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Iodine Intake and Stimulation of Thyroid Related Hormones During First Trimester of Pregnancy

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Present study was planned to investigate iodine intake and thyroid hormone concentration in normal pregnant women during first trimester of pregnancy in Lahore. Urinary iodine concentration, free T_4, T_3 and TSH of pregnant women were determined and compared with that of control women. Results showed that most of the control and pregnant women in this study were mild to moderate deficient in iodine intake. The median level of urinary iodine concentration was 70 and 75 $\mu g\ L^{-1}$ in pregnant and control, respectively. In all the pregnant women FT_4 level were towards lower limit of control's normal range. The mean T_3 level in pregnant women was raised as compared to control. 34.7% of pregnant women had T_3 levels above upper limit of normal range. The mean TSH level was slightly lower in pregnant women as compared to control. Eight percent of pregnant women had undetectable TSH (0.05 $mlU\ L^{-1}$). Only three women had TSH above upper limit of normal range.

Key words: Iodine, thyroid harmones, pregnancy

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INTRODUCTION

Iodine is a natural element present in the human body in minute amounts (15-20 mg). Humans need iodine to make thyroid hormones 3,5,3',5'-tetrathyronine or T_4 (thyroxine) and 3,5,3'-triiodothyronine or T_3. These hormones are produced by thyroid gland, a butterfly shaped gland in the front part of the neck. The major role of iodine in nutrition arises from the important part played by the thyroid hormones in the normal growth and development; function of the brain and nervous system and for maintenance of body heat and energy. Iodine deficient diet may cause a wide spectrum of illness collectively called iodine deficiency disorders (IDD), which affects people of all ages, but particularly pregnant women, the developing fetus and the neonate[1].

The major role of iodine in pregnancy arises from the important part played by the thyroid hormones in the development of a normal brain and neurological network of fetus. That is why the requirements of thyroid hormones increase during pregnancy. To accomplish this, complex changes occur in thyroid function parameters and iodine metabolism of mother during pregnancy. Most of the changes are stimulatory that take place during first trimester of gestation[2]. Pregnancy also affects iodine metabolism. Early in pregnancy there is an increase in renal blood flow and glomerular filtration, which leads to an increase in iodine clearance from the plasma[3-5]. When fetal thyroid gland becomes progressively functional by the end of the first trimester, there is a further increment in iodine requirement due to the trans-placental transport of iodide, which is required for iodothyronine synthesis. This results in decreased plasma iodide and an increased requirement for iodide in the diet. In women with iodine sufficiency, there is little impact of this obligatory increased iodide loss in terms of thyroid function. However, in geographical areas where the iodide supply is borderline or low, significant changes occur[3,4]. In 1993, almost one third of the World's population was affected by iodide deficiency[6]. In many areas of the World, iodine deficiency is a serious public health issue. Recommended dose of iodine by World Health Organization (WHO) for pregnant women is 200 μg day^{-1}[7].

Iodine deficiency and resulting maternal hypothyroxinemia has adverse effect on maternal health and pregnancy outcome. Hypothyroidism during pregnancy has been associated with goitrogenesis, pregnancy induced hypertension, placenta abruption, fetal distress, postpartum depression and an increased frequency of low birth weight babies. Recent findings suggest that adequate functioning of both maternal and fetal thyroid gland plays an important role to ensure that the fetal neurophyco-intellectual development progresses normally[6]. Pakistan is an iodine deficient country with 70% population affected by or at the risk of IDD[9]. The quantification of the extent of IDD in pregnant women and the effects of its deficiency on maternal and neonatal health are yet to be elucidated in Pakistan. Keeping these facts in view, this cross-sectional study was planned to investigate iodine intake and thyroid hormone concentration in pregnant women during first trimester of pregnancy.

MATERIALS AND METHODS

Study samples: Seventy-five pregnant women, who were in their first trimester of pregnancy, were enrolled from the Department of Obstetrics and Gyneocology, Government Mian Munshi Hospital. All the participating women were belonging to area surrounding the hospital. Blood and urine samples of these patients were collected in laboratory during March 2002 to September 2002. They were all normal pregnant women; none of them had neither goiter, nor taking any thyroid medication or had thyroid surgery. Their detailed history was taken on performa designed for this purpose.

Urine samples were collected in aseptic vials. Five millilitre of blood sample was obtained in a disposable syringe. Samples were stored in a refrigerator. The serum was separated by low speed centrifugation (2000 x g) for 5 min at room temperature with a centrifuge. Serum samples were stored at -20°C until analysis. Urine samples were also centrifuged at same speed and time and 5 mL supernatants were stored with serum.

Analytical techniques: Urinary iodine was measured by the Ammonium persulphate method provided by International Council for Control of Iodine Deficiency Disorders (ICCIDD). Estimation of thyroid related hormones was carried out at Centre for Nuclear Medicine (CENUM), Mayo hospital, Lahore. FT_4 and T_3 were estimated by radioimmunoassay (RIA) based on competitive immunoanalytical technique. TSH was estimated by immunoradiometric assay (IRMA) technique using mouse monoclonal antibodies directed against to different epitopes of TSH (sandwich type assay). All assays were carried out using commercial kits of Impotency (Beckman, Czech Republic).

RIA and IRMA methods were run with commercially derived control sera at low, medium and high concentrations. All assays were carried out in duplicate. RIA and IRMA results were expressed at less than 10% CV of imprecision profile. The analysis of T_4 and TSH levels distribution was carried out using SPSS program (SPSS Inc., Chicago, IL) on a personal computer. Levels of

urinary iodine, FT_4, T_3 and TSH in pregnant women were compared with the normal ranges of these analytes in control women. Normal range of TSH levels were determined after log transformation.

Techniques

Radioimmunoassay (RIA): Radioimmunoassay is one of the important technique in the clinical and biochemical field for the quantitative analysis of hormones, steroids and drugs. The technique is based on the competition between unlabeled antigen and a finite amount of the corresponding radiolabelled antigen for a limited number of antibody binding sites in a fixed amount of antiserum. At equilibrium in the presence of an antigen excess there will be both free antigen and antigen bound to antibody. Under standard conditions the amount of labeled antigen bound to antibody will decrease as the amount of unlabeled antigen in the sample increases.

Immunoradiometric Assay (IRMA): IRMA utilized purified radiolabelled antibody to the antigen to be estimated is often more sensitive than RIA. IRMA is a direct binding assay in which the radiolabelled antibody reagent is in excess and the amount bound to the antigen is actually measured. Increased efficiency was obtained by removing the antigen to be assayed from solution with antibody against one subunit or epitope and using radiolabelled antibody with another subunit or epitope.

Spectrophotometry: Many molecules of biochemical and biological interest absorb specific types of radiant energy when light passes through the solution containing such molecules (i.e. solutes). The principle of spectrophotometer is based on Lambart's Beer's Law, which states "The degree of absorption is directly proportional to the concentration of solute as well as length of light path". In spectrophotometer, when a polychromatic light falls on a homogenous medium, at a specific wavelength a portion of light is absorbed and a portion is reflected and remainder is transmitted through the medium.

Estimation of urinary iodine: Urine was mixed to suspend sediments. Each urine sample (250 μL) was taken into test tube. Each iodine standard was pipetted out into a test tube and then added H_2O as needed to make a final volume of 250 μL. One millilitre of ammonium persulphate (1.0 %) was added to each the tube. Heated all tubes for 60 min at 100°C. Then cooled tubes to room temperature. arsenois acid solution (2.5 mL) was added, mixed and stand for 15 min. Ceric ammonium sulphate solution (300 μL) was added to each tube 5-30 sec. Allowed to sit at room temperature and then read absorbance at 420 nm.

Estimation of Free Thyroxine (FT_4): Sample (25 μL) was added with 400 μL of tracer solution followed by 100 μL of ligand solution. Total cpm (T) was obtained by 400 μL tracer solution only. Tubes were then incubated for 90 min at room temperature (18-25 °C) with mild horizontal shaking (350 rpm). Each tube was counted in gamma counter for one-minute calibration for I^{125} after aspiration. Results were obtained from standard curve by interpolation.

Total Triiodothyronine (TT_3): Standard samples (50 μL) were added to antibody-coated tubes followed by 500 μL of tracer and mixed. Tubes were incubated for 1 h at room temperature with continuous shaking at 280 oscillations per minutes. Two tubes were prepared separately, containing 500 μL of tracer for the determination of total counts; T. Liquid contents of tubes were aspirated carefully, except tubes for T measurement. Radioactivity of all the tubes was measured and counted at least 1 min in a gamma counter adjusted for I^{125}. Results were compared from the standard curve.

Estimation of Thyroid Stimulating Hormones (TSH): Two hundred microlitre of standard, control samples and 100 μL of tracer were added and shaken gently. Two tubes were prepared separately; containing 100 μL of tracer only for the determination of total count T. Tubes were incubated for 2 h with shaking (>350 rpm) at room temperature (18-25°C). The contents of tubes except of two tubes T were aspirated and washed with approximately 2 mL of wash solution. The radioactivity of all tubes determined by gamma counter. Results were obtained from the standard by interpolation.

RESULTS AND DISCUSSION

Average age of pregnant women (n=75) was 24.6±4.7 years. Average parity of these women was 3.4±1.9 pregnancies. Sixteen women (21%) were in primary gravida and 59 women (79 %) were of higher gravida. Average fetus age was 9.6±2.6 weeks, ranging from 4-13 weeks. The control women (n=54) had slightly more average age (26.3±6.7 years) but of similar parity (3.2±2.3 pregnancies). Parity ranged from 1 to 9 pregnancies in control women. The mean urinary iodine (UI) concentration level of control women was 72.8±15 μg L^{-1} (range: 28-98 μg L^{-1}). The median level of urinary iodine concentration was 75 μg L^{-1}. Four women (7.4 %) had urinary iodine level below 50 μg L^{-1}. They

Table 1: Urinary iodine concentration (μg L^{-1}) in control and pregnant women

Subjects	Number (n)	Range (median) (μg L^{-1})	Mean±SD (μg L^{-1})
Pregnant women	75	34-100 (70)	66.1±18.1
Control	54	28-98 (75)	72.8±15.0

Table 2: FT_4 concentration (pmol L^{-1}) in control and pregnant women

Subjects	Number (n)	Range (median) (μg L^{-1})	Mean±SD (μg L^{-1})
Pregnant women	75	8.6-20.9	12.4±2.4
Control	54	9.6-32.1	16.1±3.9

Table 3: T_3 concentration (nmol/L) in control and pregnant women

Subjects	Number (n)	Range (median) (μg L^{-1})	Mean±SD (μg L^{-1})
Pregnant women	75	1.2-4.3	2.3±0.7
Control	54	0.8-2.9	1.6±0.4

Table 4: TSH concentration (ml U/L) in control and pregnant women

Subjects	Number (n)	Range (median) (μg L^{-1})	Mean±SD (μg L^{-1})
Pregnant women	75	0.05-12.4	1.1±1.5
Control	54	0.1-6.1	1.5±1.0

were severe iodine deficient according to criteria of International Council of Iodine Deficiency Disorders (ICIDD). All the rest women (92.6 %) had UI levels between 50-98 μg L^{-1}. They were mild to moderate iodine deficient. None of the control women had UI above 100 μg L^{-1} (a level of iodine sufficiency). In pregnant women the mean urinary iodine level was 66.1±18.1 μg L^{-1} ranging from 34 to 100 μg L^{-1}. The median level of urinary iodine concentration was 70 μg L^{-1}. The urinary iodine levels in 17 pregnant women (22.7%) were below 50 μg L^{-1}. Fifty-seven pregnant women (76.0%) had urinary iodine levels within 50-100 μg L^{-1} range. Only one woman (1.3%) had urinary iodine level of 100 μg L^{-1}. She was the only iodine sufficient woman of this cohort (Table 1). The mean values of free T_4, T_3 and TSH in control women were 16.1±3.9 pmol L^{-1} (range: 9.6-32.1 pmol L^{-1}), 1.6±0.4 nmol L^{-1} (range: 0.8-2.9 nmol L^{-1}) and 1.5±1.0 ml IU L^{-1} (range: 0.1-6.1 ml U L^{-1}), respectively. The normal ranges of these thyroid related hormones in control women were 8.4-23.9 pmol L^{-1}, 0.8-2.47 nmol L^{-1} and 0.3-3.5 ml IU L^{-1}, respectively.

In all pregnant women FT_4 levels were within normal range of controls. Mean level of FT_4 in pregnant women was 12.4±2.4 pmol L^{-1} and ranged from 8.6 to 20.9 pmol L^{-1}. The overall trend of FT_4 in pregnant women was towards the lower limit of control's normal range. Compared to control women, the mean FT_4 level was also below in pregnant women (Table 2). The mean value of T_3 in pregnant women was 2.3±0.7 nmol L^{-1} and ranged from 1.2 to 4.3 nmol L^{-1}. Compared to control women mean T_3 value in pregnant women was raised. Twenty-six pregnant women (34.7 %) had T_3 levels above upper limit of normal range (2.4 nmol L^{-1}) (Table 3). The mean value of TSH in pregnant women was 1.1±1.5 mI IU L^{-1} and ranged from 0.05 to 12.4 ml IU L^{-1}. Compared to control women mean TSH value in pregnant women was slightly lower. Thirty-three women (44%) have TSH levels below the normal range. Six pregnant women (8%) had undetectable TSH (0.05 ml IU L^{-1}). Only three women had TSH above upper limit of normal range (Table 4).

In summary, urinary iodine intake and thyroid related hormones were affected due to pregnancy. Compared to non-pregnant women, urinary iodine intake and T_3 had increased while FT_4 and TSH were decreased in pregnant women. In normal subjects with a daily iodine intake of 150 μg/day, the thyroid clearance rate for iodide is 10-25 mL/min (average: 17 mL/min). The renal iodide clearance is 30 mL/min resulting chiefly from glomerular filtration with no evidence for tubular secretion or active transport. In pregnancy, the renal clearance of iodide increases significantly because of an increased glomerular filtration rate. This begins in the early weeks of pregnancy and persists until term. When fetal thyroid gland starts thyroid hormone production, a part of available iodine is diverted to it. Thus when iodine deprivation exists during the first trimester, it tends to become more severe in later trimesters. Present results showed that most of the control and pregnant women in this study were mild to moderate deficient in iodine intake. Although iodine deficiency is a well-known public health problem all over the world but we have not come across any study related to determination of iodine intake levels in Lahore. However, for overall country, 40% women and 72% newborns are reported iodine deficient[10]. Ours is probably the first study in Lahore related to evaluation of this problem in Lahore. Present figures of median urinary iodine intake in pregnant and non-pregnant women are 70 and 75 μg L^{-1}, respectively. This level of Iodine intake is below 100 μg L^{-1} (iodine sufficiency) and corresponds to mild iodine deficiency in Lahore.

As much as there is no endemic goiter in the population of Lahore, this restricted level of iodine intake is presumably sufficient to cover the usual needs of thyroid hormone production in normal adult subjects, at least as long as no physiological condition like pregnancy intervenes. Pregnancy acts as an indicator of the underlying iodine restriction by its increased hormonal demands and obligatory iodine losses and results in a relative iodine-deficient state[2]. Present results are in confirmation to this fact. While only 7.4% non-pregnant women were severe iodine deficient this figure for pregnant women during first trimester were 22.7%. Present results are similar to Glinoer[11] who have studied iodine intake and thyroid hormone regulation during first half

pregnancy in Belgium (median iodine intake 56 μg L^{-1}). The corresponding figures for severe iodine deficiency was 43%. During pregnancy when iodine supply is restricted, thyroid gland has to adapt to the relative deficient state, which leads to chronically enhance thyroidal stimulation through the pituitary-thyroid feedback mechanism[11]. Thyroidal alterations, mainly relative hypothyroxinemia and goitrogenesis frequently accompany this increased thyroidal stimulation. Gestational goiterogenesis is directly correlated with the degree of iodine restriction[12]. A goiter formed during gestation may only partially regress after parturition and also affects progeny[2,5]. The thyroidal stimulation can be assessed by four simple biochemical parameters. They are relative hypothyroxinemia, preferential T_3 secretion, and increased TSH level and enhanced levels of Thyroglobulin (Tg). Failure to this adaptation may results in maternal hypothyroidism, which has harmful effects for mother and fetus. The mean low FT_4 levels in pregnant women during first trimester of pregnancy and the overall trend towards lower limit of normal range is in parallel to the low iodine intake of these women. This is in accordance to many studies carried out in iodine deficient countries like Belgium, Hungary, Italy, Hong Kong and Zaire[3,13,14]. Early in pregnancy mother's iodine is not diverted towards fetus as fetal thyroid is not functional as yet. Moreover, during first trimester maternal thyroid is under additional stimulation by placental factors like hCG. So the FT_4 levels are in maximum concentration available for fetus brain and nervous system development. Deficiency of thyroxine at this critical period has grave consequences for the child in the later life. Hypothyroxinemia per se during first trimester is associated in children[15,16]. That is why damaged reproduction is termed as the most important consequence of iodine deficiency[17].

During pregnancy maternal thyroid adaptation to the iodine deficiency is reflected by increased production of T_3. In severe iodine deficient women T_3 levels exceed the normal limit. An increased T_3/T_4 molar ratio in maternal serum is reported by several workers[3,12,13]. Present results are in agreement to this adaptation to low iodine intake and T_3 levels in 34.7% of the pregnant women were above the upper limit of normal range. Decreased levels of TSH in 44% of pregnant women as compared to control women provide the further evidence of thyroid adaptation to the iodine deficiency during first trimester. The first observation of a transient fall in serum TSH during the first trimester of normal pregnancy was reported in 1976. Since then a bulk of compelling evidences are there to prove that this partial suppression is associated with the elevation in circulating hCG. Glinoer *et al.*[3] has shown that the lowering of TSH corresponds to a transient and partial blunting of the pituitary-thyroid axis associated with an increased hormonal output by the thyroid gland. In this study, 18.7% women had TSH levels ≤0.2 mI IU L^{-1}. Glinoer *et al.*[4] has reported the similar figure (18%). They have observed that among such women 11% had transiently increased FT_4. In this study, however, FT_4 levels in these women were within normal limit. This study has a lot of implications for maternal and neonatal health.

In women living in iodine deficient areas such as Papua New Guinea, Central Africa and the Himalayan several epidemiological surveys have shown an increased risk of spontaneous abortions, stillbirths and neonatal deaths[18-20]. Similarly a number of reports since early sixty's regarding the increased proportion of unsuccessful or complicated pregnancies (i.e. spontaneous abortions, premature births, major complications at delivery, perinatal deaths, congenital malformations and congenital hypothyroidism) in hypothyroxinemic women confirmed the important role of the thyroid hormones during pregnancy[21-23]. Iodine deficiency contributes to the occurrence of hypothyroidism in pregnant women, which may affect the outcome of pregnancy[5,11,15,24]. The incidence of gestational hypertension and low birth weight infants is increased in cases of overt and subclinical maternal hyperthyroidism[26]. Keeping in view these repercussions of low iodine intake it is recommended that iodine intake should be increased and monitored particularly pregnant women. This cross-sectional study points to the urgent need of a comprehensive large longitudinal study of iodine intake and thyroid hormone regulation in pregnant women of Lahore.

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