Study of thyroxine and triiodothyronine in female population using enzyme-linked immunosorbent technique

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Abstract

A case-control study designed with 175 normal pregnant women selected from the first , the second , and the third trimesters and 160 non-pregnant healthy female controlsto study the thyroid funtions. Thyroid function tests carried out by measuring thyroxin and triiodothyronin by enzyme linked immunoassay technique. We found that T4 increased progressively during pregnancy. Our study showed increase of T3 in the second trimester and then declining during the third trimester compared with non-pregnant women. We found that non pregnant women have T3 level 0.43 to 1.28 ng/ml. This study showed that the serum T4 level depends on nutrition factor . T4 levels of non-pregnant women are 4.3 µg/dl to 5.1 µg/dl .The serum T4 of pregnant mother during third trimester was significantly higher 7.5 to 9.81 µg/dl compared to that of non-pregnant women. The serum T4 of pregnant mother during first trimester was found to be 4.10 to 4.9 µg/dl and second trimester was 4.40 to 5.15 µg/dl. The thyroid function tests in pregnancy should be interpreted against gestational intervals to avoid mis-interpretation of thyroid function during pregnancy.

Keywords: thyroxin and triiodothyronin, enzyme-linked immunosorbent technique, hormonal parameters.

Introduction

There are two basic forms of thyroid hormone: T4 (3,5,3',5'-tetraiodothyronine) and T3 (3, 3',5-triiodothyronine). The two forms of thyroid hormone (T3, T4) are produced and secreted by the follicular cells of the thyroid gland. Thyroid hormones mediate their action on different types of cells (plasma).

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Membrane, nucleus, cytoplasm and in the mitochondria) in different ways. The thyroid hormone receptors and steroids belong to the same family with similar molecular structure, but having different transcriptional functions which define their genomic actions. Pregnancy is associated with significant, but reversible changes in thyroid function studies,which are among the most profoundly seen as a result of a normal physiologic state [1,2,3].Furthermore, human chronic gonadotropin (hCG) can stimulate the thyroid gland during first trimester because of its structural similarity to thyrotrophin (TSH) . Thyroid hormones have important role in embryogenesis and fetal development

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during pregnancy . Therefore, thyroid status is frequently assessed during pregnancy, both to evaluate suspected thyroid abnormalities, and to monitor the status of pre-existing thyroid disease. However, the usual clinical and laboratory assessment can be potentially misleading[4,5,6,7].The findings associated with the hypermetabolic state of normal pregnancy can overlap with the clinical signs and symptoms of thyroid disease. Both normal pregnancy, and pregnancy complicated by conditions such as hyperemesis gravidarum (HG), can be associated with thyroid function study changes that are strongly suggestive of hyperthyroidism, in the absence of primary thyroid disease [8,9,10]. So an accurate interpretation of thyroid hormone status in complicated pregnancies is needed. Literature survey showed only one single such study of female population in Bangladesh with in Dhaka city by Khandaker M A et al[10]. They used radioimmunoassay method as measurement technique which is not available to general diagnostics and only available at the Atomic Energy Commission. Experimental values of hormone vary with analytical method[5]. Enzyme-linked immunosorbent assay method which ismost widely used in Bangladesh was employed in our present study to find hormonal values so that results may be used for general diagnostic interpretation. It also important to gather test subjects from different zones of Bangladesh so that the values may have greater acceptance. Thyroid hormones are produced from protein rich foods and iodine is required. Deficiencies in any of these will affect thyroid hormone production.Therefore, the values of thyroxin and triiodothyronin may also differ with the nutrition factor and not a single study was found with nutrition based parameters .We designed a crossectional case-control study with systematic random sampling to find out alterations in thyroid function tests in each trimester in normal pregnant women as compared to non-pregnant women in Bangladesh with nutrition based factors.

Origin of thyroxin and triiodothyronin

The thyroid gland is the endocrine gland responsible for producing thyroid hormone,a regulator of growth, development, and basal metabolic rate, and calcitonin, a regulator of calcium homeostasis .The structures of the two thyroid hormones, triiodothyronin (T3) and thyroxin(T4), are shown in Figure 1. In effect, T3 is the actual hormone, since T3 has a much higher affinity for the thyroid hormone receptor than does T4; T4 is thought to act primarily a precursor for T3. Each T3 molecule contains three iodines, and each T4 contains four. This implies that the thyroid must be able to obtain iodine to incorporate into the thyroid hormones it produces. The thyroid has an active uptake mechanism that concentrates iodine about 30-fold over the serum levels. The thyroid is very efficient: 20-40% of an administered dose of iodine is trapped by the gland, and the gland contains over 90% of the total amount of iodine present in the body [11].

Triiodothyronine (T3) = *3,5,3´-triiodothyronine* Thyroxine (T4) = *3,5,3´,5´-tetraiodothyronine*

Figure 1. The structures of the thyroid hormones.

Materials and methods

Blood samples were collected from 12 different cities of Bangladesh and they are: Dhaka, Mymensingh, Rajshahi, Dinajpur, Rangpur, Khulna, Barisal, Faridpur, Bogra, Comilla and Coxs Bazar .In total 335 subjects were seleced ,without thyroid history, divided into two groups; A and B. Group A as control with healthy individuals. While group B are pregnant women. Blood samples (5 ml) were taken and analyzed to estimate the levels of serum T3 (tri-iodothyronin) and T4 (thyroxin) hormones on a monthly basis. A case-control study designed with 175 normal pregnant women selected from the first , the second , and the third trimesters and 160 randomly selected non-pregnant healthy female controls with theage range of 22-38 years. Thyroid function tests were carried out by measuring serum levels and total thyroxin and triiodothyronine by enzyme linked immunoassay technique All subjects were fed iodide salt. To ensure no one of the subjects had iodide deficiency problem. A and B groups were divided into three sub class diet group such as low, mid and high protein diet.

Blood sampling and hormone analysis

Five milliliters ofblood were collected between 9:00- 10:00 am in the morning from (after breakfast) both patient and control group weekly basis. Then, thyroid function tests were carried out by measuring serum levels of total thyroxin (T4), and total triiodothyronine (T3) using enzyme-linked immunosorbent technique with Thermoelectron Multiscan EX microplate reader.

Thyroxine estimation:

Thyroxine (T4) is measured by competitive immunoassay techniques. A sample of serum or plasma containing the T4 to be quantified was mixed with labeled T4 and T4 antibody.The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins. A fixed amount of labeled T4 competes with the unlabeled T4 in the sample for a fixed number of binding sites on the specific T4 antibody. Antibody to thyroxin (T4) is coated on a solid phase (microtiter well). A measured amount of serum and a constant amount of thyroxine (T4) labeled with horseradish peroxidase are added. During incubation, T4 in the sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody. After a 60 minute incubation at room temperature, the wells were washed 5 times with water to remove unbound thyroxin(T4) conjugate. A solution of tetramethylbenzidine (TMB) reagent was added and incubated for 20 minutes,resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Thyroxine (T4) in the sample. Absorbance is measured spectrophotometrically at 450 nm withThermoelectron Multiscan EX microplate reader .

Triiodthyronine estimation:

The assay system utilizes a highly specific T3 monoclonal antibody bound to a polystyrene well coated with goat anti-mouse antibody and an enzyme-labeled analyte.

Test sample, T3 antibody solution, and a buffer containing chemical blocking agents and T3-enzyme conjugate were added to each antibody coated well. The blocking agents, 8-anilino-1-naphthalene sulfonic acid (ANS) and sodium salicylate,cause a release of T3 from the serum binding proteins and allow the T3 to bind to the antibody-coated well. During a 60 minute incubation, T3 in the patient's serum competes with the T3-enzyme conjugate for binding sites on the coated wells. The number of binding sites on the well are limited; as more of them are occupied by T3from the sample, less of the T3 enzyme conjugate can bind. The amount of T3 in the patient serum is inversely proportional to the amount of T3-enzyme conjugate bound to the well. After a short incubation, the wells were washed to remove any unbound T3-enzyme conjugate. An enzyme substrate-chromogen (hydrogen peroxide, H_2O_2, and tetramethylbenzidine, TMB) was added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color.The addition of 1.0 N H_2SO_4 stops the reaction and converts the color to yellow and increases the absorbance by a factor of approximately 3. The intensity of the yellow color is inversely proportional to the concentration of T3 in the sample. The concentration of T3 in the patient sample was interpolated from a standard curve relating the absorbance, measured spectrophotometrically at 450 nm, of each calibrator to the concentration of T3.

Results and discussion

The thyroid gland secretes thyroid hormones having important role in embryogenesis and fetal development during pregnancy. The present study was carried out to find out alteration in the blood serum for thyroid hormonal levels (T4, T3) in normal pregnant women as compared to non-pregnant women in Bangladesh (Table 1). The study showed that the serum T4 level of non-pregnant women was normal from lower limit was 4.3 μg/dl to higher limit of 5.1 μg/dl and the serum T4 of pregnant mother during third trimester was significantly higher 5.8 to 8.81 μg/dl compared to that of non-pregnant women. The serum T4 of pregnant mother during first trimester was found to be 4.10 to 4.9 μg/dl second trimester 4.40 to 5.15 μg/dl. The serum T3, levels of non-pregnant women were found to be 0.43 to 1.28 ng/ml. For pregnant women in first trimester it was found to be 0.83 to 1.24 ng/ml , for second trimester it was found to be 0.90 to 1.30 and in the third trimester it was found to be 0.89 to 1.28 ng/ml within normal range . It can be concluded from the study that they attained euthyroid status as observed by increased production of serum total thyroxin (T4) by thyroid to fulfill the maternal requirement.Table 1 shows the measured thyroid function tests in pregnant women and the non- pregnant control group. We found that mean T4 levels decreased insignificantly in the first and the second trimester and then increased significantly during the third trimester relative to the non-pregnant women. In the first trimester, the mean T3 values showed declining , which then was increasing during the second trimester and declined in the third trimester relative to the control group.

Table 1: *Comparison of thyroxin and triiodothyronin level in pregnant and non-pregnant women.*

Test subjects	Thyroxin level(T4) μg/dl	Triiodothyronin level(T3)ng/ml
Normal healthy non pregnant women	Lower level-4.3 Upper level-5.1	Lower level-0.43 Upper level-1.28
First trimester	4.10 to 4.9	0.83 to 1.24
Second trimester	4.40 to 5.15	0.90 to 1.30
Third trimester	5.8 to 8.81	0.89 to 1.28

Overall significant difference was observed in mean values of serum thyroid hormones (T3, T4) level in all groups.During pregnancy several hormonal changes and metabolic demands occur, resulting in complex effects on thyroid function [12,13,14,15]. Alterations in the pituitary-thyroid axis include an increase in thyroid hormone-binding globulin along with increases in total T4, T3 as well as serum thyroglobulin (TG). Additionally, iodine clearance by the kidneys is enhanced during gestation, while the mild thyrotropic effects of rising -hCG may exert negative feedback on TSH secretion wrongly suggesting hyperthyroidism in normal pregnant women of the 1st trimester[16-21] . The values of hormonal parameter differs with analytical methods. Most studies are done with RIA method and our study is performed with enzyme-linked immunosorbent assay method which is most commonly used in Bangladesh . As we compare these values with others it seems our results of thyroid hormone changing pattern are close to Nosratollah Z etal's[5] work on Iranian pregnant women (T4 for first trimester is 87.98+/- 40.87 nmol/L, second trimester is 94.30+/-41.70 nmol/L and for third trimester 123.80+/-50.5 nmol /L) . Thevarajah M etal [16] also showed alteration in thyroid hormone pattern in Malaysian pregnant women. It seems like thyroid hormone pattern in the pregnant woman may vary with geographic position, food habits and other variables.[16-21].

To generate thyroid hormones, a number of key nutrients are required: Vitamins E, A, C, B2, B3, B6 and the minerals selenium, iron, zinc, and the amino acid tyrosine derived from protein rich foods and iodine. Deficiencies in any of these will affect upon thyroid hormone production[10,21,22,23]. From our present study, the effect of nutrition in thyroxine production in non pregnant and trimesters are shown in figure 2,3,4 and 5. Since triiodothyronine differs in very small value only thyroxine is compared in different nutritional factor.

Our study shows that thyroxin hormonal parameter is significantly different in different diet status. However in third trimester thyroxin levels increases in all levels of diet status. So we may assume that for low diet pregnant women their own body fat and protein is used to produce the additional thyroxin hormone which may lead mother and child to mortal danger. The nutritional status of women when becoming pregnant and during pregnancy can have significant influence on fetal, infant and maternal health.

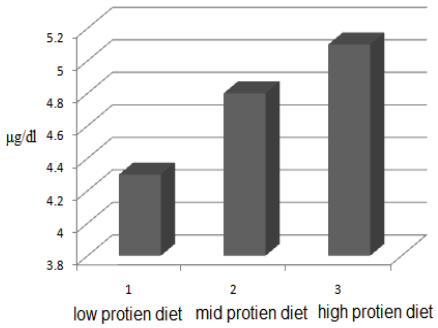

***Figure 2**: T4 level; non pregnant women*

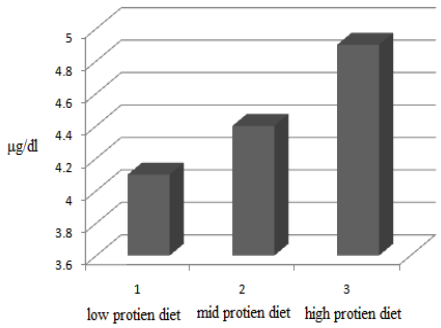

***Figure 3**: T4 level; first trimester*

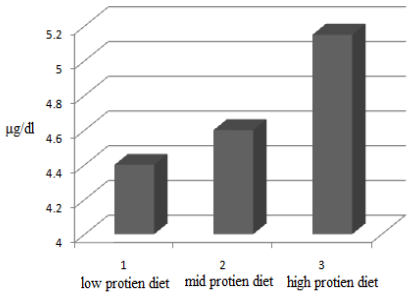

***Figure 4:** T4 level; second trimester*

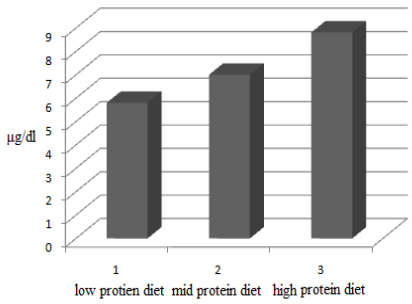

***Figure 5:** T4 level; third trimester*

Micronutrient deficiencies such as calcium, iron, vitamin A and iodine can lead to poor maternal health conditions and pregnancy complications which put the mother and baby at risk. Poor maternal weight gain in pregnancy due to an inadequate diet increases the risk of premature delivery, low birthweight and birth defects. [21-23]

Conclusion

The thyroid gland secretes thyroid hormones having important role in embryogenesis and fetal development during pregnancy. The present study was carried out to find out serum thyroid hormonal levels (T4, T3) in normal pregnant women as compared to non-pregnant women from 12 different places of Bangladesh. The study revealed a significant difference of thyroxin level in both pregnant and non-pregnant women in different nutritional status. The study showed that the serum T4 level of non-pregnant women was normal and the serum T4 of pregnant mother during third trimester was significantly higher compared to that of non-pregnant women. Our study showed increasing T3 in

serum levels in the second trimester and then declining during the third trimester compared with non-pregnant women It can be concluded that their euthyroid status is affected by increased production of serum total thyroxin (T4) by thyroid to fulfill the maternal requirement, with changed T3 secretion. Our study shows that thyroxine hormonal parameter is significantly varies in diet status. However in third trimester thyroxin levels increases in all levels of diet status. For low diet pregnant women the production of excess thyroxin hormone may lead to poor maternal health.

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