Trimester specific reference intervals of thyroid function tests among Nigerian pregnant women

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Abstract

Background*: Thyroid disorders are common among women of childbearing age with associated adverse pregnancy outcomes. A normal pregnancy results in a number of important physiological changes that alter thyroid function with considerable variations per trimester. American Thyroid Association recommended each laboratory to establish its thyroid function tests reference interval for each trimester. Lack of these reference intervals makes management of thyroid disorders during pregnancy difficult.*

Objective: *To establish trimester-specific thyroid function reference values in pregnancy.*

Method: *Three-hundred apparently healthy pregnant women were recruited into the study. Thyroid function tests (TSH, free T_4, free T_3, Thyroxin Binding Globulin (TBG) were assayed using ELISA kits. Trimester-specific reference intervals (2.5th and 97.5th centiles) were calculated for 75, 125 and 100 pregnant women in first, second and third trimesters' respectively using Microsoft Excel software 2007.*

Results: *Mean ages ± Standard deviation was 25.4 ± 5.98 years respectively (p>0.05). First trimester TSH, Free T_4, Free T_3 and TBG reference intervals were (0.03 – 2.41) µIU/L, (0. 84 – 2.06) ng/mL, (1.92–3.51) pg/mL, and (11.32 – 43.17) µg/mL respectively. Second trimester reference intervals for TSH, Free T_4, Free T_3 and TBG were (0.14 – 3.55) µIU/L, (0.76 – 2.08) ng/mL, (1.65 – 3.96) pg/mL, and (14.51–72.86) µg/mL respectively. Third trimester reference intervals for TSH, Free T_4, Free T_3 and TBG were (0.21– 3.12) µIU/L, (0.70–1.70) ng/mL, (1.74 -3.65) pg/mL, and (20.33–72.55) µg/mL respectively.*

Conclusion: *Thyroid function Reference intervals established in keeping with international recommendations.*

Keywords: Trimester, Reference intervals, Pregnancy, Thyroid

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Introduction

Thyroid disorders are common especially in women of childbearing age[1]. Gestational thyroid diseases are associated with adverse maternal and foetal outcomes. Pregnancy is seen as a risk factor in the occurrence of thyroid dysfunctions[2]. Recently, more frequent association between pregnancy and thyroid gland pathology has been observed[2] .Hypothyroidism is commoner and most serious disorder of those occurring during pregnancy, and it might go unnoticed as some 'non-specific' problem[1]. Some women with subclinical hypothyroidism are absolutely asymptomatic. Weight gain, fatique, sleepiness, lowered performance are common to both hypothyroidism and normal pregnancy[1]. Anxiety, palpitations, dyspnoea, sweating, heat intolerance, weakness, irritability and cardiac systolic murmurs are seen in both normal pregnancy and hyperthyroidism[1]. This makes diagnosis difficult, as high index of suspicion is required to differentiate between the symptoms of pregnancy related hyper or hypometabolism, and mild obstetrical thyroid disease.

Hormonal changes and increased metabolic demands during pregnancy produce complex alterations in thyroid hormones concentrations[3]. Such changes are more pronounced during first trimester but additional changes occur in both second and third trimesters[3]. These include increase in oestrogen and human chorionic gonadotropins (hCG). Oestrogen increases the hepatic synthesis of thyroxin binding globulin (TBG) in the serum, while hCG has a weak thyroid stimulating activity [3, 4, 5-7]. Consequently, the serum levels of total T_4 and T_3 increase with a reciprocal reduction in resin uptake. Also hCG and TSH have similar ã-subunit and slightly different â-subunit. This results in marked suppression of TSH by hCG in pregnancy especially in the first trimester during the rapid surge of hCG [8]. Thyroid stimulating hormone is suppressed by 20 – 50 % per week by 10^{th} week of pregnancy. During pregnancy, therefore, the results of conventional biochemical tests will be in the hyperthyroid range, so that free hormone levels must be used as true indices of function [4, 6, 7].

Early in pregnancy, there is also an increase in the renal excretion of iodide with a corresponding fall in plasma levels [4, 8]. In response, thyroidal trapping of iodide is increased and, unless there is a liberal supply in the diet, there is decrease in thyroid hormones production with goitre formation [4, 6, 7, 8].

These changes prompted the American Thyroid Association (ATA) to recommend establishment of trimester specific reference intervals for TSH and Free T_4 by each laboratory since thyroid function reference intervals varies with geographical conditions and ethnic group[9]. These reference intervals must be established in iodine sufficient environment.

Subjects and Methods

Four hundred subjects were recruited for this study. These consisted of three hundred apparently healthy pregnant women and one hundred apparently healthy non pregnant age-matched controls. Their ages ranged from 14 to 40 years. These subjects were drawn from various antenatal care centres in Zaria, a city in Northern Nigeria. Controls were drawn from the township community. Only pregnant women who were sure of their last menstrual period which was confirmed from their booking ultrasound scan and not on any drug that affects thyroid function were recruited for the study after written consent was obtained. Approval to carry out this study was obtained from the Ethical and Scientific Committee of Ahmadu Bello University Teaching Hospital, Zaria.

Ultrasensitive Enzyme Linked Immunosorbent Assay (ELISA) kits for Thyroid stimulating hormone, Free T_4, Free T_3, and Thyroxin Binding Globulin (TBG) were obtained from Diagnostic Automation Inc. USA.

Data was analyzed using Epi-Info 3.5.3. The results of thyroid function tests from pregnant women were compared to non-pregnant controls using two tailed student's t-test for matched samples. The parameters of thyroid function tests were plotted as histograms and observed to be of non-Gaussian distribution. Reference intervals were therefore determined as 2.5 and 97.5 percentiles after log transformation using Microsoft excel 2007 software.

Results

Table 1 shows social and clinical characteristics of the respondents. Four hundred participants were recruited for the study. These consisted of three hundred pregnant women and one hundred non pregnant controls. Ages (Mean ± SD) for the pregnant women and controls were 25.41 ± 5.98 and 26.70 ± 5.83 years respectively ($p > 0.05$). Seventy five pregnant women were in their first trimester, one hundred and twenty five in the second trimester and one hundred in the third trimester. Parity distribution (mean ± SD) was 2.32 ± 2.24 and 2.63 ± 2.75 among the pregnant women and non pregnant controls respectively, of which the differences were not statistically significant. The mean blood pressures (BP) (both systolic and diastolic) were within normal limits. The systolic BP was 112.7±13.2 and 120.3±20.8 mmHg among pregnant and non pregnant controls respectively, while the diastolic BP was 74.9±11.9 and 79.4±14.1 mmHg for pregnant and non pregnant controls respectively ($p< 0.05$). Majority of the respondents were married but there is high disparity in their educational status with only 8% among the cases that attend university in contrast to 32 % among controls.

Table 1: Comparison of social and clinical characteristics of control subjects and pregnant subjects

Characteristics	Controls (Mean ± SD)	Patients (Mean ± SD)	p-value
Frequency	100	300	
Age	26.70 ± 5.83	25.41 ± 5.98	0.062
Parity	2.63 ± 2.75	2.32 ± 2.24	0.259
Systolic BP	120.28 ± 20.79	112.75 ± 13.30	0.000
Diastolic BP	79.39 ± 14.14	74.88 ± 11.86	0.002

Table 2: Median values and reference intervals for thyroid function test in subjects and controls {Median (2.5 and 97.5 percentiles)}

Subjects	TSH (µIU/L)	Free T_4 (ng/mL)	Free T_3 (pg/mL)	TBG (µg/mL)
Controls	0.93 (0.12 – 3.73)	1.22 (0.81 – 1.91)	2.43 (1.56 – 3.62)	11.89 (6.09 – 31.98)
Pregnant	1.01 (0.1 – 3.19)	1.22 (0.73 – 2.06)	2.46 (1.69 – 3.81)	28.56 (12.25 – 72.38)
1st trimester	0.70 (0.03 – 2.41)	1.34 (0. 84 – 2.06)	2.41 (1.92 – 3.51)	21.51 (11.32 – 43.17)
2nd trimester	1.05 (0.14 – 3.55)	1.18 (0.76 – 2.08)	2.63 (1.65 – 3.96)	28.81 (14.51 – 72.86)
3rd trimester	1.19 (0.21 – 3.12)	1.20 (0.70 – 1.70)	2.40 (1.74 -3.65)	32.47 (20.33 – 72.55)

Table 2 shows the trimester specific reference interval of thyroid function tests among the respondents. Non-pregnant thyroid hormone reference intervals 50th (2.5th – 97.5th) obtained for TSH, Free T_4, Free T_3 and TBG were 0.93 (0.12 – 3.73) µIU/L, 1.22 (0.81 – 1.91) ng/mL, 2.43 (1.56 – 3.62) pg/mL, and 11.89 (6.09 – 31.98) µg/mL respectively.

First trimester TSH, Free T_4, Free T_3 and TBG reference intervals were 0.70 (0.03 – 2.41) µIU/L,1.34 (0. 84 – 2.06) ng/mL, 2.41 (1.92 – 3.51) pg/mL, and 21.51 (11.32 – 43.17) µg/mL respectively.

Second trimester reference intervals for TSH, Free T_4, Free T_3 and TBG were 1.05 (0.14 – 3.55) µIU/L, 1.18 (0.76 – 2.08) ng/mL, 2.63 (1.65 – 3.96) pg/mL, and 28.81 (14.51 – 72.86) µg/mL respectively.

Third trimester reference intervals for TSH, Free T_4, Free T_3 and TBG were 1.19 (0.21 – 3.12) µIU/L, 1.20 (0.70 – 1.70) ng/mL, 2.40 (1.74 - 3.65) pg/mL, and 32.47 (20.33 – 72.55) µg/mL respectively. The lower reference intervals for TSH and TBG increased as pregnancy progressed while Free T_4 decreased with increase in trimester. Free T_3 has not demonstrated any specific pattern.

Discussion

Thyroid function test profile (TSH, free T_3 and T_4) in pregnant women in the present study did not show any significant difference compared with those of controls. However, pregnant women TSH increased steadily from first to third trimesters which was statistically significant, hence trimester specific reference intervals of TSH was obtained in the present study and were found to be 0.03 – 2.41 µIU/L, 0.14 – 3.55 µIU/L and 0.21 – 3.12 µIU/L for first, second and third trimesters respectively. This is consistent with the recommendation of American Thyroid Association (ATA) that each laboratory should establish its trimester specific reference intervals of TSH in pregnancy [9], but in the absence of any, it recommended the use of 0.1 – 2.5 µIU/L, 0.2 – 3.0 µIU/L and 0.3 – 3.0 µIU/L for first, second and third trimesters respectively [9]. Our findings agree with the above and the works of Haddow *et al* (2004) [9, 10], Stricker *et al* (2007) [9, 11], Panesar *et al* (2001) [9, 12], Bocos – Terraz *et al* (2009) [9, 13] and Soldin *et al* (2007) [9, 14]. Stickler *et al*, Bocos - Terraz *et al* and Soldin *et al* used 2.5th and 97.5th percentiles as in the present study, while Haddow *et al* and Panesar *et al* used 5th and 95th percentiles [9-14].

The values of free T_4 concentrations in the present study showed significant decline from first to third trimesters. In 2012, American Thyroid Association (ATA) recommended that each laboratory should establish its trimester specific reference intervals for free T_4 [15]. The reference intervals in this study are 0.84 – 2.06 ng/mL, 0.76 – 2.08 ng/mL and 0.70 – 1.70 ng/mL for first, second and third trimesters respectively. This is in agreement with what was obtained by La'ulu *et al* in 2007 who reported free T_4 second trimester reference interval of 0.70 – 1.48 among Americans [16]. The slightly higher upper reference value seen in the present study can be explained by the environmental and ethnic differences as well as difference in the methods of analysis [16, 17]. La'ulu *et al* used chemiluminescence method of analysis (Architect $i2000_{SR}$) while we used ELISA method (microplate reader RT 2100C) in the present study. Also, La'ulu *et al* used 14 – 20 weeks of gestation as second trimester population while we used 14 – 26 weeks of gestation.

Marwaha *et al* in 2008 established 0.93 – 1.51 ng/mL, 0.74 – 1.52 ng/mL and 0.88 – 1.38 ng/mL as the first, second and third trimesters reference intervals for free T_4 respectively [18], Gong *et al* in 2008 documented 0.85 – 1.48 ng/mL, 0.75 – 1.36 ng/mL and 0.63 – 1.19 ng/mL for the first, second and third trimesters respectively [19], while Bocos – Terraz *et al* in 2009 reported 0.84 – 1.38, 0.70 – 1.16 and 0.62 – 1.17 ng/mL for the first, second and third trimesters respectively [13]. The lower reference interval in the present study is in agreement

with what Marwaha [18], Bocoz – Terraz [13], La'ulu [16] and Gong *et al* [19] reported, however the upper reference interval varied. Marwaha *et al* [18] used Elecys 1010 analyzer, Bocoz – Terraz *et al* [13] used Architect $i2000_{SR}$ while Gong *et al* [19] used Roche Modular E-170 electrochemiluminescent immunoassay analyzer. Electrochemiluminescence principally emits light when an electron at an excited state or high energy level returns to ground state or low energy level. This assay method is more sensitive than ELISA but high intensity light emission can lead to pulse pile-up in photomultiplier tubes and this leads to serious underestimation of the true light emission intensity.

Other sources of errors associated with electrochemiluminescence measurements are light leaks, light piping, high background luminescence from assay reagents and reaction vessel. Difference in equipment used, methods of analysis, geographic location and ethnic variation may explain the difference in the upper reference interval [16, 17]. Silvio *et al* in 2009 compared the results of second trimester reference intervals of thyroid hormones using Elecsys E170 and Architect $i2000_{SR}$ and reported similar lower reference interval, but there was significant disparity in the upper reference interval [17].

This disparity is most conspicuous in this study as compared with others. This may be well explained by the factors described above. Both American Endocrine Society and American Thyroid Association were silent about establishment of trimester specific reference interval for Free T_3. Also, it does not exhibit significant variation within the three trimesters in this study. However, the pattern obtained in this study showed the highest mean value in the second trimester which was not statistically significant. Insignificant difference within the trimesters may be the reason why the two international bodies were silent in the establishment of trimester specific reference intervals. However, the reference intervals obtained for free T_3 in this study are 1.92 – 3.51 pg/mL, 1.65 – 3.96 pg/mL and 1.74 – 3.65 pg/mL for first, second and third trimesters respectively.

As part of the recommendation by American College of Pathologist that each laboratory must establish reference values for the society it serves[20] we hereby established trimester specific reference values for thyroid function tests in Zaria.

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