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Urinary iodine levels and thyroid hormones in first trimester pregnant women in Nigeria

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Abstract

Background/Aim: Iodine is an essential component of thyroid hormones required for the normal growth, development and functioning of the body. Its deficiency causes intellectual impairment, reproductive risks such as overt hypothyroidism, infertility, gestational hypertension, increased first trimester abortions and still births. Paradoxically, iodine deficiency disorders (IDD) are among the easiest and cheapest preventable disorders. This study therefore evaluated dietary iodine status and some thyroid parameters in first trimester (10th–12th week) pregnant women

Methods: Forty-two healthy pregnant women, mean age and gestational age of 30±5.22 years and 11.43±0.83 weeks respectively were recruited in consecutive manner for this study after obtaining their informed consents. Urinary iodine levels were analysed in casual urine samples using the ammonium persulphate method as described by Dunn *et al* while thyroid stimulating hormone (TSH) and free thyroxine (fT_4) were measured in serum using Enzyme Immunoassay technique.

Results: 40.5% of the participants had adequate dietary iodine, 47.6% had more than adequate, 9.5% had mild iodine deficiency, while 2.4% had excess dietary iodine. 92.9% of the participants had normal TSH values, 4.8% and 2.4% fell in the hypothyroid and hyperthyroid ranges respectively. 91.4% of the participants had normal values for fT_4, 8.6% had below normal while none had above. Qualitative salt analysis shows iodization of salt in all the salt samples tested.

Conclusion: This study reveals adequate iodine nutrition in the first trimester sub-population, however, screening for overt and subclinical hypothyroidism should still be strongly considered.

Keywords: *Iodine, iodine deficiency disorders, thyroid hormones, first trimester pregnant women*

Résumé

Contexte / But : L'iode est un composant essentiel des hormones thyroïdiennes nécessaires à la croissance, au développement et au fonctionnement normaux du corps. Sa carence entraîne une déficience intellectuelle, des risques de reproduction tels que l'hypothyroïdie manifeste, l'infertilité, l'hypertension gestationnelle, l'augmentation des avortements au premier trimestre et des d'enfants mort-nés. Paradoxalement, les troubles dus à la carence en iode (IDD) sont parmi les troubles évitables les plus faciles et moins chers. Cette étude a donc évalué l'état de l'iode alimentaire et certains paramètres thyroïdiens chez les femmes enceintes du premier trimestre (10ème - 12ème semaine).

Méthodes : Quarante-deux femmes enceintes en bonne santé, âge moyen et âge gestationnel de 30 ± 5,22 ans et 11,43 ± 0,83 semaines respectivement ont été recrutés de façon consécutive pour cette étude après avoir obtenu leur consentement informé. Les niveaux d'iode urinaire analysés dans des échantillons d'urine occasionnels en utilisant la méthode du persulfate d'ammonium décrite par Dunn *et al* tandis que la thyréostimuline (TSH) et la thyroxine libre (fT_4) ont été mesurées dans le sérum en utilisant la technique d'essai d'immune enzymatique.

Résultats : 40,5% des participants avaient un apport alimentaire adéquat en iode, 47,6% avaient plus qu'adéquat, 9,5 % avaient une légère carence en iode et 2,4% avaient un excès d'iode alimentaire. 92,9% des participants avaient des valeurs de TSH normales, 4,8% et 2,4% étaient à portée avec l'hypothyroïdie et l'hyperthyroïdie respectivement. 91,4% des participants avaient des valeurs normales pour l'fT_4, 8,6% avaient un taux inférieur à la normale alors qu'aucun n'avait au-delà. L'analyse qualitative des sels a montrée l'iodation du sel dans tous les échantillons de sel testés.

Conclusion: Cette étude révèle une nutrition adéquate en iode dans la sous-population du premier trimestre; cependant, le dépistage de l'hypothyroïdie manifeste et sub-clinique devrait être fortement envisagé.

Mots clés: *Iode, troubles de la carence en iode, hormones thyroïdiennes, femmes enceintes au premier trimestre*

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Introduction

Iodine is required for the synthesis of thyroid hormones, which are important for the development of foetus especially for the maturation of the central nervous system [1]. Adequate dietary iodine intake during pregnancy is essential to prevent adverse maternal and neonatal outcomes. In fact, mild to moderate degrees of iodine deficiency during foetal development have been linked to reduced intellectual function [2, 3]. Insufficient iodine intake as well as its associated thyroid disorders negatively and irreversibly affects the psychoneurotic- intellectual development of the foetus, especially when the deficiency occurs during the first trimester [4]. Furthermore, there is also evidence for an increased risk of adverse obstetrical outcomes such as pre-eclampsia, placental abruption, and negative effects on the offspring which include preterm birth, low-birth weight or even foetal death [5].

Pregnancy is associated with changes in maternal thyroid physiological function which can be viewed globally as a balance between hormone requirements and the availability of iodine [6, 7]. During pregnancy, synthesis of thyroid hormones is increased by up to 50% with an increase in the requirement of thyroxin (T_4) in order to maintain a normal global metabolism in the mother [8-10]. This increase in hormone demands is due to several factors that concur to exert stimulatory effects on the thyroid machinery. These factors include the adjustment of the thyroidal economy during the first trimester to the marked increase in the circulating levels of thyroxin-binding globulin (TBG) in response to increased oestrogen production caused by human chorionic gonadotropin (hCG) [9].

The second factor is thyrotropic action of hCG, also occurring in the first trimester, which tends to transiently elevate free thyroxin (fT_4) levels and decrease serum thyroid stimulating hormone (TSH). A third factor which intervenes later in gestation is related to modification in the peripheral metabolism of the thyroid hormones, particularly at the placenta level [9]. Other factors include the transportation of iodide and T_4 from maternal circulation to the foetus. The foetal thyroid hormone production increases during the second half of gestation [11] and after delivery; iodide is also transported into the breast milk [11].

Another factor is the increased loss of iodide through the kidney [6, 11]. The majority of dietary iodine (90%) is excreted in the urine. Urinary iodine excretion is largely a passive process dependent on glomerular filtration rate (GFR) [12]. Pregnancy results in increased loss of renal iodine. In circumstances of borderline or overt iodine deficiency, pregnancy-related increases in GFR could deplete total body iodine reserves without the capacity for replenishment if dietary intake remains low [13]. This is due to GFR increases within the first month after conception peaking by the end of the first trimester at approximately 40-50% above pre-pregnancy levels in normal pregnancy [14].

A consensus statement from the American Thyroid Association (ATA) highlights the importance of higher dietary iodine intake in pregnancy and higher pregnancy-specific urinary iodine concentration (UIC), to allow for physiological changes during pregnancy [15]. The recommendation stipulates that a median UIC of 150-249μg/L is expected in iodine sufficient pregnancy [15].Thyroid hormone synthesis is impaired when nutritional iodine intake is low. The body reacts to low iodine intake by increasing thyroidal uptake efficiency (up to 4 times) in order to maintain the T_4 output. This favours the preferential production of the more potent triiodothyronine (T_3) over T_4 for efficient utilization of the available iodine. Clinically, euthyroidism is thus maintained but biochemically, the pattern of low T_4, normal or moderately elevated TSH and normal or elevated T_3 is often observed [16, 17].

Previous reports have shown that TSH levels maybe decreased in some women with otherwise healthy thyroid glands in the first trimester. Approximately 10% of women have TSH levels below normal and up to 10% of women have suppressed levels of TSH [18]. However, the lower TSH level in the first trimester mirrors the highest level of hCG and a significant negative correlation between these levels has been reported [19]. The hCG and TSH molecules share similarities as do the hCG and TSH receptors. Consequently, hCG weakly stimulates the thyroid gland. The TSH receptor stimulation depends on the amplitude and duration of the hCG peak which may induce gestational hyperthyroidism that occurs in 2-3% of pregnancies [9]. During pregnancy, TSH level increases and reach the highest value in the third trimester, irrespective of iodine supply [20 - 23]. In mild iodine deficiency, lower levels of fT_4 and free triiodothyronine (fT_3) and higher levels of TSH, thyroglobulin and TBG were observed in the second and third trimester of pregnancy compared with the first trimester [19].Total thyroxin slightly increased in the first trimester and decreased by approximately 30% to low normal values in the second and third trimester [24].

Although Ojule and Osotimehin [25, 26] and Akanji *et al.* [27] have reported maternal iodine and thyroid status in Ibadan, none addressed specifically, the status of first trimester pregnant sub-population. More so, the finding by Pop *et al.*[28] that neurological impairment of the foetus is most critical at 12weeks gestation and that treatment may be ineffective if given after this period, it becomes imperative to understand the interplay between UIC and thyroid hormones status during the first trimester pregnancy

Materials and methods

The study group consists of 42 consecutive apparently healthy pregnant women aged between 20-40years with gestational age of 10-12weeks. They were recruited in a consecutive manner at St-Gregory Specialist Ultrasound Clinic, Ibadan. Pregnant women with previous history of thyroid diseases or any IDD and those on any medication that could affect iodine and thyroid hormones homeostasis were excluded from the study. Short structured questionnaire was administered on each participant to obtain information on education, socio economic background, medication use, dietary intake and awareness about universal salt iodization. Informed consent was obtained from each participant and study approved by the University of Ibadan/University College Hospital Joint Ethics Review Committee.

Three millilitres of venous blood samples were collected from each participant into bottles without anticoagulant. The samples were allowed to clot and retract then centrifuged at 3000rpm for 5min to obtain the serum. Similarly, 10ml of spot urine samples were collected from each participant into clean universal bottles and cooking salt samples being used at home were collected for salt iodine analysis from the participants. The sera and urine samples were stored frozen at 20°C until the day of analysis.

Determination of Urinary Iodine Concentration

Urinary iodine concentration was determined using ammonium persulphate method as described by Dunn *et al.*[29]. After an initial heating of samples at 100°C for 60 minutes, iodide was measured by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerrous ion (Ce^{3+}) coupled to the oxidation of arsenite (As^{3+}) to (As^{5+}). The absorbance was measured at a wavelength of 420 nm using a spectrophotometer.

Determination of serum TSH

The Ultra-TSH EIAgen assay was based on the one-step immune-enzymatic sandwich principle, in conjunction with the Biotin-Streptavidin technology using enzyme immunoassay test kit obtained from Adaltis Italia S.P.A., Italy and following the manufacturer's instruction.. In this method, two monoclonal anti-TSHs of high affinity and specificities were used: one was labelled with horseradish peroxidase (HRP) and the other with biotin while the microplate wells were coated with streptavidin. Samples, calibrators and controls were dispensed into the wells, followed by the mixture of the two labelled anti-TSH. During the incubation, the two monoclonal antibodies bind the TSH molecule to two different and specific sites and at the same time, the streptavidin immobilizes the forming immunological sandwich to the wells through the binding to the biotin moiety of the biotinilated antibody. After washing to eliminate the un-reacted species, the mixture of chromogen/substrate was added. The reaction was then blocked by adding the stop solution and the developed colour was measured photometrically at wavelength of 450 nm. The intensity of the colour was directly proportional, within the working range of the assay, to the concentration of TSH in the sample. The concentration of TSH in a participant's sample or control was then determined by the interpolation on the calibration curve.

Determination of serum fT4

The fT_4 was measured using enzyme immunoassay test kit obtained from Adaltis Italia S.P.A., Italy and following the manufacturer's instruction. The method is based on a solid phase competitive enzyme immunoassay. Serum samples, standards and thyroxin-enzyme conjugate working reagent were added to wells coated with monoclonal T_4 antibody, fT_4 in the specimen and the T_4 labelled conjugate compete for available binding sites on the antibody. After washing to remove unbound T_4 conjugate, the substrate solution was added resulting in a colour development. The colour development was stopped with the addition of the stop solution and absorbance read spectrophotometrically at 450 nm. The intensity of the colour formed was proportional to the amount of enzyme present and was inversely related to the amount of un-labelled fT_4 in the sample. The concentrations of fT_4 in unknown samples were quantified by reference to a series of fT_4 standards assayed in the same way.

Determination of salt iodine levels

The rapid test kit is a stabilized starch-based solution from UNICEF for the qualitative determination of iodine in salt. In this method, iodine (if present in

salt) react with starch in the solution to give a blue/purple colouration.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences, version 20.0. Values were reported as mean ± standard deviation (SD), median and range. Pearson's correlation was used to establish the relationship between the variables and Chi-square test was used to estimate the statistical difference in the level of awareness of Universal Salt Iodisation (USI) and the levels of education.

Results

The mean, standard deviation (SD), median and range of age, gestational age, serum TSH, fT4 and urinary iodine (UI) are shown in table (1). Table 2 shows the relationship between the level of education and knowledge of universal salt iodization. In this table, 32 women had tertiary education while 9 women and 1 woman had secondary and primary education respectively. Despite the increasing awareness about USI, only 12 women (28.57%) were aware of USI regardless of the educational background. In table [3], the mean, percentage and frequency distribution of the study group sub-classification according to the reference values for the measured parameters are depicted. Using WHO

Table 1: Characteristics of the studied pregnant women

Characteristics	Mean (SD)	Median	Range
Age (years)	30.00 (5.22)	30.00	20.00 – 40.00
Gestational age (weeks)	11.43 (0.83)	12.00	10.00 – 12.00
TSH (mIU/L)	1.83 (1.55)	1.40	0.20 – 8.00
fT4 (pmol/L)	11.35 (0.24)	10.32	6.45 – 20.64
UI excretion (µg/dL)	191.20 (63.00)	197.50	80.00 – 300.00

Table 2: Educational Status and Knowledge about Universal Salt Iodisation (USI)

Level of Education	Knowledge of USI n (%)	No knowledge of USI n (%)	Total n (%)
Primary	0 (0.00)	1 (2.38)	1 (2.38)
Secondary	1 (2.38)	8 (19.05)	9 (21.43)
Tertiary	11 (26.19)	21 (50.00)	32 (76.19)
Total	12 (28.57)	30 (71.43)	42 (100)

X^2 = 2.273; p-value = 0.321 and df = 2; n = number; % = percentage

Table 3: Sub-classification of the pregnant women based on reference interval

	Classification	Reference Interval	n (%)	mean (SD)
UI (µg/L)	Mild deficiency	50 – 99	4 (9.5)	88.75 (6.30)
	Adequate*	100 – 199	17 (40.5)	148.75 (29.50)
	More than adequate	200 – 299	20 (47.6)	242.20 (24.90)
	Excess	≥300	1 (2.4)	300.00 (0.00)
TSH (mIU/L)	Hyperthyroidism	<0.3	1 (2.4)	0.20 (0.00)
	Euthyroidism**	0.3 – 4.5	39 (92.9)	1.60 (0.98)
	Hypothyroidism	>4.5	2 (4.8)	7.10 (0.27)
fT_4 (pmol/L)	Low	<9.0	3 (8.6)	6.84 (0.06)
	Normal**	9.0 – 25.7	32 (91.4)	11.87 (0.23)
	High	>25.7	0 (0.0)	0.00 (0.00)

**Reference interval for the general population [30] **Reference interval for first trimester pregnancy [31]*

conventional reference interval, the sub-classification of the women revealed that none of the women had severe iodine deficiency. Thirty-nine women (92.9%) had euthyroidism while 2 women (4.8%) and 1 woman (2.4%) had hypothyroidism and hyperthyroidism respectively. An inverse relationship was observed when fT4 was correlated with TSH and UI. The correlation between fT4 and TSH was significant (Table 4).

Table 4: Pearson Correlation between fT4, TSH and UI

	FT_4 (ng/dL) r-value	p-value
TSH (mIU/L)	0.42*	0.012
UI (µg/L)	0.18	0.300

*significant at $p<0.05$

Discussion

The validity of UIC as an indicator of dietary intake is dependent on the relationship between dietary iodine intake and urinary excretion [13]. Using the conventional World Health Organization (WHO) reference criteria, our study showed that as much as 40.5% of the participants had adequate iodine intake between 100 - 199 µg/L [30]. This could be a reflection of the aggressive campaign on salt iodization by various Health Institutions in Nigeria. Likewise, it could be a pseudo-UIC since the conventional WHO reference criteria did not account for the commonly observed increased ioduria which occurs during early pregnancy as a result of increased GFR which could hitherto overestimate iodine nutrition. Similar concern was raised by Stilwell *et al.* [13].

Similarly, our findings showed that 47.6% of the participants had more than adequate iodine nutrition while 2.4% had excess iodine intake. Thus, implying that about 50% of the studied participants had iodine nutritional value greater than 200µg/L. This observation requires attention in order to prevent iodine induced hyperthyroidism as experienced in Tasmania [32], Zimbabwe [33] and Zaire [34]. Therefore, imminent solutions of regular assessment of nutritional iodine and effective quality control of salt iodization are required.

Furthermore, the observed median urinary iodine value for the entire study population is in line with the WHO criteria for median urinary iodine concentration which should be greater than 100 µg/L in 'iodine sufficient' population [30]. Although, National Health and Nutrition Studies in the United States between 1971 and 2002 put forth inconsistent reports on UIC median levels. Their reports showed that median urinary iodine excretion increased in pregnancy when compared with non-pregnant women of reproductive age[35-37]. Therefore, the absence of clearly defined reference intervals for iodine excretion in pregnancy underscores the urgent need for appropriate and specific UIC ranges in pregnancy.

Surprisingly, educational status did not affect the UIC. The result shows that 50% of the studied women with adequate UI Chad no knowledge of USI despite having attended a tertiary institution. This is a good success for USI campaign which has been in place in Nigeria and strongly supported by legislation since 1995. The result implies that majority of Nigerians consume table salt without even considering whether they are fortified or not. Thus signifying that eradication of IDDs is achievable if 100% of USI can be implemented. This observation further shows that government does not need to fund awareness campaign any longer but instead, divert the funds to USI implementation which, as shown in this study, could be more cost effective.

Equally of interest, in this study, is the finding that 92.9% and 91.4% of the studied women had normal levels of TSH and fT_4 respectively showing that thyroid homeostasis was maintained despite the various physiological changes in pregnancy. This could be due to adequate dietary iodine intake found in the pregnant women as revealed by the UIC. Surprisingly, one woman had normal value of fT_4 (1.1ng/dL) with a mildly depressed TSH (0.2mIU/L) in the presence of normal UIC (160µg/L). This might be due to transient fall in serum TSH near the end of the first trimester in normal pregnancy in association with elevated circulating hCG [38]. The hCG weakly stimulates the thyroid gland thus inducing gestational hyperthyroidism which occurs in 2-3% of the pregnancies [9]. The overt hypothyroidism observed in 2 women despite adequate urinary iodine excretion, could be due to underlying factors such as antibodies to thyroid tissues and goitrogenic factors which were not measured in this study. The significant negative correlation observed between fT_4 and TSH is in accordance with previous reports [39, 40]. This is due to the usual negative feedback mechanism.

Conclusion

Iodine deficiency poses reproductive risks such as hypothyroidism, infertility, gestational hypertension, increased first trimester abortions and still births. Although the studied pregnant women in Ibadan had adequate dietary iodine intake, screening for overt

as well as subclinical hypothyroidism during pregnancy should still, be strongly considered.

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