – 28.25 pmol/L for FT4 and 4.26 – 8.10 pmol/L for Ft3.

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc. Chicago, Illinois, U.S.A.). Frequencies and percentages were obtained for categorical variables. Differences in proportions were analyzed using the chi-squared test. Kolmogorov-Smirnov test revealed a skewed distribution of subjects' TSH, FT4 and FT3. The means of normally distributed continuous variables were compared using unpaired students t test and the means of skewed continuous variables were compared using Mann-Whitney U test. Results were expressed as mean ± standard deviation (SD) and median ±interquartile range (IQR). Spearman's correlation statistics was used to assess the relationship between variables. 2.5th and 97.5th percentiles were used to determine

upper and lower reference intervals respectively. P-values ≤ 0.05 were considered significant in all analyses.

RESULTS

The total number of pregnant women was 178 with a mean (SD) gestational age of 38.5 (2.1) weeks, ranging from 33 to 42 weeks (Table 1). Their mean (SD) age was 29.5 (5.1) years with a range of 18 to 44 years (Table 1). Overall mean (SD) and median (IQR) TSH were 1.94 (1.40) mIU/L and 1.41 (2.05) mIU/L respectively (Table 1). The mean and median FT4 were 11.98 (4.78) pmol/L and 10.58 (1.68) pmol/L respectively (Table 1). The mean and median FT3 were 3.40 (0.92) pmol/L and 3.13 (0.23) pmol/L respectively (Table 1).

Mean TSH of women older than 35 years (2.47 mIU/L) was significantly higher (p=0.020) than that of women younger than 35 years (1.84 mIU/L) but there was no difference in their FT4 (p=0.457) and FT3 (p=0.433) levels. One hundred and thirty (73%) women used iodized salt regularly in cooking meals and 48 (27%) did not make use of iodized salt. There was no significant difference between the mean TSH of those who used iodized salt and those who did not (p=0.808).

Using the ATA criteria, overall prevalence of thyroid disorders was 18.0%; twenty-two (12.4%) women had subclinical hypothyroidism, 6 (3.4%) had overt hypothyroidism, 2 (1.1%) had overt hyperthyroidism and 2 (1.1%) had isolated hypothyroxinemia. Using the assay-specific reference intervals, overall prevalence of thyroid disorders was 11.7%; 13 (7.3%) women had subclinical hypothyroidism, 4 (2.2%) had overt hypothyroidism, 2 (1.1%) had overt hyperthyroidism and 2 (1.1%) had isolated hypothyroxinemia. FT3 correlated positively with FT4 (r = 0.320, p = 0.000) and negatively with TSH (r = - 0.166, p = 0.027).

After eliminating results with thyroid disorders and outliers from the data, 154 values were eligible for reference interval analysis. Reference intervals were determined for TSH, FT4 and FT3 (Table 2). Laboratory-derived lower and upper reference intervals for TSH, FT4 and FT3 were observed to be lower than the assay-specific reference intervals and higher than the ATA third trimester TSH reference intervals.

When these laboratory-derived reference intervals were applied to the original data of 178 women in determining prevalence of thyroid disorders, overall prevalence of thyroid disorders was 13.4%; 15 (8.4%) women had subclinical hypothyroidism, 5 (2.8%) had overt hypothyroidism, 2 (1.1%) had overt hyperthyroidism and 2 (1.1%) had isolated hypothyroxinemia. This overall prevalence was higher than the prevalence determined using assay-specific reference intervals and lower than the prevalence determined using ATA third trimester TSH reference intervals.

Table 1. Demographic/Obstetric Profile of Subjects

Variable (N = 178)	Mean (SD)	Range/Median (IQR)
Maternal Age (Years)	29.5 (5.1)	18 - 44
Gestational age (weeks)	38.5 (2.1)	33 - 42
Parity (No. of children)	2.8 (1.5)	. . .
TSH (mIU/L)	1.94 (1.40)	1.41 (2.05)
FT4 (pmol/L)	11.98 (4.78)	10.58 (1.68)
FT4 (ng/ml)	0.93 (0.37)	0.82 (0.13)
FT3 (pmol/L)	3.40 (0.92)	3.13 (0.23)
FT3 (pg/ml)	2.21 (0.60)	2.03 (0.15)

N = number; SD = Standard Deviation; IQR = Interquartile Range

Table 2. Reference Intervals of Thyroid Function Tests in the Third Trimester

Test	N	Mean	SD	Median	IQR	2.5th Percentile	97.5th Percentile
TSH mIU/L	154	1.57	0.97	1.39	1.93	0.40	3.70
FT4 (ng/ml)	154	0.89	0.22	0.82	0.13	0.69	1.46
FT4 (pmol/L)	154	11.53	2.84	10.58	1.68	8.89	18.85
FT3 (pg/ml)	154	2.15	0.45	2.03	0.13	1.87	3.93
FT3 (pmol/L)	154	3.32	0.70	3.13	0.23	2.88	6.05

N = number; SD = Standard Deviation; IQR = Interquartile Range

DISCUSSION

Prevalence of thyroid disorders in this study using internationally-derived trimester-specific reference limits was 18.0% which is relatively high compared to using laboratory-derived reference intervals which gave an overall prevalence of 13.4%. Laboratory-derived lower and upper reference intervals for TSH were observed to be higher than the ATA third trimester TSH reference intervals respectively.

Gestational overt and subclinical hypothyroidism may lead to maternal obstetric complications as well as adverse neonatal outcomes including neurodevelopmental deficits.[9-11] Subclinical hypothyroidism had a higher prevalence than overt hypothyroidism, which is in agreement with previous studies that revealed that subclinical hypothyroidism occurs more commonly than overt hypothyroidism in pregnancy.[7,10] Hypothyroidism also had a higher prevalence than hyperthyroidism. Similar findings have been documented in earlier studies.[10,11] Overt hyperthyroidism has been demonstrated to be associated with placental abruption, preeclampsia and preterm delivery. Similar associations have not been observed for subclinical hyperthyroidism.[10] Isolated hypothyroxinemia, defined as low FT4 with normal TSH, occurs in 1% - 2% of pregnancies.[3] Some studies have revealed adverse fetal and maternal effects of isolated hypothyroxinemia while others have concluded that there are no adverse effects on pregnancy outcome and no benefits from levothyroxine treatment.[3]

Alkafajei and colleagues obtained a hypothyroidism prevalence of 20.8% in

Jordanian women in their first trimester using internationally-recommended TSH level ≥ 2.6 mIU/L. When the local laboratory diagnostic TSH limit of ≥ 4.6 mIU/L was used, the prevalence reduced substantially to 4.3%.[12] This is in agreement with our study where we had a total prevalence of 15.8%, 11.2% and 9.5% for both subclinical and overt hypothyroidism using internationally-recommended, laboratory derived and assay-specific reference intervals respectively.

Mbah et al obtained a hypothyroidism prevalence of 36.8% in another city in south-eastern Nigeria, using a TSH cutoff > 6.0 mIU/L.[13] This prevalence is much higher than the one from this study and it is a reflection of endemic goiter in this city. Regions of iodine deficiency usually record higher prevalence of hypothyroidism compared to iodine-sufficient regions.[1] Iodine deficiency has been identified as the most common cause of hypothyroidism in African populations unlike in developed countries with iodine-replete populations where autoimmune thyroid disease (AITD) is the predominant cause of hypothyroidism.[1]

Port Harcourt is not known to be an iodine-deficient region.[8,14] It is a coastal city located in the Niger Delta region and has access to sea foods like fish, shrimps and shellfish which are rich in iodine[8]. Seventy-three percent of women in this study made use of iodized salt but their TSH levels were not significantly different from those who did not. This corroborates earlier studies that demonstrated that Port Harcourt is an iodine-sufficient city.[8,14]

The lower and upper reference limits for TSH, FT4 and FT3 obtained in this study for pregnant women in the third trimester were clearly lower than non-pregnant values. Stricker et al determined trimester-specific reference intervals for thyroid function tests and discovered that they were different from non-pregnant assay-specific reference intervals. It was also established that 3% of TSH results and 3.3% of FT4 results in the third trimester would have been incorrectly classified if non-pregnant reference intervals were used.[7]

Significant variations have been observed in TSH, FT4 and FT3 pregnancy-specific reference intervals between populations and these have been attributed to ethnic differences and differences between methods of analysis, among other factors.[1,4,11] Medici et al reviewed several population-based studies and noted that the upper limit of TSH varied between 2.15 and 4.68 mU/L and that 90% were higher than the recommended fixed TSH cutoff limits of 2.5 and 3.0 mU/L for the first and second trimesters respectively.[4] They also observed that the use of population-based pregnancy-specific intervals identified increased risk of adverse maternal and child outcomes whereas the use of fixed TSH cutoffs did not, thus highlighting the significance of determining pregnancy-specific reference intervals specific to each population rather than relying on fixed universal cutoffs, which may lead to misclassification of thyroid disease and overtreatment.[4]

Black and Asian women have TSH values that are slightly different from that of Caucasian women during pregnancy as well as in the non-pregnant state.[1,11] This study has shown

that third trimester TSH reference intervals in our population are higher than those of Caucasian population. Population-based studies conducted among Chinese, Korean, Indian and Iranian pregnant women revealed significantly higher TSH reference ranges than their Caucasian counterparts for each trimester.[6,15-18] It was also demonstrated that about 28% of pregnant Chinese women would be misclassified as having hypothyroidism using the suggested 0.1 – 2.5 mIU/L as reference range in the first trimester compared to 4% if the ethnically specific reference range were used.[6] Population-based data from African countries are sparse but available evidence show that universal TSR, mostly derived from Western populations, though useful, may not be appropriate for application to all populations worldwide.[6]

Some researchers have linked maternal age with the incidence of hypothyroidism. Casey and colleagues observed that women with SCH aged 35 years or more were older than controls.[9] Our study revealed similar findings; women older than 35 years had higher TSH values than younger women. Mbah et al also discovered that hypothyroid pregnant women in their study were older than non-hypothyroid pregnant women.[13] The prevalence of thyroid disorders, particularly subclinical hypothyroidism, has been demonstrated to increase with age.[15]

CONCLUSION

The prevalence of thyroid disorders using internationally-derived trimester-specific reference limits is relatively high in this environment. Taking differences in population-specific characteristics into consideration, there was a need to determine laboratory trimester-specific reference intervals for TSH and thyroid hormones in our environment for proper classification and management of thyroid disease in pregnancy.

Laboratory-derived lower and upper reference intervals for TSH, FT4 and FT3 in the third trimester were 0.40 – 3.70 mIU/L, 8.89 – 18.85 pmol/L and 2.88 – 6.05 pmol/L respectively. These were observed to be lower than the assay-specific reference intervals and higher than the ATA third trimester TSH reference intervals.

Limitations

Thyroid ultrasound was not done in these subjects and thyroid auto-antibodies (antibodies to thyroid peroxidase and thyroglobulin) were not analysed in this study and therefore were not included in the exclusion criteria. Exclusion of subjects with antibody-positivity and ultrasound evidence of thyroid dysfunction could further improve reference interval estimations[1]. In addition, due to the nature of the study, sequential evaluation of thyroid function in the first and second trimesters was not done as well, which limits the clinical utility of the study. Further studies are therefore required in this regard.

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