

THYROXINE LEVELS IN DIABETIC AND NON-DIABETIC PATIENTS

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ABSTRACT

Thyroxine levels in diabetic and non-diabetic patients in Nigeria are not well described. To determine the concentration of thyroxine in diabetic and non-diabetics fasting blood sample from 45 diabetics and 45 non-diabetic patients were analysed. Total thyroxine (T₄) concentrations (11.50 ± 3.50) in diabetic males between 1-29 years of age were significantly (p ≤ 0.05) higher than that of the non-diabetic males (6.75 ± 0.47), concentrations (8.50 ± 0.29) in diabetic males between 30-59 years of age were lower than that of non-diabetic males (8.71 ± 0.42) and concentrations (7.60 ± 0.68) in diabetic males between 60 years and above, were also lower than that of non-diabetic males (8.60 ± 0.25). T₄ concentrations (9.00 ± 0.31) in diabetic females between 1-29 years of age were higher than that of non-diabetic females (7.33 ± 0.62), concentrations (8.56 ± 0.34) in diabetic females between 30-59 years of age were also higher than that of non-diabetic females (8.30 ± 0.47) and concentrations (6.50 ± 0.50) in diabetic females between 60 years and above, were lower than that of non-diabetic females (6.67 ± 0.33). Note that values are mean and standard deviations.

Keywords: Thyroxine, diabetics, non-diabetics, fasting blood glucose, thyroid stimulating hormone, hypothyroidism, hyperthyroidism.

1. Introduction

Thyroxine (T₄) can be defined as one of the hormones produced by the thyroid gland that helps regulate the adrenal system, control the rate of oxidation in cells, play a role in energy metabolism, normal growth and metabolism as well as ability to maintain a healthy weight and in mood stability (Ekholm, *et al.*, 1997).

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced (Chinaka, *et al.*, 2012).

1.1 Diabetes and thyroid disorders

Thyroid disease is common in the general population, and the prevalence increases with age. The assessment of thyroid function by modern assays is both reliable. Screening for thyroid dysfunction is indicated in certain high-risk groups such as neonates and the elderly.

Hypothyroidism is by far the most common thyroid disorder in the adult population and is more common in older women. It is usually autoimmune in origin, presenting as either primary atrophic hypothyroidism or Hashimoto's thyroiditis. Thyroid failure secondary to radioactive iodine therapy or thyroid surgery is also common.

By contrast, hyperthyroidism is much less common with a female-to-male ratio of 9:1.

Graves' disease is the most common cause and affects primarily young adults. Toxic multi-nodular goiters tend to affect the older age groups. Diabetic patients have a higher prevalence of thyroid disorders compared with the normal population, because patients with one organ-specific autoimmune disease are at risk of developing other autoimmune disorders and thyroid disorders are more common in females. It is not surprising that up to 30% of female type 1 diabetic patients have thyroid disease.

The rate of postpartum thyroiditis in diabetic patients is three times that in normal women. A number of reports have also indicated a higher than normal prevalence of thyroid disorders in type 2 diabetic patients, with hypothyroidism being the most common disorder. There are several implications with patients with both diabetes and hyperthyroidism.

In hyperthyroid patients, the diagnosis of glucose intolerance needs to be considered cautiously, since the hyperglycemia may improve with treatment of thyrotoxicosis.

Understanding hyperthyroidism should be considered in diabetic patients with unexplained worsening hyperglycemia. In diabetic patients with hyperthyroidism, physicians need to anticipate possible deterioration in glycemic control and adjust treatment accordingly. Restoration of euthyroidism will lower blood glucose level.

In young women with type 1 diabetes, there is a high incidence of autoimmune thyroid disorders. Transient thyroid dysfunction is common in the postpartum period and warrants routine screening with serum thyroid-stimulating-hormone (TSH) 68 weeks after delivery. Glucose control may fluctuate during the transient hyperthyroidism followed by hypothyroidism typical of the postpartum thyroiditis.

It is important to monitor thyroid function tests in these women since approximately 30% will not recover from

the hypothyroid phase and will require thyroxine replacement. Recurrent thyroiditis with subsequent pregnancies is common.

Poorly controlled diabetes, with or without its complications, may produce changes in thyroid function tests that occur in non-thyroidal illnesses. Typical changes include a low serum T_3 due to impaired extra-thyroidal T_4 to T_3 conversion, a low serum T_4 due to decreased protein binding, and an inappropriately low serum TSH concentration.

2. Materials and Method

T_4 standard; 6 vials (0.7ml each), Conjugate diluents, TMB substrate, Stop substrate, T_4 enzyme, (HRP) conjugate concentration; 1 vial (10×1.5ml), and Wash concentrate.

Microwell strips coated with T_4 MAb (12×18×1 wells),

Absorbent paper towels,

Electric centrifuge (B. Bran Scientific and Instrument Company, England),

Micropipettes (500 μ L, 200 μ L, 100 μ L and 50 μ L),

ELISA washer (Stat Fax 2600),

ELISA reader (Stat Fax 2100).

2.1 Sample Collection

Blood samples were collected from 45 diabetic patients and 45 non-diabetic patients who visited University of Port Harcourt Teaching Hospital. All diabetic patients were confirmed diabetic, who previously had fasting blood glucose levels above 6.8mmol/L on more than two occasions and were receiving treatment. All subjects both diabetics and non-diabetics were Nigerians residing in Rivers State and neighbouring States. The samples (venous blood) were collected from patients, centrifuged, separated and labeled. The labeled samples were then stored at 10°C for nine days.

2.2 Reagent preparation

Dilute T_4 -HRP enzyme conjugate 1:11 with total T_4 conjugate buffer in a suitable container. Dilute 960 μ l of conjugate with 9.6ml of buffer for 96 wells. A slight excess of solution is made. After constitution of reagents, use within 24hrs for maximum performance of assay.

2.3 General formula

Amount of buffer required = number of wells * 0.1ml

Quantity of T_4 -enzyme necessary = number of wells * 0.01ml

$96 \times 0.1 = 9.6$ ml for total T_4 conjugate buffer

$96 \times 0.01 = 0.96$ ml for T_4 -enzyme conjugate.

Prepare one was buffer by adding the contents of the bottle (25ml, 20×) to 475ml of distilled water.

Store at room temperature.

2.4 Assay procedure

Prior to assay, samples and reagents were allowed to stand at room temperature so as to thaw completely, thereafter they were gently mixed and placed in the desired number of coated strips into the holder.

50 μ L of T_4 standards were pipette into patients' sera. 100 μ L of working T_4 -enzyme conjugate was added to all wells and incubated for 60mins at room temperature (18-26°C).

All wells were emptied and filled with working wash buffer and washing was done three times after which wells were blotted on absorbent paper towels. 100 μ L of TMB substrate was added to all wells and incubated for 15mins at room temperature.

50 μ L of stop solution was added to all wells and gently mixed with the solution. Absorbance was read on ELISA reader at 450nm wavelength within 15mins after addition of the stop solution.

2.5 Principle

The samples, assay buffer and T_4 -enzyme conjugate are added to the wells coated with anti- T_4 monoclonal antibody. T_4 in the patient's serum competes with T_4 -HRP conjugate for binding sites. Unbound T_4 and T_4 -enzyme conjugate is washed off using washing buffer. Upon the addition of the substrate, the intensity of colour is inversely proportional to the concentration of T_4 in the samples. A standard curve is prepared relating colour intensity to the concentration of T_4 .

3. Result

The result of the analysis of T_4 concentration in both diabetic and non-diabetic males is represented in table 1.1 and figure 1.1 below, while the result of the analysis of T_4 concentration in both diabetic and non-diabetic females is represented in table 1.2 and figure 1.2 respectively. The result shows the mean level of concentration

for both male and female with their respective age differences.

4. Discussion

Table 1.1 indicates that the concentrations of T_4 in diabetic males within ages 1-29 were higher than the concentrations in non-diabetic males within the same age range (11.50 ± 3.50 and 6.75 ± 0.47 respectively). From the table, the concentrations of T_4 in non-diabetic males within ages 30-59 were also higher than the concentrations in diabetic males within the same age range (8.71 ± 0.42 and 8.05 ± 0.29 respectively), and the concentrations of T_4 in diabetic males within ages 60 and above were lower than the concentrations in non-diabetic males within the same age range (7.60 ± 0.68 and 8.60 ± 0.25 respectively).

Table 1.2 shows that the concentrations of T_4 in diabetic females within ages 1-29 were higher than the concentrations of in non-diabetic females within the same age range (9.00 ± 0.31 and 7.33 ± 0.62 respectively). The table also shows that the concentrations of T_4 in diabetic females within age 30-59 were higher than the concentrations in non-diabetic females within the same age range (8.56 ± 0.34 and 8.30 ± 0.47 respectively), and the concentrations of T_4 in diabetic females within age 60 and above were lower compared to the non-diabetic females within the same age range (6.50 ± 0.50 and 6.67 ± 0.33 respectively).

The thyroid hormones, tri-iodothyronine and tetraiodothyronine are insulin antagonists that also potentiate the action of insulin indirectly (Granner, 2000). TRH synthesis decreases in diabetes mellitus (Suzuki, *et al.*, 1994). These facts could be responsible for the occurrences of low thyroid hormone levels in some diabetics. This finding is not consistent with the report of Celani, *et al.*, 1994, Smithson 1998 who recorded varied levels of thyroid hormones in diabetic subjects.

Majority of the non-diabetic subjects showed euthyroid status. This observations is in agreement with the report of Smithson, 1998, Suzuki, *et al.*, 1994 and Celani, *et al.*, 1994 who in separate studies found altered thyroid hormone levels of different magnitudes in diabetic patients. The abnormal T_4 levels may be the outcome of the various medications the diabetics were receiving. Some oral hypoglycaemic agents such as the phenylthioureas are known to suppress the levels of T_4 , while causing raised levels of TSH (Smithson, *et al.*, 1998). Some of the diabetics were on oral hypoglycaemic agents alone and some were on both insulin injections and oral hypoglycaemic agents. These situations may explain the finding of low or raised thyroid hormone levels in some of the euthyroid diabetics.

Never the less, the situation in these diabetics does not seem to follow the pattern previously recorded in other non-thyroidal diseases such as liver diseases and Cushing syndrome where low thyroid hormone levels were recorded (Sacks, 1999). The presence of both raised and low levels of thyroid hormone in diabetics in this study may also be due to modified TRH synthesis and release (de-Greef, *et al.*, 1992) and may depend on the glycaemic status of the diabetics studied. Glycaemic status is influenced by insulin, which is known to modulate TRH and TSH levels (Reusch and Tomsa, 1999).

The incidence of hyperthyroidism was lower in females than in males, but the number of subjects in hypothyroid state was higher in females than in males. These observations agree with the report of Radetti, 1994, Sacks and Celani, *et al.* and the finding is probably associated with the higher prevalence of obesity recorded in female diabetics (Sacks, 1999).

Finally, this study has shown that diabetics in Port Harcourt had abnormal thyroid hormone levels. Some of the diabetics had low levels of thyroid hormone while some had raised levels.

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Table 1.1: Shows the mean values and S.D. of Diabetic and Non-diabetic males with concentration of T₄.

Age	Diabetic	Non-diabetic
1-29	11.50 ± 3.50	6.75 ± 0.47
30-59	8.05 ± 0.29	8.71 ± 0.42
60-Above	7.60 ± 0.68	8.60 ± 0.25

Table 1.2: Shows the mean values of Diabetic and Non-diabetic females with concentration of T₄.

Age	Diabetic	Non-diabetic
1-29	9.00 ± 0.31	7.33 ± 0.62
30-59	8.56 ± 0.34	8.30 ± 0.47
60-Above	6.50 ± 0.50	6.67 ± 0.33

APPENDIX

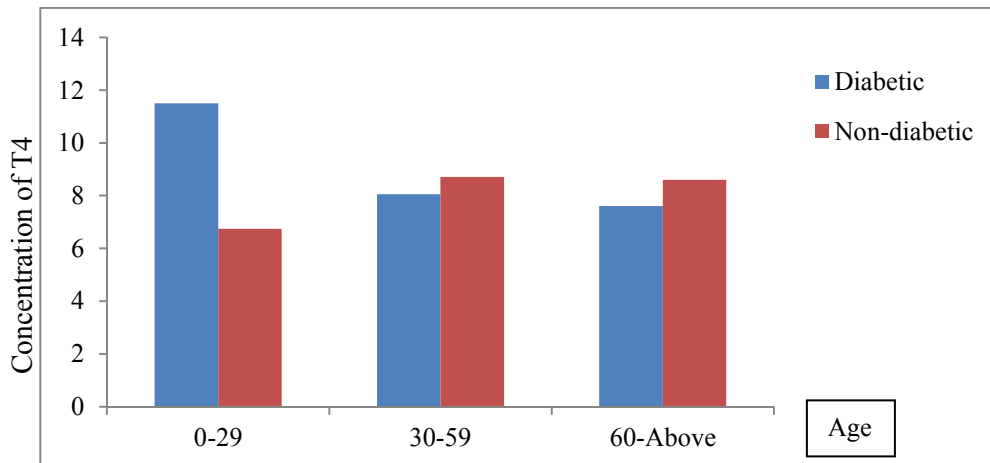


Fig. 1.1: Showing the mean values of Diabetic and Non-Diabetic males with concentration of T₄.

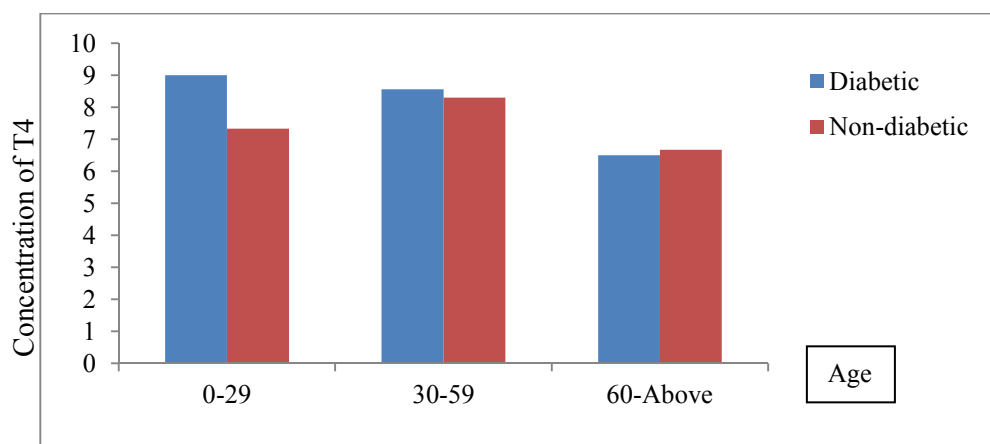


Fig. 1.2: Showing the mean values of Diabetic and Non-Diabetic males with concentration of T₄.

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