

Salt iodization and thyroid function of pregnant women in early pregnancy in Owerri-southeast Nigeria

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Studies have shown that one of the greatest challenges in the global fight to eliminate IDD is ensuring sustainability of effective iodization programs. Regular assessment of iodine status of the most vulnerable population and monitoring the progress of salt iodization programs are cornerstones of IDD control strategy. This study measured the iodine content of common table salts sold to the population studied and assessed the thyroid activity and urinary iodine concentration of pregnant women in their first trimester of Pregnancy. The results of the iodine content of the common table salts showed that all the salt sampled from the markets and shops contained iodine at concentration range of 12.7 ± 0.60 mg/kg – 74.1 ± 4.00 mg/kg. Majority of common salts sampled met the required iodine content. Biochemical assessment showed that the median serum concentrations of TSH, T4, T3 and Urinary iodine excretion were 1.35 μ IU/ml, 10.45 μ g/dl, 189.6ng/dl and 175.6 μ g/l respectively. These values indicated optimal iodine nutrition and healthy thyroid status.

Keywords: Thyroid function, thyroid hormones, urinary iodine, common salt, hypothyroidism, iodine deficiency, hyperthyroidism, first trimester.

INTRODUCTION

The first trimester of pregnancy should be under specific and particular medical care, due to fetal physiological demand, which involves physical, mental and brain developments. The evaluation of thyroid functions during pregnancy is of great importance to prevent the fetal IDD (LeBeau and Mandel, 2006; Springer et al., 2009; Hallengren et al., 2009). The consequence of iodine deficiency during pregnancy is impaired synthesis of thyroid hormones by the mother and the fetus. An insufficient supply of thyroid hormones to the developing brain may result in mental retardation (Morreale et al., 2004; Auso et al., 2004; Koibuchi and Chin, 2000; Delange, 2000). It has been established that, manifestation of iodine deficiency disorders (IDD) in fetuses could be as a result of low thyroxine level in the

blood of the iodine deficient mother. The lower the level of thyroid hormone of the pregnant women, the greater the threat to fetal development (WHO, 1996).

The main consequence of maternal iodine deficiency is fetal and neonatal hypothyroidism (Liesenkotter et al., 1996). Such damage can be prevented by maternal iodine supplementation, if initiated before conception or early within the first trimester. The fetal thyroid gland commences the active secretion of thyroid hormone at about 18 weeks of gestation (Contempre et al., 1993; Iskaros et al., 2000). Maternal T4 is however present in the coelomic fluid from 6 weeks (Contempre et al., 1993) and in fetal brain at 9 weeks of gestation (Bernal and Pekonen, 1984; Ferreiro et al., 1988; Costa et al., 1991).

Salt iodization is one of the strategies used in delivering iodine in the study population. Though, the importance of salt iodization for the eradication of IDD is undisputed (Pandav, 2000) the monitoring of salt iodine content aids in detecting adequately and inadequately iodized and

uniodized common salts. It also identifies the quality of salt production level in a given population (Vander, 2002). In monitoring salt iodization, one of the objectives is to ensure that the community consumes adequately iodized salt (WHO et al., 1994). It is noteworthy that despite the remarkable progress in the control of iodine deficiency disorders (IDD), they remain a significant proportion global public health problem. The present study was undertaken to assess the impact of the continued universal salt iodization program in southeast Nigeria and the biochemical effect on the thyroid function of women in early pregnancy.

EXPERIMENTAL DESIGN

Common Salt Sample Collection

The common salt samples used in performing these tests were collected in different markets and shops in the sample areas. The samples were packaged common salt not exposed to direct sunlight, packaged common salt exposed to direct sunlight, unpackaged common salt samples and unbranded salts. The manufacturers of the branded salts sampled are; Annapurna, Royal Salt, Dangote, Uncle Palm, and Mr. Chef. The markets in which the samples were collected are; old market- Douglas Road, Owerri, New market- Douglas Road Owerri, Umuokochi market- Nekede Owerri. Ihiagwa market, Ihiagwa Owerri Umuokoto market, Nekede, Owerri, all in Imo State Nigeria

Serum and Urine Collection

This was a hospital based study conducted amongst pregnant women attending antenatal clinics of Federal Medical Center Owerri, Imo state, Nigeria. Eighty-four pregnant women, with mean age of 29.4 years, were involved in the study. Eighty-four urine and thirty-two blood samples were obtained from the women after seeking their consent and the approval of the hospital ethical committee. The pregnant women were clinically euthyroid and were not on thyroid medication. Other selection criteria were the absence of chronic disease such as thyroid disease, diabetes mellitus, anemia, hypertension and coronary artery. Early morning urine samples and fasting blood samples were obtained from the women for the determination of urinary iodine excretion (UIE), triiodothyronine (T3), tetraiodothyronine (T4), and thyroid stimulating hormone (TSH). All blood samples were collected by experienced medical laboratory scientists using conventional venipuncture. The blood samples were allowed to clot before separation by centrifugation at 3000 g for 15 min. All the serum samples were stored frozen until testing, at the

end of the collection period.

Determination of Iodine content in Common Salt

This was carried out by carefully weighing 10 g of the common salt sample into a 250 ml conical flask with a stopper. Approximately 30 ml of distilled water was added, and then swirled to dissolve the salt sample. Then 20 ml of distilled water was added to make up the volume to 50ml. A pipette was used to deliver 1.0 ml of 1M H_2SO_4 into the salt solution. Then 5.0 ml of 10 % potassium iodide (KI) was added to the salt solution and the solution turned yellow. This indicated the presence of iodine. The flask was then covered with a stopper and was put inside a dark cupboard for 10 mins. The burette was rinsed and filled with 0.005 M $\text{Na}_2\text{S}_2\text{O}_3$ (Sodium thiosulphate solution), then the level in the burette was adjusted to Zero. After 10 minutes, the flask was removed from the dark cupboard and some $\text{Na}_2\text{S}_2\text{O}_3$ from the titration burette was added slowly until the solution turned pale yellow. Then, 2.0 ml of starch solution was added and the solution turned dark purple. Titration was continued until the solution became pink and finally colourless. The level of thiosulphate in the burette was recorded and converted to parts per million using the conversion table

Measurement of Serum Thyroid Hormones

The Micro-well TSH, T4 and T3 enzyme immunoassay (EIA) was used. Serum TSH, T4 and T3 concentration was measured by the enzyme-linked immunosorbent assay commercial kits (Syntron Bioresearch, Inc. Carlsbad, CA - USA). The normal range of TSH, T4 and T3 concentrations determined with this kit were 0.5 - 4.10 $\mu\text{IU/ml}$, 5.0 - 13.0 ug/dl and 70 - 184 ng/dl respectively. The micro-well EIA is a solid-phase enzyme immunoassay based on the "sandwich" principle. Two separate antibodies directed against distinct antigenic determinants of the thyroid hormone molecule were utilized in the assay. The thyroid hormone present in the test sample reacts simultaneously with one antibody immobilized on the micro-well surface and with another antibody conjugated to horseradish peroxidase enzyme. So an Ab-Ag-Ab-Enzyme complex is formed on the micro-well surface.

Determination of urinary iodine excretion

The iodine in the urine is measured by a modification of the traditional colorimetric method of (Sandell and Kolthoff, 1937). This was done using the Ammonium Persulfate Method as described by (Pino et al., 1996).

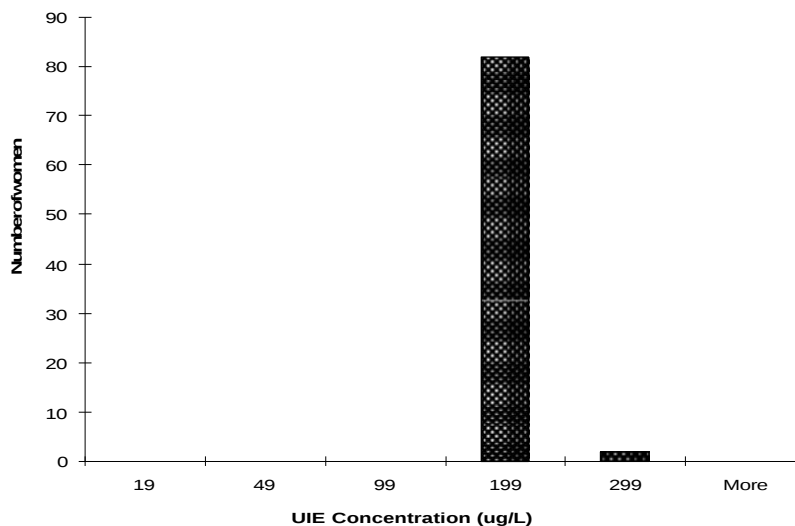


Figure 1: Concentration of Urinary Iodine Excretion in pregnant woman (n=82)

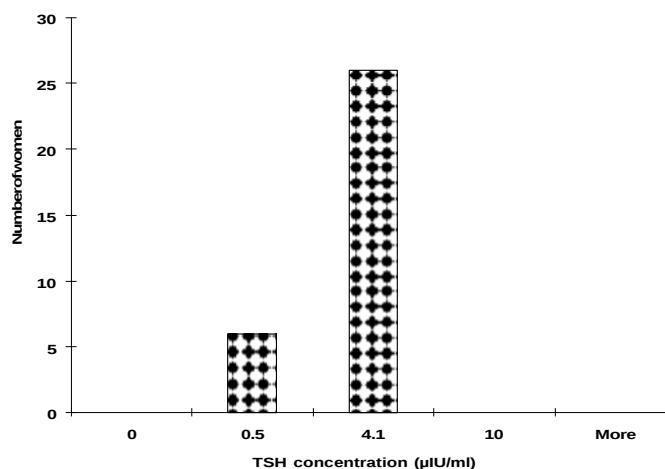


Figure 2: TSH Concentration in the serum of pregnant woman (n=32)

Urine was digested with ammonium persulphate. The iodine in the urine samples catalyses the reduction of ceric ammonium sulphate (yellow colour) to the cerous form (colourless) in the presence of arsenious acid. The degree of reduction in colour intensity of the yellow ceric ammonium sulphate is proportional to the iodine content in the urine sample. This method was applied to all the urine samples.

Statistical analysis

The data obtained were subjected to statistical analysis using the statistical software package, Statistical Analysis Software (SAS). The mean, median, range and regression of the data were determined.

RESULTS

Figure 1 shows the result of Urinary Iodine Excretion (UIE) of 82 pregnant women in their first trimester. The result showed that none of the women had severe ($< 20 \mu\text{g/L}$) or moderate ($20\text{--}49 \mu\text{g/L}$) or mild ($50\text{--}99 \mu\text{g/L}$) iodine deficiency, 98 % had optimal ($100\text{--}199 \mu\text{g/L}$) iodine nutrition and 2 % had more than adequate ($200\text{--}299 \mu\text{g/L}$). From the result, the mean and median UIE values were $172.38 \pm 18.66 \mu\text{g/L}$ and $175.6 \mu\text{g/L}$ respectively. The values of the UIE ranged from $125.6 \mu\text{g/L}$ to $200.6 \mu\text{g/L}$.

Figure 2 shows the result of concentration of Thyroid Stimulating Hormone (TSH) of 32 pregnant women in their first trimester of pregnancy. The result showed that 19% of the pregnant women had TSH values between

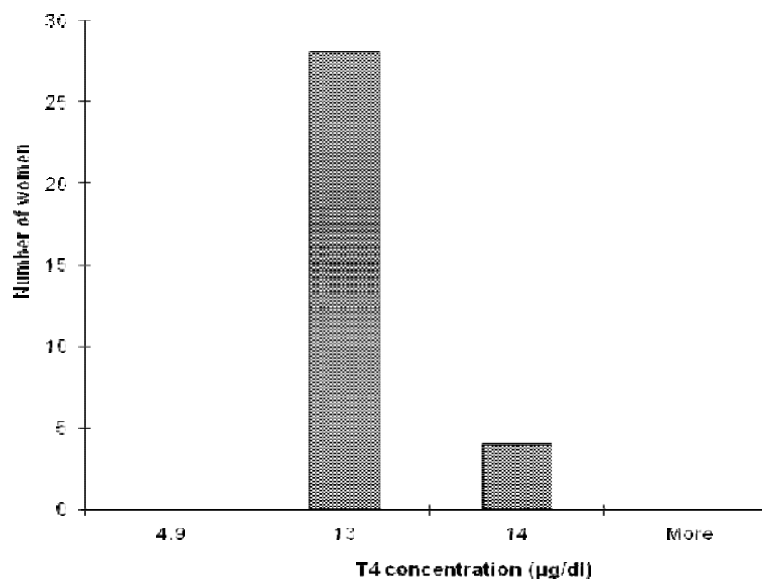


Figure 3: T4 Concentration in the serum of pregnant woman (n=32)

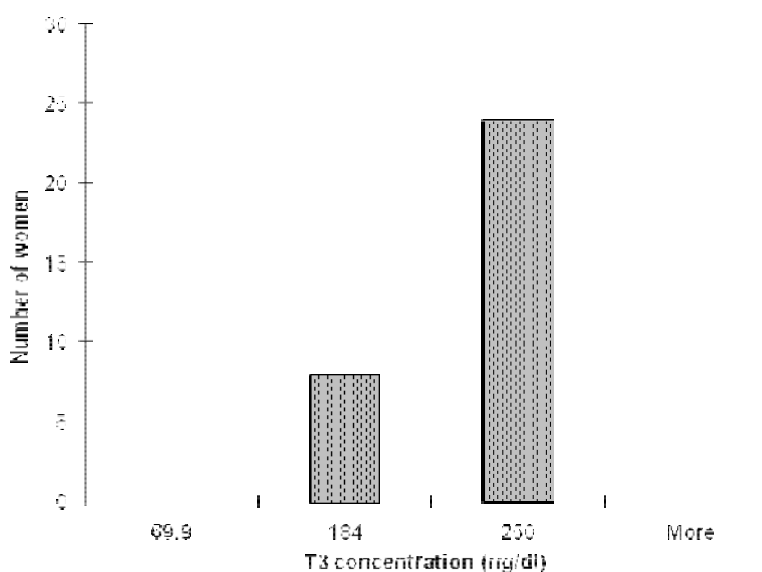


Figure 4: T3 Concentration in the serum of pregnant woman (n=32)

0.0-0.5 µIU/mL (Hyperthyroid status) while 81% of the pregnant women had TSH level between 0.6 – 4.10 µIU/mL (normal thyroid status). The result also showed that none of the pregnant women had TSH value > 4.10 µIU/mL (Hypothyroid condition). From the result, the range, mean and median TSH were 0.2 µIU/mL to 3.2 µIU/mL, 1.48 ± 0.97 µIU/mL and 1.35 µIU/mL respectively.

Figure 3 shows the result of enzyme immunoassay of thyroxine hormone (T4) of 32 pregnant women in their first trimester of pregnancy. From the result none of the women had T4 level between 0.0 – 4.9 µg/dL (below

normal thyroid status). The result also showed that 88 % of the pregnant women had T4 values between 5.0-13.0 µg/dL (normal thyroid status). Also 12% of the pregnant women had T4 values > 13.0 µg/dL (above normal). From the result, the range, mean and median T4 are 7.4 µg/dL to 13.8 µg/dL, 110.36 ± 1.77 µg/dL and 10.45 µg/dL respectively.

Figure 4 shows the result of the quantitative determination of Triiodothyronine (T3) of 32 pregnant women in first trimester of pregnancy. The result showed that none of the pregnant women had T3 values < 70

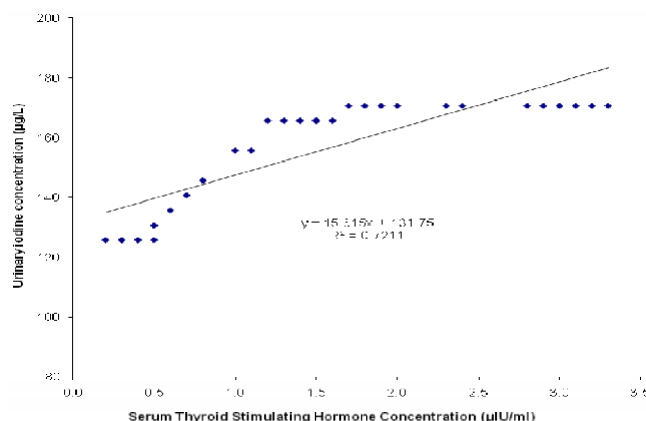


Figure 5: Relationship between urine iodine Concentration and serum TSHC Concentration (n=32)

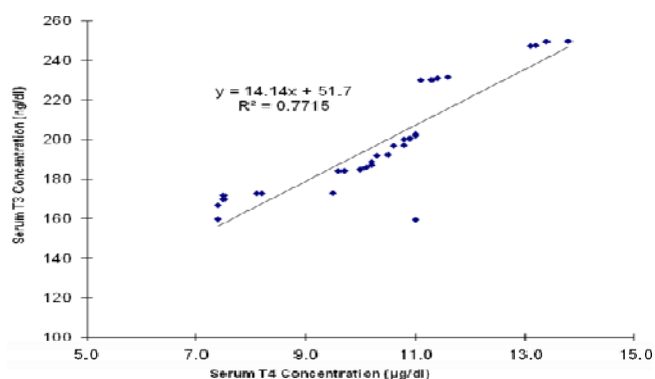


Figure 6: Relationship between serum T3 Concentration and T4 Concentration

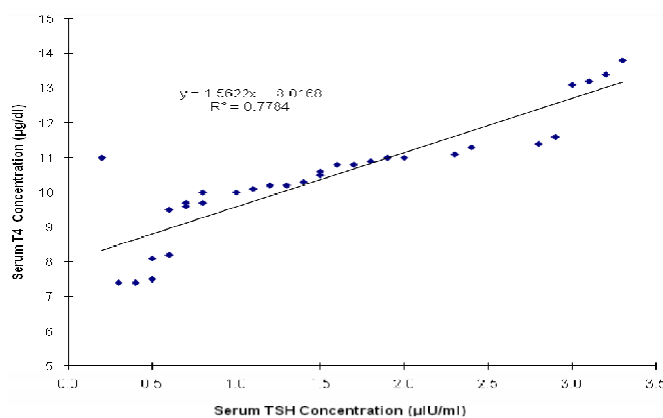


Figure 7: Relationship between serum T4 and TSH Concentration

ng/dl (below normal), while 25% of the pregnant women had T3 level between 70 – 184 ng/dl (normal thyroid status). The result also showed that 75% of the pregnant women have T3 value > 184 ng/dl (above normal). The mean and median T3 values were 197.7 ± 28.00 ng/dl

and 189.6 ng/dl respectively and the T3 values ranged from 159.7 ng/dl to 249.6 ng/dl

Figures 5 - 7 show the relationships between the parameter (TSH, T4, T3 and UIE). The results showed that there are direct relationship between UIE and TSH

Table 1: Iodine concentration of packaged and properly stored common salts

Samples	Name of Product	Month of Manufacture	Number sampled (n)	Iodine (mg/kg)
1	AP	May	8	38.1±0.70
2	AP	June	8	46.6±3.76
3	AP	January	8	36.1±2.46
4	AP	July	8	46.6±1.80
5	DG	March	8	57.1±3.95
6	DG	January	8	54.0±1.20
7	DG	May	8	57.1±3.10
8	DG	August	8	54.0±3.80
9	MC	August	8	44.4±3.80
10	MC	February	8	44.4±3.80
11	MC	July	8	74.1±4.00
12	UP	December	8	22.2±2.85

Table 2: Iodine concentration from packaged salts exposed to sunlight

Samples	Name of Product	Month of Manufacture	Number sampled (n)	Iodine (mg/kg)
1	AP	May	8	31.7±1.7
2	AP	June	8	32.8±1.4
3	AP	January	8	19.0±1.2
4	AP	July	8	33.9±2.4
5	DG	March	8	24.3±1.2
6	DG	January	8	21.1±0.9
7	DG	May	8	28.6±1.2
8	DG	August	8	38.1±2.1
9	MC	February	8	12.7±1.8
10	MC	July	8	49.7±2.9
11	MC	August	8	15.9±2.0
12	UP	January	8	15.9±2.0

($r^2 = 0.7211$), T3 and T4 ($r^2 = 0.7715$) and T4 and TSH ($r^2 = 0.7784$).

Table 1 shows the results of iodine concentration of packaged and properly stored common salts sold in Owerri. The result showed that the iodine contents of the salt samples ranged from 22.2±2.85 mg/kg in Uncle Palm's salt manufactured in December 2007 to 74.1±4.00 mg/kg in Mr. Chef salt manufactured in July 2008. The results also showed that apart from Uncle Palm's salt other common salts; Annapurna, Dangote, Uncle Palm, and Mr. Chef, sampled had iodine concentration above 30 mg/kg. The result also showed that these common salts were manufactured in 2008.

Table 2 shows the results of the iodine concentration from packaged salts exposed to sunlight. The result showed that the iodine contents of the salt samples ranged from 12.7±1.8 mg/kg in Mr. Chef Salt manufactured in February, 2008 to 49.7±2.9 mg/kg in Mr. Chef Salt manufactured in July 2008. The results also

showed that 64% of the sampled salts had iodine concentration less than 30 mg/kg

Table 3 shows the results of the iodine concentration from unpackaged salts exposed to sunlight. The result showed that the iodine contents of the common salt sampled, ranged from 12.7±0.60 mg/kg in Royal Salt manufactured in January 2008 to 33.9±2.18 mg/kg in Royal salt manufactured in September 2008. The results also showed that 71% of the sampled salts had iodine concentration less than 30 mg/kg.

DISCUSSION

The UIE values obtained from the pregnant women showed none of the women had severe, moderate or mild iodine deficiency, suggesting that all obtained iodine in their diets. Previous studies have shown urinary iodine concentrations as highly sensitive to recent changes in

Table 3: Iodine concentration from unpackaged salts exposed to sunlight

Samples	Name of Product	Month of Manufacture	Number sampled (n)	iodine (mg/kg)
1	DG	August	8	19.0±0.60
2	DG	January	8	25.4±1.55
3	DG	May	8	25.4±2.65
4	DG	July	8	19.0±1.85
5	GD	January	8	29.4±0.55
6	PM	July	8	27.5±2.55
7	RY	April	8	28.6±2.25
8	RY	January	8	12.7±0.60
9	RY	June	8	28.6±1.85
10	RY	September	8	33.9±2.18
11	UB	January	8	26.5±0.40
12	UD	January	8	22.2±2.35

iodine intake (WHO et al., 2001), while changes in thyroid status lag behind changes in urinary iodine concentration (Zimmermann et al., 2003). This result corroborates the values obtained from the iodine content of common table salts sold in the area under study. The results of the iodine content of the common table salts showed that all the salt sampled from the markets and shops contained iodine, though in varying concentrations. Majority of properly packaged common salts sampled met the required iodine content of 30mg/kg at the point of retailing (WHO et al., 2001; SON; Egbuta and Onyezili., 2002; Vander, 2002). This finding reinforces the report of UNICEF MICs Survey which indicated that 98% of Nigerian households consumed adequately iodized salt (UNICEF, 2001).

The values of the UIE obtained indicated that the pregnant women had other sources of iodine apart from the table salt. This is supported by the earlier report of (Ujowundu et al., 2011) on the presence iodine in some of the plant foods consumed in the area under study. This population consumes lots of fresh vegetables harvested from nearby farms and brought to the markets on daily basis. This assumption of the contribution of supplementary sources of dietary iodine to the pregnant women was based on the UIE value which showed that none were within the deficiency status range, despite the presence of iodine below the recommended concentration in some of the common salts. Iodine concentration in water, soil and plants reflects the environmental iodine distribution, and is also an important index of human natural iodine intake (Ying-li et al., 2005). Naturally, the iodine content of foods and total diets differ and is heavily influenced by the presence of iodine in the soil (Koutras et al., 1985; WHO, 1996; Souci et al., 2000).

Our results showed that the low iodine content was due to poor storage, especially exposure to direct sunlight and improper packaging. Report has shown that iodine volatilizes when poorly packaged and exposed. The

concentrations of iodine in the unpackaged and exposed salts suggest that, not packaging and exposing iodized salts to direct sunlight makes useless, the efforts and resources put in iodizing common salts. This may also endangers the health of the end users who are unaware that the common salt consumed lacks the adequate amount of iodine required to maintain proper thyroid function (Hetzl, 1983; WHO et al., 2001). The values of iodine concentrations in the iodized salts samples indicate that the concentration of the iodine in common salts was time dependent. This assertion is drawn from our results which showed higher iodine concentration in the common salts recently manufactured prior to the period of analyses. It could also result from inconsistency by salt manufacturers in maintaining standard iodine concentration in each batch of salt manufactured.

The high iodine content of some of the common salts sampled may also contribute to iodine toxicity, which has adverse consequences for proper thyroid function (Roti and Vagenakis, 1996). It has also been reported that disturbed thyroid gland activity results from excessive iodine intake may manifest either as goitre or hypothyroidism with/without goitre or as hyperthyroidism (Joint FAO/WHO, 1989; Cavalieri, 1997). While greater excessive intake inhibits the formation of iodinated tyrosine, lowers the T4 and T3 plasma levels and raises the plasma TSH (Wolff-Charkoff iodide effect) (Braverman, 1991; Expert Group on Vitamins and Minerals (EGVM), 2000).

Studies have shown that iodine deficiency markedly alters thyroid metabolism (Delange, 2000), and thyroid hormones are especially important for adequate fetal, neonatal central nervous system development and enzyme system activities (Rosychuk, 1982; Plumb, 1999). Our study measured the consequences of inadequate iodine intake by estimating the serum TSH, T4 and T3 concentrations. Iodine deficiency disorders arise from a depletion of the thyroid iodine stores of the

body with consequent fall in daily T4 and T3 production and their plasma levels, which trigger an increased secretion of TSH and hyperactivity of the thyroid gland coupled with thyroid epithelial cell hyperplasia (WHO, 1996). The mean and median urinary iodine concentrations from our study suggest that, the population under study consumes adequate iodine, though the values were below the RDA of 200-250 µg/day for pregnant and lactating women (WHO et al., 2001). The higher RDA for iodine during pregnancy is as a result of increased glomerular filtration rate. The increased renal blood flow, alter the normal clearance of renal iodide to be increased. Therefore pregnancy demands additional iodine in the diet to protect the maternal and also to provide the iodine required by fetus thyroid, which become gradually active at the end of first trimester of pregnancy. Also, studies have shown that maternal iodide can cross the placental barrier to assist in building the thyroxine pool in fetal circulation during pregnancy (Dussault et al., 1977).

One of the initial biochemical signs of poor iodine nutrition is below normal serum TSH concentration (Smith and Bold, 2005). In our results the values of TSH showed 81% with normal thyroid status, this indicates that most of the women consume adequate amount of iodine needed for optimal thyroid function. Previous reports have shown that under optimal iodine presence, the thyroid releasing hormone (TRH) secreted by the hypothalamus, stimulates the production of TSH, which leads to the synthesis of thyroid hormones (Burrow et al., 1994; Fathzadeh et al., 2005). The percentage of the women with normal TSH value corroborating the values obtained in the UIE which showed that 98% of the women had optimal iodine nutrition. Figure 5 showed that, there was a positive correlation between UIE and TSH ($r^2 = 0.7211$). Also, the TSH values tallies with the result obtained from the measure of common table salts which showed that all the common salt sampled were iodized. Equally intriguing in our result is that 19% of the women showed hyperthyroid status. Considering the values of UIE and concentration of the iodine in salts sampled, the hyperthyroid activity observed in the few women could be as a result of other metabolic and physiological consequences associated with pregnancy. Similarly TSH values showed that none of the women are in the hypothyroid range, indicating that iodine was constantly supplied, resulting in the maintenance of normal concentration of serum T3 and T4 which triggers a feedback via TRH to maintain homeostasis in the human system (Burrow et al., 1989).

Results from the estimation of T4 showed that 88 % of the pregnant women had normal T4 values. This result supports the values obtained for T3, suggesting that greater percentage of the T4 may be converted to T3, and could have resulted in high percentage of the pregnant women having T3 values above normal. Human

studies also indicated that thyroid hormones (especially T4) can cross placenta in appreciable quantities (de Escobar, 1988; Braverman, 1988), whereas TSH cannot (Braverman, 1988; Das and Isichei, 1994). The result from the T3 assay showed that 75% of the pregnant women had T3 values above normal. Metabolically, most T3 are formed outside the thyroid gland by deiodination of T4 (Greco and Stabenfeldt, 1997; Braverman and Utiger, 2000). Our result from the T3 assay suggests that large amount of T4 was converted to T3 and figure 6 shows a positive correlation between T3 and T4 ($r^2 = 0.7715$). T3 is considered the most important thyroid hormone at the cellular level, because it is about three to five times more potent than T4 (Ferguson, 1986). The large percentage of the women with above normal T3 values could also be due to the elevated concentration of estrogen which occurs during normal pregnancy. This leads to increase in the liver serum level of Thyroxine Binding Globulin (TBG). The consequence of increasing amount of TBG, leads to elevated concentration of thyroid hormones of T3 (Chen and Jhon, 2002; Idris et al., 2005; Kooistra et al., 2006). This assertion is supported by results obtained from this study, which showed that greater percentage of the women had optimal iodine nutrition and thyroid function assay showed normal thyroid status.

The values of iodine content in common salts, urinary iodine excretion and thyroid hormone activity have shown that, there has been remarkable progress in the control of iodine deficiency disorders through the iodization of salt in Nigeria. This assertion is supported by results obtained from this study which showed that greater percentage of the women have optimal iodine nutrition and thyroid function assay showed normal thyroid activity. Since iodized salt is considered as the most appropriate measure for iodine supplementation, Nigeria has to maintain and intensify her efforts in salt iodization and utilization programs.

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