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Thyroid Stimulating Hormone Assay as the First Line Biochemical Parameter to Determine Thyroid Gland Abnormalities

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Abstract: Increased cellular catabolic activities observed in hyperthyroid state had been established. This is consequent to excessive hormones secreted by the thyroid gland during this condition. A total of 60 subjects comprising of 45 females mean age 43.02±1.90 (range 22-70 years) and 15 males mean age 50.40±3.59 (range 25-68) and 60 controls comprising of 45 females mean age 41.18±1.68 (range 22-68 years) and 15 males mean age 40.53±2.88 (range 25-65) were recruited for the study. The plasma level of T4, T3 and TSH were determined in both the experiment group and the controls. A significant increase ($p<0.05$) in plasma T4, T3 and a significant decrease ($p<0.05$) in plasma TSH were observed in the experiment group in comparison to the controls. Also, an inverse relationship was noted to exist between the plasma T4 and T3; and TSH in primary hyperthyroid state.

Key words: Hyperthyroidism, triiodothyronine, thyroxine, thyroid stimulating hormone, thyroid gland

INTRODUCTION

Hyperthyroidism is a clinical condition of the thyroid gland characterized by high plasma levels of hormones; thyroxine (T4) and triiodothyronine (T3). The secreted hormones circulate in the blood either in a protein bound form (bound to Thyroxine Binding Globulin (TBG), Thyroxine Binding Prealbumin (TBPA) also called Transthyretin (TTR) and Albumin) or free non-protein bound form. The observed changes in cellular metabolic activities are as a result of the active free unbound form of the hormones. Hyperthyroidism is a state of increased catabolic activities affecting all cellular activities and resulting in utilization of biological fuel in the form of carbohydrates, proteins and lipids, hence accumulation of metabolic products and excretion of wide varieties of metabolites. Due to these high catabolic activities, there may be associated loss of body fat and weight and thus serve to regulate pace of metabolism in body tissues. The thyroid hormones regulates the body metabolism, the rate at which the body uses fats and carbohydrates, burn calories, regulate body temperature, influence heart rate and controls the protein synthesis (Huether and McCance, 2007).

In evaluating abnormality of the thyroid gland, the total (free+protein-bound) concentrations of the thyroid hormones (TT4 and TT3) are generally used because

these are easier to measure than the free moieties as they circulates at nanomolar concentrations unlike the free hormone moieties (FT4 and FT3) that circulate in picomolar range.

Total T4 represents the blood levels of the hormone secreted by thyroid gland into the plasma but not the hormone that gets to the cells. The plasma T4 level is determined by plasma thyroid binding proteins and albumin levels which in turn, are affected by factors such as pregnancy, exogenous estrogens, drugs (such as birth control pills), chronic non-thyroid illness and congenital factors. These altogether affect thyroid binding proteins to give an impression that the patients are hyperthyroid when in fact they are not. The diagnostic accuracy of total hormone measurements had been found by Stockigt to be proportional to that of free hormone tests if all patients had similar binding protein concentrations (Stockigt, 2001). Total hormone measurements is diagnostically unreliable because some patients have abnormal thyroid hormone binding proteins such as thyroid hormone autoantibodies, some uses steroids (such as glucocorticoids) and some have TBG abnormalities that distort the proportionality of the total to free thyroid hormone relationship.

The use of TT4 and TT3 measurements alone is rarely done but together with an estimate of thyroid binding proteins. Some of the methods available for evaluating

thyroid gland abnormalities are serum-based immunoassay, tandem mass spectrometric methods (to measure total T4 and total T3 and free hormone moieties (FT4 and FT3) (Thienpont *et al.*, 2005); thyroid peroxidase antibodies, thyroglobulin antibodies and Thyroid Stimulating Hormone (TSH) receptor antibodies (Ajjan and Weetman, 2008) and measurements of Thyroxine Binding Globulin (TBG), thyroglobulin, prealbumin, albumin and TSH (Stockigt, 2001).

In hyperthyroid state, the plasma TSH is expected to be low due to negative feedback inhibition from excess T4 and T3. Assay interference from a human anti-mouse antibody (HAMA) or an endogenous TSH antibody is one of the causes of a falsely high TSH level in hyperthyroid state (Verhoye *et al.*, 2009). Also, plasma levels of total hormone, free hormone or Thyroid Hormone Binding Ratio (THBR) can be falsely raised depending on the method of measurement (Massart *et al.*, 2009; Zouwail *et al.*, 2008).

The prevalence of thyroid hormone autoantibodies ranges from two percent in the general population to as much as thirty percent in patients with autoimmune thyroid disease (Sakata *et al.*, 1994). However, despite this prevalence, significant interference by these autoantibodies is considerably less common but depends on the quality of the autoantibody present (i.e., its affinity for the test reagents).

The use of TSH as the first-line test in most clinical settings while relegating FT4 or FT3 measurements to second or third-line tests to investigate situations in which TSH abnormalities are found and to detect both overt and subclinical hyperthyroidism in ambulatory patients with stable thyroid status and intact hypothalamic-pituitary function had been advocated (Baloch *et al.*, 2003; Baskin *et al.*, 2002), except in those conditions where TSH may be diagnostically unreliable. Whenever this occurs, FT4 becomes the first-line test of choice. The TSH test is ultrasensitive as it can detect tiny amounts of TSH in the blood and can thus be useful in detecting mild hyperthyroidism. Hence, this study is to corroborate and affirm the call of other researchers in using TSH measurement as the first line biochemical parameter to determine thyroid gland abnormalities.

MATERIALS AND METHODS

Study design: A total of 60 subjects and 60 controls (comprising of 45 females and 15 males in each group) that attend outpatient department of a private clinic in Sagamu were used for the study. The study was done between

August 2010 and September 2012. The controls were those that attend the clinic but without features of hyperthyroidism. Informed consent was obtained from both the subjects and the controls.

Clinical examination was done on both the subjects and controls to determine associated features of hyperthyroidism. The Hypothalamo-pituitary-thyroid axis was assessed using measurements of TSH, T4 and T3 hormones.

Biochemical parameters: Ten milliliters of blood was collected from both the patients and controls by venepuncture into heparinized bottle and centrifuged at 5000 rpm for 5 min. The clear serum was separated and kept at 20°C till assayed. The hormonal assays were done by Radioimmunoassay (RIA) technique using the kits supplied by Immunometrics (UK) Limited, London. The presence of β -HCG in the serum was used to rule out pregnancy in all the female subjects and controls. The blood pressure of the hyperthyroid patients and controls was determined using mercurial sphygmomanometer in the sitting position with the arm rested. The first and fifth phases of Korotkoff sounds were used for systolic and diastolic respectively. Hypertension was defined as systolic and diastolic blood pressure of 140 and 90 mm Hg, respectively (Chobanian *et al.*, 2003), on two occasions, at least 6 h apart.

Statistical analysis: The descriptive characteristics of the group variables were expressed as mean values and standard deviation. Mean values between the groups were compared using paired T tests. Statistical analysis was done using SPSS version 17 and the level of significance was put at $p < 0.05$.

RESULTS

The percentage distribution of hyperthyroidism by sex is as expressed in Fig. 1. This figure shows that 15 out of 60 (25%) of the studied subjects were male while 45 out of 60 (75%) were female. This result indicates that females are more predisposed to thyroid gland abnormalities than males.

Figure 1 shows the percentage distribution of hyperthyroidism by sex amongst the hyperthyroid subjects. 15 (25%) of the studied subjects were male while female constituted the larger percentage of 45 (75%).

The plasma level of T3, T4 and TSH in male and female subjects shows a significant ($p < 0.05$) difference between the subjects and controls (Table 1, 2). While

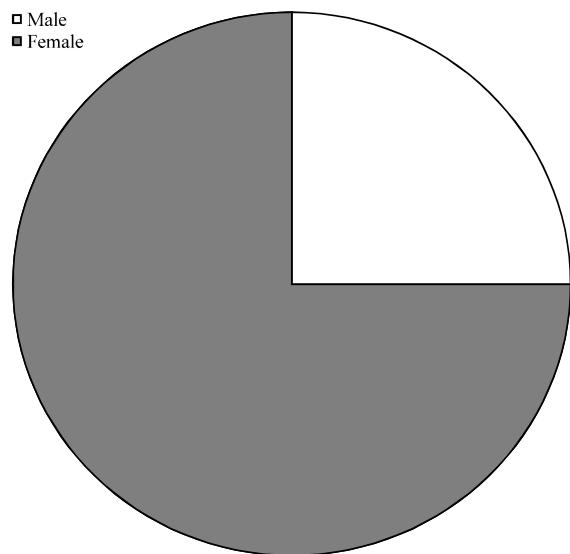


Fig. 1: Percentage distribution of hyperthyroidism by sex

Table 1: Plasma T3, T4 and TSH levels in both female subjects and control

	Subjects	Controls	't-value'	'p-value'
T3 (nmol L ⁻¹)	5.44±0.66	1.50±0.04	5.99	0.000
T4 (nmol L ⁻¹)	259.7±15.03	86.04±1.43	11.40	0.000
TSH (mU L ⁻¹)	0.66±0.06	2.49±0.11	14.70	0.000

Mean±SEM

Table 2: Plasma T3, T4 and TSH levels in both male subjects and control

	Subjects	Controls	't-value'	'p-value'
T3 (nmol L ⁻¹)	6.01±1.27	1.55±0.07	3.481	0.000
T4 (nmol L ⁻¹)	230.07±21.71	88.53±3.10	6.577	0.000
TSH (mU L ⁻¹)	0.66±0.11	2.20±0.16	8.716	0.000

Mean±SEM

there was a significant ($p < 0.05$) increase observed in plasma T3 and T4, a significant ($p < 0.05$) decrease in plasma TSH level was observed. An inverse relationship was noticed to exist between plasma T4, T3 and circulating TSH.

Table 1 showed the hormonal characteristics of the female subjects and controls. The level of statistical significance was considered at $p < 0.05$. Serum T3 and T4 were significantly higher in the subjects while TSH was significantly lower compared to the control group.

Table 2 showed the hormonal characteristics of the male subjects and controls. The level of statistical significance was considered at $p < 0.05$. Serum T3 and T4 were significantly higher in the subjects while TSH was significantly lower compared to the control group.

DISCUSSION

From this study, 25% of the studied subjects were males while females constituted the larger percentage of 75% (Fig. 1). This is in agreement with the earlier findings

of a higher hyperthyroidism incidence in females (AACE, 2006). From Table 1 and 2 it could be seen that the plasma level of thyroxine (T4) and triiodothyronine (T3) were significantly higher ($p < 0.05$) in the subjects (both in the male and female subjects) than the controls while there was a significant decrease ($p < 0.05$) in the plasma levels of thyroid stimulating hormones in the subjects (both in the male and female subjects) than the controls. The significant decrease in the TSH levels is due to negative feedback effect of either excess T3 or T4 or both T3 and T4 levels in the plasma which the anterior pituitary gland interpreted as to mean there was sufficient hormones and thus no basis to secrete TSH into the plasma.

Meanwhile, the physiologic inverse relationship between plasma T4, T3 and circulating TSH had been established and this gives a diagnostic superiority of TSH measurement (Benhadi *et al.*, 2010). The low circulating levels of TSH with high T4 and T3 ruled out sick euthyroidism as the cause of the observed low TSH levels. The low TSH levels may indicate primary failure of the pituitary gland or temporary inhibition of the pituitary gland due to other illnesses. In patients with endogenous subclinical hyperthyroidism, a relatively higher T3 production and low TSH levels might be expected (Belaya *et al.*, 2007). A transient suppression of TSH and stimulation of triiodothyronine may normally be observed during pregnancy (Kol *et al.*, 1996) but this is taken care of in this study by determining the presence of β -HCG in the serum which was found to be negative in all the female subjects and controls.

Isolated systolic hypertension (Bp = 140/<90 mm Hg) was observed in 28% (17 out of 60) of the subjects. This is in support of the earlier work of Prisant *et al.* (2007). The observed hypertension is as a result of the hyperthyroid state in which case the thyroid hormone increases the force of contraction of the cardiac muscle and increase the amount of nitric oxide in the lining of the blood vessels. Also, T3 dilates resistant arterioles, reduces systemic vascular resistance and increases cardiac output and pulse pressure. The forceful cardiac contraction increases the systolic blood pressure while the increased dilation of the blood vessels (caused by the nitric oxide and T3) reduces the diastolic blood pressure (Klein and Ojamaa, 2001).

CONCLUSION AND RECOMMENDATION

It could be concluded and recommended that serum TSH should be used as the first-line test for detecting both overt and subclinical hyperthyroidism in ambulatory patients with stable and intact hypothalamo-pituitary-

thyroid axis base on its sensitivity and ability to detect $<0.01 \text{ mU L}^{-1}$ concentrations. Such individuals must not be receiving drug therapies that alter TSH secretion. Though, it may fail to detect the presence of hypothalamic and pituitary diseases in which case commuted tomographic scan could be of good use. It is however, worthy to note that no one single laboratory test is 100% accurate in diagnosing all types of thyroid disease; however, a combination of two or more commonly used thyroid diagnostic tests can usually detect even the slightest abnormality of thyroid function.

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