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EVALUATION OF THYROMETABOLIC DISORDERS ON THE BASIS OF SDS-PAGE AND ITS COMPARISON WITH BIOCHEMICAL PARAMETERS

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Abstract: In the present study, the elevated values in thyroid disorders showed a higher percentage of electrophoretic patterns and a significantly higher in comparison with euthyroid. The regulation of biochemical analytes for evaluating the thyrometabolic disorders (TMD) has a profound effect on the pathogenesis and the proliferation of thyroid disease and the correlation between biochemical analytes and thyroid disorders are important in the development and prognosis of TMD. Our findings suggest that the evaluation of biochemical analytes in thyroid disorder if not interpreted might lead to complications and the pathogenesis of the disease. The interpretations in time may provide a useful tool for biochemical diagnosis of thyroid disorders. In addition, a novel way of diagnosis and timely intervention may help to control and manage further spread of the disease.

Key words: Electrophoretic pattern, thyrometabolic disorders, thyroid disease.

INTRODUCTION

The thyroid gland is one of the largest endocrine organ. It is first recognized at about one month after conception (Attia *et al.,* 1991). The normal thyroid is made up of two lobes joined by a thin band of tissue-the isthmus. The right lobe of thyroid gland is normally more vascular than the left. The formation of normal quantities of thyroid hormones ultimately depends upon the availability of adequate quantities of exogenous iodine (Fattu *et al.,* 1982; Alexander, 1984). Thyroid hormones are necessary for the development of the central nervous system during the late fetal and early post-natal life. In their absence, there is irreversible

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mental retardation. Thyroid hormone is also necessary for the proper functioning of the cardiovascular system, gastro-intestinal tract, immune mechanisms and growth in general. The hormones are also important in the adult as a deficiency results in mental sluggishness and torpor development. The thyroid gland stores sufficient hormone to last about three months. The endocrine system consists of a number of ductless glands which manufacture certain chemical substances that take an essential part in the life processes of the body (Ingbar, 1985; Guyton, 1991). The Lab's parameters and profiles of different tests are utilized all over the world to acquire vital information of diagnostic importance which may not be apparent from the history, routine biochemistry, hematology or urinalysis (Galbwell *et al.,* 1985). Certain parameters are very valuable in identifying diseases which may not have been detected by routine diagnostic work up. The abnormalities in lab patterns are better correlated with the patients' clinical data. The main purpose of this study was to look into the pattern of major types of thyroid disorders on the basis of thyroid profiles, biochemistry analysis and SDS-PAGE electrophoreses in the patient population attending the Sheikh Zayed Hospital, Lahore.

MATERIALS AND METHODS

Equipment

1. Packard Gamma Counter (Minaxi Gamma – Autogamma 5000 Series, Model 5550
2. Refrigerated Centrifuge (CRU – 5000 Damon / IEC Division, England)

Random blood samples were drawn during the day time. Most of the patients were outpatients of Sheikh Zayed Hospital, Lahore. The samples were immediately centrifuged at 20 °C. Serum thus separated was stored in three parts of 2-3 ml aliquots at -80 °C until assayed in the ultra cold freezer available. The first part/aliquot was used to determine glucose, albumin, hemoglobin, triglycerides, cholesterol, creatin kinase, alkaline phosphatase and total protein (Kingsley, 1939) was tested for biochemical analyses. The second part was used in thyroid profile which was determined by employing radio-immunoassay methodology for total T3, total T4 and ultra TSH by using kits of Coat-a-Count from Diagnostic

Product Corporation, Los Angeles, CA, USA. The third part was used for electrophoretic pattern on the basis of SDS-PAGE which was developed by Tiselius (1939) and Laemmli (1970). Polyacrylamide gel (PAG) 12%, was prepared using thyroid disordered samples. PAG of 8 % and 15 % were prepared for high molecular (HMW) weight and low molecular weight (LMW) fractions.

RESULTS

A total of 425 consecutive new patients (July 1989 to June 1990) were referred to our advanced diagnostic research and radio-immunoassay (ADRIA) Lab., at Shaikh Zayed Post-graduate medical complex at Sheikh Zayed Hospital, Lahore. Out of 425 patients, 187 were found euthyroid, 157 were hyperthyroid and 81 were hypothyroid. Instead of taking the whole lot, we took only ten representative samples of each group to run for analyzing in terms of biochemical and SDS-PAGE.

Hyperthyroid group showed a total of 21 fractions having a molecular weight of 87-16 kDa which appeared in both groups (euthyroid and hyperthyroid) except one fraction of 35 kDa which played a moderate elevation of 29%. Most of the fractions in hyperthyroid group were markedly reduced when compared to euthyroid subjects.

In the hypothyroid group, a total of 49 fractions were detected, 23 fractions of high molecular weight and 26 fractions of low molecular weight. Among euthyroids, 17 fractions of HMW and 21 fractions of LMW appeared. Sixteen fractions were common in both groups (euthyroid and hypothyroid). One fraction of euthyroid did not appear in the hypothyroid group. However, 7 new fractions with a molecular weight of 325 - 60 kDa were detected in the hypothyroid group which were absent in the euthyroid groups shown in Table 3. Among LMW proteins, 21 fractions appeared in euthyroid group, of which 20 fractions are found to be common in both groups *i.e.,* euthyroid and hypothyroid subjects. One fraction of the euthyroid, on the other hand, could not be detected in hypothyroid group. Twenty six LMW fractions were detected in hypothyroid subjects, of which 6 were new fractions. The molecular weight of these new fractions ranged from 55kDa to 12kDa. These fractions were non-existent in the euthyroid subjects except 26kDa fraction which was undetected following the development of hypothyroidism but appeared in euthyroid subjects.

Biochemical Profile

We took only ten representative samples of each group and analyze them for glucose, albumin, hemoglobin, triglycerides, cholesterol, creatin kinase, alkaline phosphatase and total protein. The biochemical analysis of the three groups and compare to euthyroid groups as control groups. Highly significant differences were found in these groups. The biochemical analysis was determined to see the metabolic changes due to the faster rate of catabolism and anabolism.

Table I: Details of the fractions seen on SDS-PAGE in hypothyroid cases.

Variable	Hypothyroid		Euthyroid	
	LMW	HMW	LMW	HMW
No of fractions appearing	26	23	21	17
Common fractions	20	16	20	16
Fractions unappeared	-	-	01	-
New Fractions	06	07	-	-

LMW, low molecular weight; HMW, high molecular weight.

Table indicates that the ratio of albumin, triglycerides, cholesterol, creatin kinase and alkaline phosphatase were increased in hypothyroid case while decreased in the cases of hyperthyroid. The levels of glucose and total protein was decreased in hypothyroid case and increased in hyperthyroid. The level of alkaline phosphatase remains normal in euthyroid and hyperthyroid case. The hemoglobin remains normal in euthyroid, hypothyroid and hyperthyroid. By simply having the elevated values of albumin, triglycerides, creatin kinase and alkaline phosphatase in suspected cases; we can see the complications of this disease if not controlled. The study focused its objectives on the assessment of the biochemical analysis and thyroid profiles to predict the early diagnosis on any emerging abnormal patterns of fractional changes which were analysed biochemically by comparing with the healthy subjects. From this study and by comparing the biochemical analytes of TMD were found to be either present or absent or significantly increased or decreased depending on the grading and severity of the disease of TMD. From these findings, it may be

said that the biochemical analysis and thyroid profiles can be of significant importance in the early diagnosis of TMD.

Table II: The biochemical analysis of the three groups.

PARAMETERS	EUTHYROID	HYPOTHYROID	HYPERTHYROID
Albumin	N	I	D
Hemoglobin	N	N	N
Glucose	N	D	I
Triglycerides	N	I	D
Cholesterol	N	I	D
Creatin Kinase	N	I	D
Alkaline Phosphat	N	I	N
Total Protein	N	D	I

N, Normal; I, Increased; D, Decreased.

SDS-PAGE

Different proteins have different molecular weights (sizes) at a desired pH (which plays a critical role) have different electrical charges. The advanced techniques of electrophoresis can help in predicting the very early onset of metabolic disorders, and disorder usually associated with the non-specific alterations of serum proteins. As far as the importance of electrophoresis is concerned it has emerged as a powerful diagnostic tool in clinical biochemistry. Serum proteins electrophoresis separates normal serum proteins into five major fractions; albumin, α-1 globulin, α-2 globulin, β-globulin and γ-globulin. The albumin comprises 55 % and the globulin make up the remaining 45 % of the total proteins. Electrophoretic patterns are invaluable in the study of many pathological conditions with relations to thyrometabolic disorders, in which distribution and amounts of proteins constituents are altered. The normal human serum proteins have been compared with the altered proportions of the protein in the diseased states of thyroid disorders. Decreased serum albumin has been found to result from increased degradation caused by the over-activation of thyroid hormones. The present investigation is carried out to determine the protein fractions resolved by the SDS-PAGE among euthyroid, hypothyroid and hyperthyroid individuals. Among LMW proteins, 21 fractions appeared in euthyroid group, of which 20 fractions are found to be common in both groups *i.e.,* euthyroid and hypothyroid subjects. One fraction of the euthyroid/healthy subjects, on the other hand, could not be detected in

hypothyroid group. Twenty six LMW fractions were detected in hypothyroid subjects of which 6 were new fractions ranged from 55-12 kDa. These fractions were non-existent in the euthyroid subjects except 26 kDa fraction which was undetected following development of hypothyroidism but appeared in euthyroid subjects.

DISCUSSION

In the present study, the elevated values in thyroid disorders showed a higher percentage of electrophoretic patterns and a significantly higher in comparison with euthyroid. The regulation of biochemical analytes for evaluating the thyrometabolic disorders (TMD) has a profound effect on the pathogenesis and the proliferation of thyroid disease and the correlation between biochemical analytes and thyroid disorders are important in the development and prognosis of TMD (Fattu *et al.,* 1982). Our finding suggests that the evaluation of biochemical analytes in thyroid disorder if not interpreted might lead to complications and the pathogenesis of the disease. The interpretations in time may provide a useful tool for biochemical diagnosis of thyroid disorders. In addition, a novel way of diagnosis and timely intervention may help to control and manage further spread of the disease. Besides clinical acumen, diagnostic facilities play an important role in good medical practice. The development of newer laboratory techniques or newer uses of available techniques is of paramount importance in the field of medicine (Galbwell *et al.,* 1985). The use of electrophoresis in the diagnosis of thyroid disorders is a new avenue. There is a paucity of the literature on the subject. Since very little work has been undertaken the present study might open to many criticisms. Since no such work was ever undertaken as well as due to paucity of the literature, the work produced was quite open the new avenues for the better clinical management of the patients which would help to diagnose the early symptoms of the disease. The findings presented in this study suggest that SDS-PAGE may be a useful tool in the diagnosis of thyroid disorders while the other studies elsewhere support our findings (Laemmli, 1970; Fraklin, 2009).

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