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RESEARCH ARTICLE

ESTIMATION OF THYROID STIMULATING HORMONE, TOTAL THYROXIN AND TOTAL TRIIODOTHYRONINE DURING PREGNANCY, GEZIRA STATE, CENTRAL SUDAN

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ABSTRACT

Thyroid disease is the second most common endocrine disease affecting women of reproductive age. The current study aimed to assess the effect of pregnancy on thyroid function among Sudanese pregnant women at Wad Medani Police Hospital, Gezira State - Central Sudan. 115 pregnant women, at different stages of pregnancy, were randomly selected as cases. The gestational age was (28 first trimester, 32 second trimester, and 55 third trimester). 30 apparently age-matched and healthy non-pregnant women were selected as control group. 5 ml of venous blood sample were collected from all the recruited cases and controls. Serum was obtained for thyroid stimulating hormone (TSH), total thyroxin (TT4), and total triiodothyronine (TT3) analysis. TT4 concentrations in pregnant women were significantly higher during the second and third trimester ($P< 0.05$) compared to control group. TSH concentrations showed significant decrease in pregnant women compared to control group, particularly in the third trimester. No significant change was shown in TT3 concentrations. The effect of pregnancy on elevation of T4 and lowering TSH, may lead to misclassification of maternal thyroid function.

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INTRODUCTION

The thyroid gland is a butterfly-shaped gland composed of two lobes. It straddles the trachea in the front of the neck. The hypothalamic-pituitary axis is responsible for the maintenance of thyroid hormone production. Thyrotropin releasing hormone (TRH) produced by the hypothalamus stimulates production and release of thyroid stimulating hormone (TSH), which then stimulates the thyroid gland to secrete thyroxin (T4) and triiodothyronine (T3) (Gardner, 2007). The thyroid gland undergoes series of metabolic changes during pregnancy and lactation, thus resulting in increased requirement of iodine in the mother due to the transfer of T4 and iodide from mother to fetus, and the loss of iodide in breast milk during lactation. These two processes are required in order to ensure normal brain development and prevention of mental retardation in the offspring. Even in iodine-replete areas, iodine deficiency is common during pregnancy, and increases as pregnancy progresses (Ainy, *et al.,* 2007; Stilwell, *et al.*, 2008; Moleti, 2008). Iodine deficiency is more common in pregnant and lactating women and neonates, because of the impact of maternal, fetal and neonatal hypothyroxinemia. In areas of endemic deficiency, special programs of salt iodization are needed to these particular groups (Zoeller, 2003)**.** Thyroid hormones are unique in that they require the trace element iodine for biological activity. Iodine is a scarce component of soil, and hence there is little in food. A complex mechanism has evolved to acquire and retain this crucial element and to convert it into a form suitable for incorporation into organic compounds (Granner, 2003). Physiological changes associated with pregnancy require the thyroid gland to increase thyroid hormones production or administration by 40% to 100% in order to meet the needs of mother and fetus. The fetus requires T4 for normal development of neurological and perhaps other organ systems (Robert, *et al.,* 2005). Thyroid disease is the second most common endocrine disease affecting women of reproductive age; both hyperthyroidism and hypothyroidism may initially manifest during pregnancy (Idris, *et al.,* 2006). Medical therapy with anti-thyroid medications is the treatment

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of choice for hyperthyroidism in pregnancy. Surgery is considered for patients who suffer severe adverse reactions to anti-thyroid drugs. It is preferred to be performed in the second trimester of pregnancy (Mestman, *et al.*, 2004). In hypothyroid mothers, if treatment is started at birth, the prognosis for normal growth and development is good, and mental retardation can generally be avoided; for this reason, screening tests for congenital hypothyroidism are routine in all states of the USA and most other developed countries (Barrett, *et al.*, 2010). Recent studies focused on the screening of all pregnant women for thyroid disease. The current study was designed to estimate the normal alterations in thyroid function that can occur during pregnancy and may lead to misinterpretation of thyroid test values that will help recognize the importance of the gestational age-specific reference values.

MATERIALS AND METHODS

Study subjects, area and design

One hundred and fifteen Sudanese pregnant women attended The Wad-Madani Police Hospital, as part of their routine antenatal care, were randomly selected as cases (age ranged between 18-45 years) the gestational age was (28 first trimester, 32 second trimester and 55 third trimester). 30 apparently age-matched and healthy non-pregnant women were selected as control group. Pregnant women, with uncomplicated pregnancy and willing to enroll in the study were included in this study. Women with Overt thyroid disease, on thyroid medication, has been treated for hyperthyroidism, those with family history of endocrine diseases, diabetes mellitus, hepatic disease, tuberculosis and cancer were excluded from the study. The study protocol was approved by the ethics committee of Faculty of Medicine, University of Gezira.

Methods

Five ml of venous blood sample were collected from all the recruited patients and controls. Serum was stored at –20°C for thyroid hormones assay.

ELISA test for the quantitative determination of TSH

Serum levels of TSH were measured by a classical sandwich ELISA technique, using the ELISA Kit according to the manufacturer's instructions (Stat fax-2600 micro plate washer USA) and (ELISA Awareness micro plate readers USA) capable of measuring absorbance at 450 nm was used to measure the intensity of color developed in each well. All assays were done in duplicate. The detection range of the assay was 0.3 - 12 micro IU/ml, and 0.15 micro IU/ml Sensitivity. Serum TSH concentrations were measured using a commercial kits by the Human® chemical company. Max-plank-Ring-21 - D-65205 Wiesbaden-Germany, used a highly specific monoclonal anti-TSH antibody coated on the surface of the micro titer wells. In each well; specimen or calibrators (50µl) and enzyme conjugate (100µl) (peroxidase–labelled anti-TSH, PH 6.25±0.1) were mixed (incubated 60 minutes at 20C°, washed 5 times) to form the sandwich complex which bound to the surface of the wells by the interaction with the immobilized antibody. After washing with the wash solution (pH 7.2±0.2, 10 mmol/l Tris-Buffer, 8 g/l NaCL) a 100µl substrate reagent (1.2 mmol/l TMB, <6.0 mmol/l Hydrogen peroxide) a 100µl was added and incubated 15 minutes at 20C° and finally a 100µl of the stop solution (0.5 mol/l Sulphric acid) a 100µl was added, turned the color into yellow. The intensity of colour was proportional to the TSH concentration in the sample.

ELISA test for the quantitative determination of total Thyroxine (T_4)

Serum T_4 concentrations were measured using a competitive enzyme immunoassay (commercial kits by the Human® chemical company. Max-plank-Ring-21-D-65205 Wiesbaden-Germany), based on the principle of competitive binding between T4 in a test specimen and T4-peroxidase conjugate for limited number of binding site on the anti-T4 coated well. Thus the amount of T_4-peroxidase conjugate bound to the well is inversely proportional to the concentration of T_4 in the specimen. In each well; 50µl specimen or calibrators (human, for thyroxin at concentration of 0, 2, 5, 10, 15, and 25 ug/dl) and 100µl working conjugate solution (T4-HRP-Conjugate, pH 7.5±0.1 (diluted 1+10 with Phosphate buffer pH 7.42±0.1)) were mixed and incubated 60 minutes at 20C°, washed 5 times). After washing with the working wash solution (diluted to 1000 ml with fresh, deionized water) unbound enzyme conjugate was removed. A 100µl working substrate solution (mixed equal volumes of (TMB 4 mmol/l, sodium acetate buffer 0.05mol/l (pH 3.5±0.1)) and (Urea hydrogen peroxide10 mmol/l, sodium acetate buffer 0.05mol/l (pH 4.5±0.1)) was added and incubated for 15 minutes at 20C°, finally 50µl stop solution (0.5 mol/l Sulphric acid) was added. The absorbance was measured at 450 nm within 10 minutes using ELISA micro plate readers.

ELISA test for the quantitative determination of Total Triiodothyronine (T_3)

Serum T_4 concentrations were measured using a competitive enzyme immunoassay (commercial kits by the Human® chemical company. Max-plank-Ring-21- D-65205 Wiesbaden-Germany), based on the principle of competitive binding between T_3 in a test specimen and T_3-peroxidase conjugate for limited number of binding site on the anti-T_3 coated well. Thus the amount of T_3-peroxidase conjugate bound to the well is inversely proportional to the concentration of T_3 in the specimen. In each well; 50µl specimen or calibrators (human, for thyroxin at concentration of 0, 2, 5, 10, 15, and 25 ug/dl) and 100µl working conjugate solution (T_3-HRP-Conjugate, pH 7.5±0.1 (diluted 1+10 with Phosphate buffer pH 7.42±0.1)) were mixed and incubated 60 minutes at 20°C, washed 5 times. After washing with the working wash solution (diluted to 1000 ml with fresh, deionized water) unbound enzyme conjugate was removed. A 100µl working substrate solution (mixed equal volumes of (TMB 4 mmol/l, sodium acetate buffer 0.05mol/l (pH 3.5±0.1)) and (Urea hydrogen peroxide10 mmol/l, sodium acetate buffer 0.05mol/l (pH 4.5±0.1)) was added and incubated for 15 minutes at 20°C, finally 50µl stop solution (0.5 mol/l Sulphric acid) was added. The absorbance

was measured at 450 nm within 10 minutes using ELISA micro plate readers.

Data management and statistical analysis

All data was analyzed using Statistical Package of Social Software (SPSS) computer program. For statistical analysis t-test and ANOVA were used for comparing means and *p* value ≤0.05 was taken as the level of significance.

RESULTS AND DISCUSSION

Common thyroid diseases have a strong predominance in women of childbearing age. Correct diagnosis and treatment of thyroid dysfunction is important to prevent both maternal and fetal complications.

Detection of Total T_3

Total T_3 concentration showed no significant difference between pregnant women and control group throughout all trimesters (*p*= 0.21) (Table 1).

Table 1. TSH, TT_3 and TT_4 concentrations in pregnant group and control group

Parameter	Control(30)	Pregnant(115)	p-value
TSH mIU/l	1.49±0.77	1.155±0.71	0.0244*
TT_4 µg/dl	7.683±1.56	9.162±1.86	<0.0001***
TT_3 ng/ml	1.34±0.54	1.537±0.64	0.2107

(*) significant at p= 0.05; (**) significant at p= 0.01; (***) significant at p= 0.001

Table 2. TSH, TT_4 and TT_3 in pregnant women and controls in the three trimesters

Parameter	Controls (n=30)	1st trimester pregnant (n=28)	2ndtrimester pregnant (n=32)	3rd trimester pregnant (n=55)	p-value
TSH mIU/l	1.49±0.77	1.18±0.86	1.27±0.66	1.08±0.64*	0.0897
TT_4 µg/dl	7.68±1.56	8.54±2.01	9.50±1.49 ***	9.28±1.93**	0.0002
TT_3 ng/ml	1.34±0.54	1.47±0.57	1.42±0.66	1.64±0.65	0.1381

(*) significant at p= 0.05; (**) significant at p= 0.01; (***) significant at p= 0.001

Detection of TSH

The mean concentrations of TSH in the first, second and third trimester was 1.18±0.86 mIU/I, 1.27±0.66 mIU/I and 1.08±0.64 mIU/I respectively, and 1.49±0.77ng/ml for the control group, which were decreased in pregnant women compared to control. The TSH mean serum concentrations in the third trimester showed a significant difference between pregnant women and control (*P*= 0.0897) (Table 2), and that might be due to the negative feedback control system of hypothalamic pituitary thyroid axis functions normally during pregnancy. Similar findings were shown by Bloch, *et al.,* (2003) who observed decrease in serum TSH in the first trimester.

Detection of total T_4

Total T_4 showed a significant increase in concentration (*p*= 0.0001) between pregnant women and control group (Table 1). The mean concentration of T_4 during the second and third trimester was significantly higher than that in first trimester and control group, which was consistent with the study by Glinoer, *et al.*, 1997, who reported increased circulating total T_4 and TBG concentrations by 6–8 weeks of gestation and remained high until delivery. The increase of T_4 might be due to the progressive increase of TBG in the first trimester of pregnancy, as what stated by Gardner, 2007 that the concentration of TBG increases in pregnancy because of reduced hepatic clearance and estrogenic stimulation of TBG synthesis. The results of the current study was also in accordance with those who reported increase in total T_3 and total T_4 due to increased TBG concentrations, due to decrease plasma clearance of TBG and increased plasma volume along with increased TBG, which results in an increased production of total T_4 (Guillaume, *et al.*, 1985; Gilnoer, *et al.*, 1990, Burrow, *et al.*, 1994). The results of the study carried out by Glinoer, *et al.,* 1990 showed that TBG levels were highest in the second and third trimester of pregnancy and the same holds true for thyroid-hormone binding ratio.

The study was in disagreement with the studies in which the levels of total T_4 and total T_3 decline whereas the TSH levels increase as pregnancy progresses (Stricker *et al*. 2007; Gong and Hoffman, *et al.*, 2008).

Conclusion

There was a significant elevation in total T_4 in the second and third trimester, accompanied by significant lowering in TSH concentration in the cases of the study compared to control women, particularly in the third trimester. No significant difference was observed in total T_3 level in pregnant women throughout the three trimesters. The effect of pregnancy on elevation of total T_4 and lowering TSH, may lead to misclassification of maternal thyroid function. The study recommends that Sudanese medical field workers should adopt pregnancy trimester specific reference intervals for thyroid function tests to diagnose thyroid dysfunction during pregnancy so as to prevent both maternal and fetal complications. Further studies with large sample size in different gestational periods should be adopted.

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