MEASUREMENT OF SERUM T_3, T_4 AND TSH FOR THE DIAGNOSIS OF THYROID DYSFUNCTION BY EMPLOYING ELISA METHODOLOGY

Muhammad Abdul Rehman, Muhammad Ashraf and Faiz Ahmed Khan

Thyroid hormone profile was determined by ELISA method in 593 clinically suspected patients of thyroid disorders attending Bahawal Victoria Hospital Bahawalpur during 1988- 1995. 402 (67.7%) Patients proved euthyroid, 110 (18.6%) hyperthyroid and 81 (13.7%)) hypothyroid. The results revealed measurable thyrometabolic disturbances and the measurement of total serum thyroxine using thyrotrophic hormone (TSH) were the most useful in vitro tests for assessing thyroid status.

INTRODUCTION

The prevalence of thyroid disease in the general population is 0.5% but it can be much higher in selected groups of patients[1]. The diagnosis of thyroid diseases is often suspected on the basis of the history and physical examination of the patient. The active circulating hormones of the thyroid are triiodothyronine (T_3) and thyroxine (T_4). the synthesis and release of which are controlled by thyroid stimulatory hormones (TSH) secreted by the anterior pituitary gland'. Diseases of thyroid gland usually manifest themselves through symptoms resulting from excessive or insufficient quantities of these thyroid hormones. Thyrometabolic disorders like hyperthyroidism or hypothyroidism and their subtypes can be detected on clinical measurements assisted by abnormal serum levels of thyroid hormones and other related parameters"[4]. In sub-clinical disease status, definitive diagnosis becomes difficult. However, American Thyroid Association in 1990 and 1991 issued guidelines for use of laboratory tests in thyroid disorders[6,7]. The measurement of serum TSH complemented by free thyroxine provides the efficient combination for diagnosis and follow-up of thyroid disorders.

Bartalena et al.,[8] have reported that the measurement of free T_3 (FT_3) and free T_4 (FT_4) levels represent a more useful index of thyroid status than the measurement of total thyroid hormones. However, TSH levels act as an endogenous indicator of FT_4 and therefore considered best to evaluate the thyroid status[9]. Serum TSH measurement gives the sensitive assessment of primary hypothyroidism during early diagnosis or maintenance of therapy.

Muhammad Abdul Rehman, Asstt. Professor, Department of Pathology, Quaid-i-Azam Medical College, Bahawalpur.

Muhammad Ashraf, Asstt. Professor. Department of Chemistry, Islamia University, Bahawalpur.

Faiz Ahmed Khan, Medical Technologist, Department of Pathology, Quaid-i-Azam Medical College, Bahawalpur.

Bauer and Brown[10] reported that detection of thyroid dysfunctions using specific thyroid tests is controversial. It was suggested that TSH alone and not the combination of TSH and FT_4 tests, should be ordered in most outpatients for the measurement of thyroid disorders. However, if TSH levels are normal, then FT_4 test is recommended. The decision to initiate therapy should be based both on clinical and laboratory findings and not solely on the results of a single laboratory test.

Several sensitive methods have been employed for the measurement of thyroid hormones like RIA (radioimmuno assay), ELISA (enzyme linked immunosorbent assay), IRMA (immunoradiometric assay) etc.[11,13]'. The I-[131] (in the form of NaI^{131}) thyroid uptake and serum levels of thyroid hormones help in achieving a definite diagnosis. However, depending upon the facilities available here at BVH, ELISA was employed as a method to determine levels of thyroid hormones inpatients. The main objectives of this study were, therefore, to compile and assess the pattern of major types of thyroid diseases in the patient population attending Bahawal Victoria Hospital (BVH), Bahawalpur and to analyze the usefulness of these laboratory tests.

MATERIALS AND METHODS

A total of 593 patients (1988-1995) were referred to Chemical Pathology Laboratory, Pathology Department, Quaid-i-Azam Medical College Bahawalpur with suspected thyroid disease. Blood samples were drawn in the day and centrifuged and serum stored until used. Samples were tested for the total T_3, total T_4 and TSH by using the BioMerieux kits.

T_3, T_4 and TSH diagnostic kits were purchased from BioMerieux SA au capital de 45 90 000 F/RCS Lyon B 673 620 399 69280 Marcy-I Etoile (France). Labsystem Uniskan II photometer was used for assays.

For the analysis of triiodothyronine (T_3) and thyroxine (T_4), the assay was based on the ELISA method on a competitive reaction principle. TSH assay was based on ELISA employing monoclonal antibodies. All steps for assay were performed according to the kit manufacturer's instructions.

RESULTS

The results of this study are summarized in tables 1-4.

Table-1: Distribution of T_3, T_4 and TSH Levels Among Patients Attending BVH During 1988-95

Sex	T_3 (ng/mL)	T_4 (µg/dL)	TSH (µIU/mL)
Male	l.473 ± 0.11 range = 0.07-8 (n = 169)	8.109+0.38 range = 0.5-35 (n = 168)	4.213± 1.428 range = 0.2-40 (n = 40)
Female	1 .68 ±10.018 range = 0.005-12 (n= 416)	8.595+0.29 range= 0.01-65 (n = 416)	4.746±1.247 range= 0.14- 53 (n = 81)

Table 2. Percent of Males and Females at Different Stages of Thyrometabolic Disorders

	Male	Female
Euthyroid	107(62.9%)	295(69.8%)
Hyperthyroid	32(18.8%)	78(18.4%)
Hypothyroid	31(18.3%)	50(11.8%)
Total	170(100%)	423(100%)

Table-3: Expected Values of T_3, T_4 and TSH[17]

	T_3	T_4	TSH
Euthyroid	1.02.8 nmol/L	50-137 nmol/L	0.25-5 µlU/ml.
Hyperthyroid	>3.0 nmol/L	>137 nmol/L	<0.15 µlU/mL
Hypothyroid	<1.0 nmol/L	<50 nmol/L	>7.0 µIU/ml.

Table-4: Ratio of T_3:T_4: TSH Measured front the Same Serum

Sex	T_3	T_4	TSH
Male	1 .786+	9.3171	4.2131
(n= 80)	0.246	0.962	1.428
Female	1.315±	7.2691	4.7461
(n= 81)	0.169	0.629	1.247

Total number of studied patients were 593 including 170 (28.7%) males and 423 (71.3%) females. The data indicated 402 (67.7%) patients of euthyroid, NO (18.6%) were hyperthyroid and 81 (13.7%) as hypothyroid as per laboratory criteria. The mean ±S.E. levels of serum T_3 were 1.473±0.1 ng/mL in males and 1.68±0.018 ng/mL in females. Serum T_4 levels were estimated to be 8.109±0.387 ug/dL in males and 8.595±0.29 µg/dL in females. Mean serum TSH levels in males were 4.2l3±1.428pIU/mL and in females these levels were 4.746±1.247pIU/mL (Table-). Maximum value of serum T_3 was 8.00 ng/mL in males and 12.00 ng/mL in females whilst minimum values were 0.07 ng/mL and 0.005 ng/mL. respectively.

The minimum value of serum T_4 was 0.5 pg/dL in males and 0.01 µg/dL in females and the maximum values were 35.0 µg/dL and 65.0 µg/dL, respectively. The minimum level of serum TSH was 0.2 µlU/mL in males and 0.14 µIU/mL in females and maximum levels were 40.0 µIU/mL and 53.0 µlU/mL. respectively (Table-1).

These results show that higher number of females (71.3%) were suggested to have thyroid functions tests done than their male counterparts (28.7%). Females exhibit higher mean levels of T_3, T_4 and TSH than the males and also the minimum and maximum level ranges of these hormones are more variable in females than males (Table I). For example, the minimum value of T_3 in females is 0.005 ng/mL whilst this value in males is 0.07 ng/mL.

When the same data was expressed in terms of the thyrometabolic disorders, 69.8% females were euthyroid than 62.9% whilst 11.8% females exhibited hypothyroidism than 18.3% in males. However, hyperthyroidism frequency was similar in both the sexes (i.e. ca. 18%) (Table 2). All this data was computed based on the normal values of the thyroid hormones given in Table 3.

Among the patients, 40 males and 81 females were suggested to have tested for levels of all the three thyroid hormones, i.e. T_3, T_4 and TSH. Table-4 reveals the patterns of these hormones from the same serum. T_4/TSH ratio in males is 2.21 and in females this ratio is 1.53. where T_4 indicates the total T_4 levels. Levels of all these hormones fall in the category of euthyroidism. This relationship among hormones establishes the usefulness of ELISA for the diagnosis of thyrometabolic disorders.

DISCUSSION

In general, our findings confirm the commonly held belief that the measurement of the total T, in serum still occupies a prominent position for its efficient performance during the initial stages of screening patients with suspected thyroid disease. The view is supported by the fact that the highest correlation of TSH is observed with serum total T_4. Since the TSH level serves as an endogenous indicator of the biologically active free T_4 fraction. In addition, TSH levels have other advantages over free T_4 estimates in confirming the presence of thyroid disease[14].

An ideal test for thyroid functions should give an accurate measure of thyroid hormonal activity. The findings confirm the experience of others that the measurement of total T_4 in serum is still useful and thus prevalent in screening for suspected thyroid disease[13]. The correlation of thyroid with serum total T_4 was the most useful parameter. Further evaluation of other well established tests (T_3 and TSH) allowed certain aspects of mildly disturbed function to be more clearly defined. In our study, the pattern of various thyroid diseases in referred patients appears to be similar to that seen in other Countries 15-16.

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