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SERUM PROTEIN FRACTIONS IN HYPERTHYROID WOMEN

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Abstract: Serum samples of healthy women and hyperthyroid patients were obtained alongwith the radioimmunoassay values of triiodothyronine, thyroxine and thyroid stimulating hormone. Samples were diluted in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye. SDS-PAGE was employed for studying the protein profile of healthy and hyper thyroid subjects. Gels were photographed and their images were stored for quantification of protein fractions by UVP Gel Base Software Programme that provides the data of molecular weights and percent areas covered by each of the fractions. Data was employed in finding the enhancement or reduction and the appearance or disappearance of particular protein fractions for comparison between healthy and hyperthyroid groups. A total of 21 fractions were detected in normal subjects, all of which were expressed in hyperthyroid group. Most of the fractions in hyperthyroid group were found to be markedly reduced when compared to healthy group. No new fractions were encountered in hyperthyroid compared to healthy group.

Key words: Hyperthyroidism, protein fractions, electrophoresis.

INTRODUCTION

The widespread metabolic role of thyroid hormones, thyroxine and triiodothyronine, commonly called T_4 and T_3, the diverse processes involved in the synthesis, secretion and metabolism of the hormones, and the complex mode of regulation of thyroid function indicate that a great many factors could influence one or more aspects of thyroid hormone economy. Formation of normal quantities of thyroid hormone ultimately depends upon the availability of adequate quantities of exogenous iodine. Although efficient mechanisms exist to conserve iodine in the presence of iodine deficiency, they do not entirely succeed in preventing depletion of iodine stores, ultimately this may lead to insufficient hormone production. The resulting marginal iodine deficiency does, however, predispose to the development of hyperthyroidism upon exposure to sources of additional iodine. The activity of the iodide transport mechanism is influenced by a variety of physiological factors, the most important of which is the level of TSH stimulation. TSH is the major regulator of the morphological and functional state of the thyroid. An increase in both the serum T_4 and T_3 concentrations is the usual pattern in patients with hyperthyroidism. Serum T_4 concentration ranges from values that are only slightly elevated in patients with mild disease to values in excess of 20 μg/dl in the most severe cases. Concentrations of T_3 are almost invariably increased, sometimes to levels that are many times the mean normal

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value. Usually the increase in T_3 concentration is proportionately greater than the increase in serum T_4, so that the T_3/T_4 ratio in serum is almost always elevated (Ingbar, 1985).

The physiologically observable effect of thyroid hormone is due to the new or increased protein synthesis resulting from a new or increased amount of specific mRNA (Rall, 1978). An increase in enzyme activity due to an increase in enzyme protein is the result of a decreased rate of degradation of that protein rather than increased synthesis (Shimke and Doyle, 1970). An increase in the rate of synthesis of serum albumin in man caused by thyroid hormone was clearly demonstrated 20 years ago (Schwartz, 1955; Rothschild *et al.*, 1957; Lewallen *et al.*, 1959). Subsequently, overall protein synthesis was shown to be increased by thyroid hormone and this was considered to be the main effect (Krause and Sokoloff, 1967; Sokoloff and Kaufman, 1961; Tata, 1962; Tata *et al.*, 1963).

Muscle weakness occurs in most patients with hyperthyroidism (thyrotoxic myopathy) and when the hyperthyroidism is severe and prolonged, the myopathy may be severe. The muscle weakness may be due to part of increased protein catabolism (Ganong, 1995). Thyroxine increases the rate of metabolism of all cells, and as a result, indirectly affects protein metabolism. If insufficient carbohydrates and fats are available for energy, thyroxine causes rapid degradation of proteins and uses these for energy. On the other hand, if adequate quantities of carbohydrates and fats are available and excesses of amino acids are also available in the extracellular fluid, thyroxine can actually increase the rate of protein synthesis. Conversely, in growing animals deficiency of thyroxine causes growth to be greatly inhibited because of lack of protein synthesis. In essence, it is believed that thyroxine has little specific direct effect on protein metabolism but does have an important general effect in increasing the rates of both normal anabolic and normal catabolic protein reactions (Guyton, 1991).

Attempts to manipulate various metabolites by endocrine intervention have been made for last several years and protein metabolism in relation to thyroid hormones has been extensively studied. However, the studies regarding electrophoretic protein profile in response to thyroid pathophysiology are meagre and almost non-existent. By keeping in view the importance of proteins in so many physiological phenomenon and the role thyroid hormones play in protein metabolism, the present investigation is carried out to emphasize the effect of thyroid hormone excess on serum protein fractions of female subjects resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Serum samples of healthy women and hyperthyroid patients were obtained from the Institute of Nuclear Medicines and Oncology and Sheikh Zayed Hospital, Lahore along with the radioimmunoassay values of T_3, T_4 and TSH (Fig.1). Samples were diluted in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye.

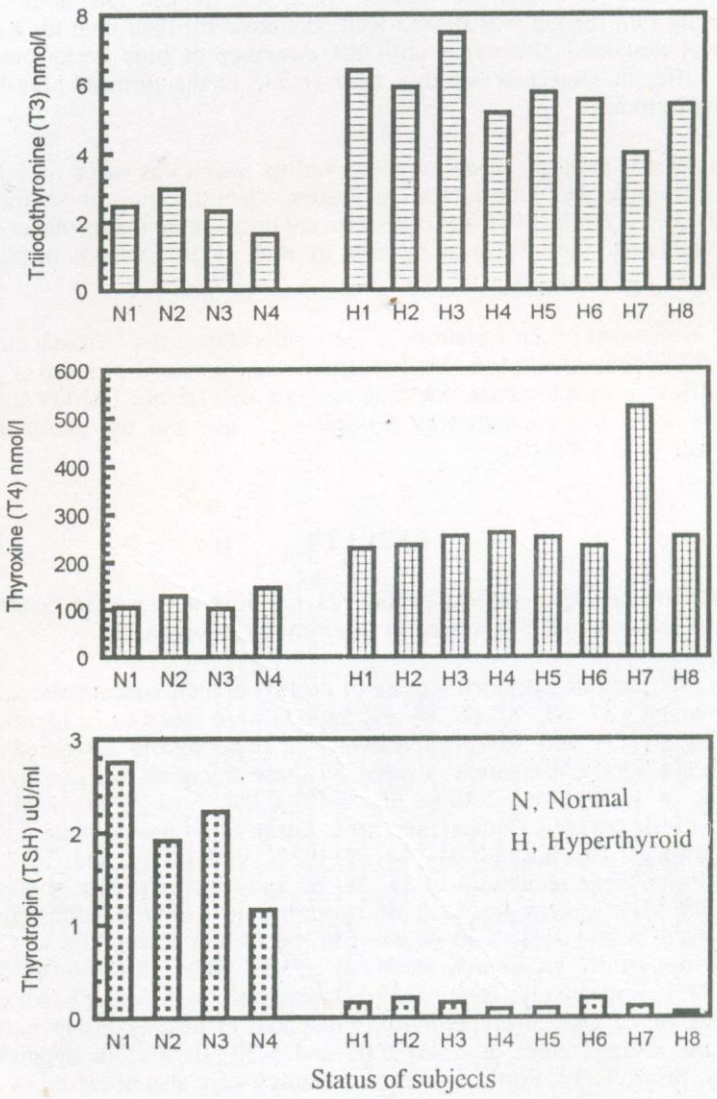

Fig. 1: **Serum hormonal levels of healthy and hyperthyroid women collected from hospitals.**

Polyacrylamide gel (12%) was prepared using the method of Laemmli (1970). Protein size markers and each of the samples were loaded in separate wells and gel was electrophoresed at a current supply of 12 mA and voltage of 150 V, in a cooling chamber maintained at 4°C, for almost three and a half an hour. Following electrophoresing run, the gel was stained with coomassie brilliant blue for a duration of two hours and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gel was photographed afterwards and its image was saved on a floppy disk with image store 5000 Gel Documentation System. Quantification of separated protein fractions was carried out by UVP gel base software programme that provides the data of molecular weights and the total areas covered by each of the fractions displayed in the form of peaks in the histogram.

For the assessment of the variations in the results among the different stages within a phase, values of individual goats were averaged and expressed as mean ± SEM. For level of significance in the means one way analysis of variance (ANOVA) was used. Sample means were also compared by Student's "t" test and the minimum level of significance was set at $P<0.05$.

RESULTS

In an overall picture of protein profile, 21 fractions appeared in healthy subjects and all of these fractions were expressed in hyperthyroid subjects.

Most of the fractions exhibited a trend of decline in their concentrations. Fractions of molecular weights 87, 84, 70, 66, 64 and 58 kDa were found to be highly depressed by 38, 51, 49, 51, 51 and 36%, respectively, in hyperthyroid compared to healthy subjects. The average percent areas covered by these fractions in hyperthyroid group were observed at 1.18±0.06, 2.19±0.06, 3.43±0.07, 7.19±0.08, 4.60±0.15 and 6.86±0.06%, respectively. Comparable areas in healthy group were estimated at 1.90±0.12, 4.45±0.10, 6.75±0.04, 14.78±0.22, 9.40±0.34 and 10.70±0.35%, respectively. Pronounced reductions of 37, 38, 51 and 28% were also observed by 56, 52, 43 and 39 kDa protein fractions of hyperthyroid group covering the average respective areas of 2.26±0.04, 3.40±0.04, 1.86±0.11 and 3.36±0.06%. Comparable areas covered by healthy group were observed at 3.58±0.11, 5.48±0.08, 3.83±0.06 and 5.43±0.74%, respectively. A single 35 kDa fraction, on the other hand, displayed a moderate elevation of 29% in hyperthyroid compared to healthy group indicating the coverage of the average areas of 6.86±0.07 and 5.30±0.78%, in hyperthyroid and healthy group, respectively. Further, marked decreases were also observed in 25, 22, 19 and 16 kDa protein fractions covering the average areas of 3.40±0.08, 2.14±0.11, 1.08±0.05 and 1.14±0.07% in hyperthyroid and 5.45±0.04, 3.43±0.06, 2.23±0.20 and 2.83±0.11% in healthy group, respectively. The reductions were found to be 38, 38, 52 and 60%, respectively, in hyperthyroid compared to healthy group. Fractions of molecular weights 100, 95, 48, 33, 28 and 14 kDa, however, did not seem to be affected appreciably (Fig.2).

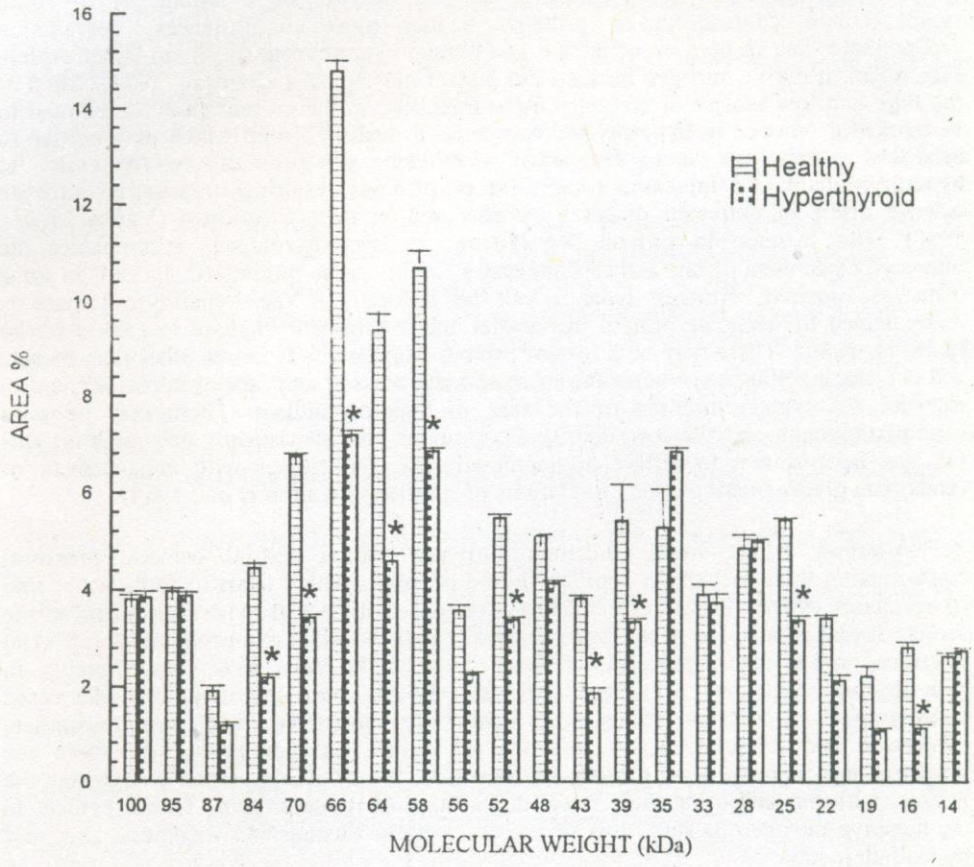

Fig. 2: Average percent area covered by various protein fractions resolved by SDS-PAGE, in healthy and hyperthyroid groups. Values are mean ± SEM. *Significance at $P < 0.05$.

DISCUSSION

The thyroid hormones increase the metabolic activities of all or almost all of the tissues of the body. The basal metabolic rate can increase to as much as 60 to 100% above normal when large quantities of the hormones are secreted. The rate of utilization of foods for energy is greatly accelerated. Although the rate of protein synthesis is increased, at the same time, the rate of protein catabolism is also increased (Guyton, 1991).

Hyperthyroidism, in experimental animals, results in a stimulation of both synthesis and degradation of proteins. Under most circumstances, degradation predominates and in both experimental and human hyperthyroidism, there is net protein catabolism, negative nitrogen balance and loss of muscle mass (Ramsay, 1974). Most of the high and low molecular weight protein fractions, in the present study, are found to be markedly reduced in hyperthyroid compared to healthy group which may be due to increased catabolism and decreased anabolism of proteins in response to hyperthyroidism. An increased catabolism of proteins resulting in negative nitrogen balance occur in untreated diabetes mellitus and in hyperthyroidism (Varley *et al.*, 1980). The increase in protein degradation, in hyperthyroidism, accompanies the increased catabolism of fats and carbohydrates, but it can be minimized. Indeed, in some instances, positive nitrogen balance can be induced, if the hyperthyroid state is accompanied by adequate protein in the diet and a sufficient increase in caloric intake (DuBois, 1936). There may be a loss of protein from the body stores other than muscle and decreased collagen synthesis and increased degradation may, for example, account in part for the typical thinning of the skin in hyperthyroidism. There may be mild hypoalbuminemia and the low density lipoproteins characteristically fall slightly; this fall has been shown to reflect on increased rate of turnover with enhancement of catabolism predominating over stimulations of synthesis (Walton *et al.*, 1965).

Albumin is the main contributor to the plasma colloid osmotic pressure, counteracting the effect of the capillary blood pressure, which tends to force water into tissue spaces (Varley *et al.*, 1980). Most pronounced impact of hyperthyroidism, in the present investigation, has been observed on albumin (66 kDa) and proalbumin (58 kDa) fractions, indicating appreciable reductions of 52% and 36%, respectively, in hyperthyroid compared to normal subjects. It has been reported that decreased concentration of plasma albumin is commonly associated with hyperthyroidism (Mckenzie and Zakarija, 1989). Ingbar (1985) has also commented that both the synthesis and degradation of protein are increased, the later to a greater extent than the former, with the result that there is net degradation of tissue protein. This is evident in the negative nitrogen balance, loss of weight, muscle wasting and weakness, and mild hypoalbuminemia.

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