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ELECTROPHORETIC ANALYSIS OF SERUM PROTEIN FRACTIONS IN HYPOTHYROID WOMEN

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Abstract: Clinical facility, for the present study, was available at the Institute of Nuclear Medicines and Oncology (INMOL) and Sheikh Zayed Hospital (SZH), Lahore. Serum samples of healthy women and hypothyroid patients were obtained along with the radioimmunoassay values of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH). Samples were diluted in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), based on the method of Laemmli (1970), was employed for studying the protein profile of healthy and hypothyroid subjects. Gels were photographed and their images were stored for quantification of various 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 hypothyroid groups. Thirty-eight fractions were detected in normal whereas 49 were detected in hypothyroid subjects. Most of the fractions in hypothyroid group were found to be significantly enhanced when compared to healthy group. Fractions of molecular weights 156 and 26 kDa, detected in healthy group, could not be expressed in hypothyroid group, however, thirteen new fractions of molecular weights 325, 123, 91, 68, 65, 62, 60, 55, 50, 40, 31, 17 and 12 kDa were found to appear in hypothyroid group which could not be detected in healthy group.

Key words: Gel electrophoresis, hypothyroidism, protein fractions.

INTRODUCTION

The thyroid gland secretes two significant hormones thyroxine and triiodothyronine, commonly called T_3 and T_4, that have profound effect of increasing the metabolic rate of the body (Guyton, 1991). Different consequences follow a primary alteration in the rate of hormone supply. For example, in hyperthyroid states, hypersecretion of hormone leads to an increase in total hormone concentration. As a result, the concentration of unoccupied binding sites on total binding globulin decreases and the concentration of both free and bound hormone

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rise. Converse consequences occur when the supply of hormone is decreased, as in hypothyroidism (Ingbar, 1985).

Inadequate synthesis of thyroid hormone leads to hypersecretion of TSH, which in turn produces both goiter and stimulation of all steps in hormone biosynthesis capable of response. This compensatory response may be inadequate and goiter with hypothyroidism or cretinism results. Hence, hypothyroidism follows upon an inadequate supply of active thyroid hormone to the peripheral tissues. The term implies failure of adequate production of hormone within the thyroid gland.

Thyroid hormone increases the metabolic rate of almost all tissues, one important exception being the adult brain. Through their stimulating effect on protein, carbohydrate and fat metabolism, thyroid hormones profoundly affect the functions of almost every organ system of the body. A protein anabolic effect of thyroxine has been demonstrated in the young hypophysectomized rat. The growth of nearly all tissues may be stimulated by thyroxine in the absence of pituitary hormones. The protein content of pelt, including hair is greatly increased.

Physiologic doses of thyroxine must be employed in order to demonstrate protein anabolic effects; toxic amounts of the hormones, however, do not stimulate growth (Scow, 1955). In hypothyroid children, small doses of thyroid hormones cause a positive nitrogen balance because they stimulate growth, but large doses cause protein catabolism similar to that produced in the adult. 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).

Polyacrylamide gel electrphoresis (PAGE) of serum proteins has an important role as a diagnostic investigation. 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 hypothyroid 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 ultrafiltered in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye.

Polyacrylamide gels, 8% for high and 15% for low molecular weight protein fractions, were prepared using the method of Laemmli (1970). Protein size markers and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 12 mA and voltage of 150 V, in a cooling chamber maintained at 4°C

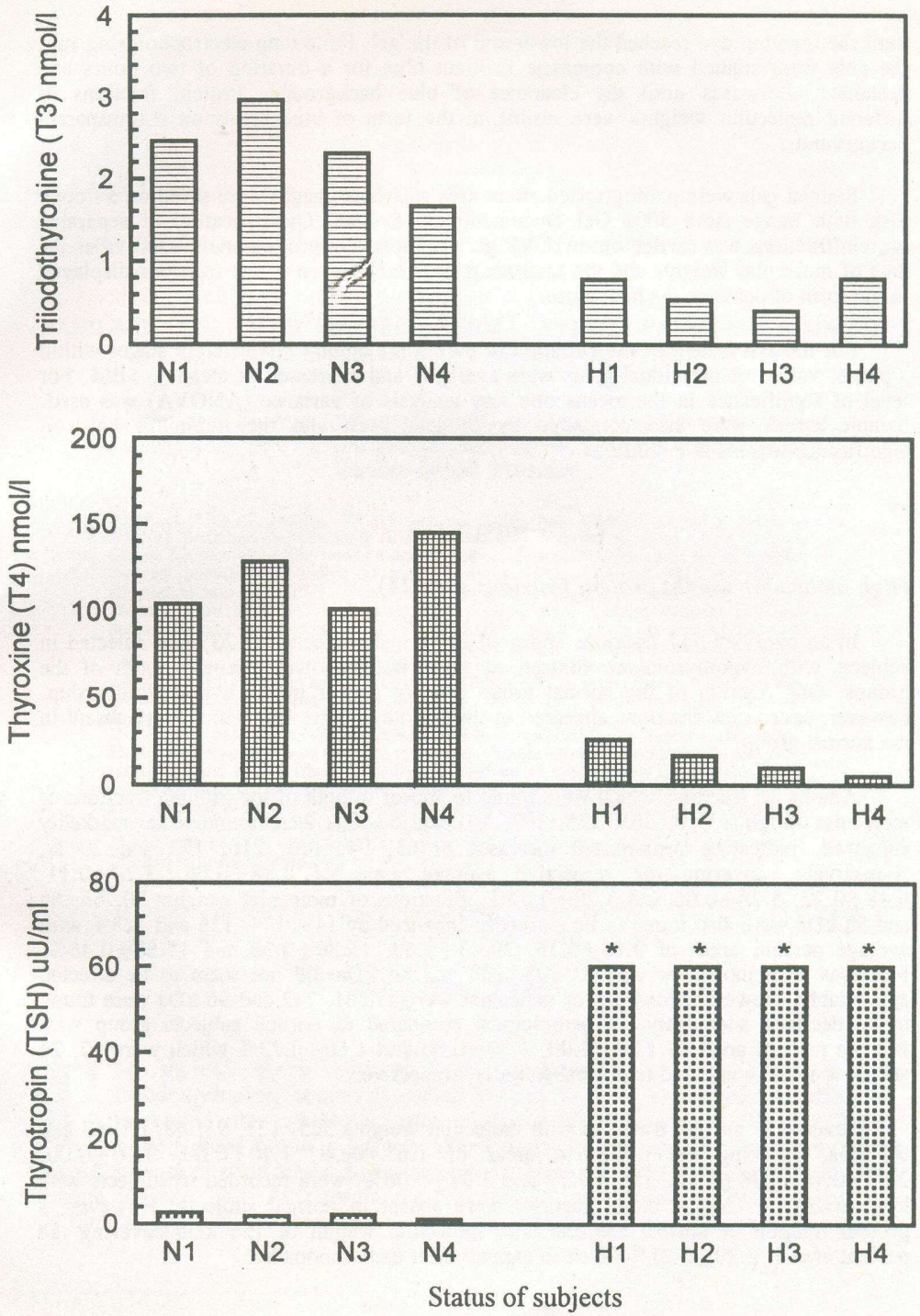

Fig. 1: Serum hormonal levels of healthy and hypothyroid women collected from hospitals. Abbreviations used: N = Normal; H = Hypothyroid, * = TSH level >60 μU/ml.

until the tracking dye reached the lower end of the gel. Following electrophoresing run, the gels were 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 gels were photographed afterwards and their images were 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 variation in the results among the different stages within a phase, values of individual goats were averaged and expressed as mean $\pm$ SEM. For level of significance in the means one way analysis of variance (ANOVA) was used. Sample means were also compared by Student t-test and the minimum level of significance was set at $P<0.05$.

RESULTS

High molecular weight protein fractions (Fig.2A)

In an overview, 17 fractions appeared in normal subjects and 23 were detected in subjects with hypothyroidism. Sixteen of these fractions were same in both of the groups. One fraction of the normal group did not appear in the hypothyroid group, however, seven new fractions appeared in the hypothyroid group, which were absent in the normal group.

Among 16 fractions which were found to appear in both of the groups, fractions of molecular weights, 310, 265, 225, 177, 141 and 88 kDa were found to be markedly enhanced, indicating pronounced increases of 63, 94, 110, 216, 173 and 204%, respectively, covering the respective average areas of 3.18 ± 0.06, 3.20 ± 0.11, 6.48 ± 0.22, 5.28 ± 0.06 and 3.28 ± 0.07%. Fractions of molecular weights 70, 66, 65 and 58 kDa were also found to be markedly enhanced by 143, 174, 126 and 125% with average percent areas of 9.85 ± 0.15, 29.43 ± 0.60, 15.30 ± 0.36 and 17.88 ± 0.48%. Fractions with molecular weights 198, 100 and 84 kDa did not seem to be affected appreciably. However, fractions of molecular weights 281, 247 and 96 kDa were found to be declined adequately, in pathological compared to normal subjects group with average percent areas of 1.20 ± 0.00, 1.50 ± 0.00 and 1.60 ± 0.23% which were 40, 24 and 34% lower compared to normal subjects, respectively.

Seven new protein fractions with molecular weights 325, 123, 91, 68, 65, 62 and 60 kDa, covering the respective areas of 1.65 ± 0.08, 1.10 ± 0.00, 3.10 ± 0.00, 2.93 ± 0.11, 3.38 ± 0.06, 5.45 ± 0.29 and 2.98 ± 0.06%, were recorded in subjects with hypothyroidism. All of these fractions were absent in normal subjects. However, a protein fraction of normal subjects with molecular weight of 156 kDa covering the percent area of 2.20 ± 0.21% failed to express after endocrinopathy.

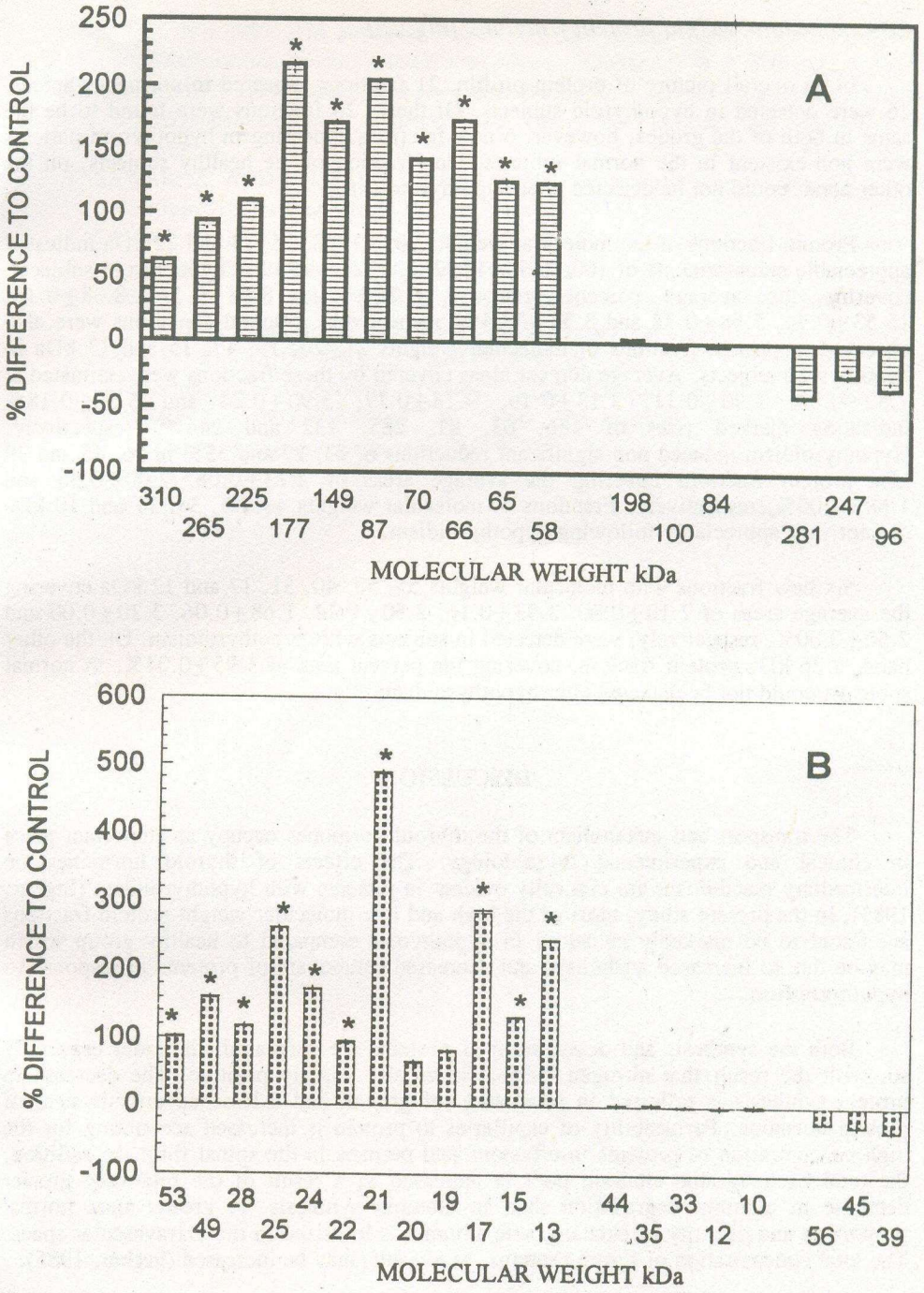

Fig. 2: **Percent difference to control of high (A) and low (B) molecular weight protein fractions resolved by SDS-PAGE, in healthy and hypothyroid groups. Values are mean ± SEM. *Sigificance at $P < 0.05$.**

Low molecular weight protein fractions (Fig.2B)

In an overall picture of protein profile, 21 fractions appeared in normal, whereas, 26 were detected in hypothyroid subjects. Of these, 20 fractions were found to be the same in both of the groups, however, 6 new fractions appearing in hypothyroid patients were non-existent in the normal subjects. One fraction of the healthy subjects, on the other hand, could not be detected after hypothyroidism.

Protein fractions of the molecular weights 53, 49, 28, 25, 24 and 22 kDa indicated appreciable enhancements of 100, 159, 117, 261, 172 and 93% in hypothyroid subjects, covering the average percent areas of 8.25 ± 0.18, 8.88 ± 0.11, 8.08 ± 0.18, 15.53 ± 0.36, 5.58 ± 0.22 and 3.38 ± 0.04%, respectively. Marked elevations were also observed in protein fractions of molecular weights 21, 20, 19, 17, 15 and 13 kDa in hypothyroid subjects. Average percent areas covered by these fractions were estimated at 7.80 ± 0.08, 3.90 ± 0.11, 3.13 ± 0.19, 9.23 ± 0.17, 3.90 ± 0.24 and 5.50 ± 0.18% indicating marked rises of 486, 63, 81, 285, 132 and 244%, respectively. Hypothyroidism induced non-significant reductions of 23, 27 and 35% in 56, 45 and 39 kDa protein fractions covering the average areas of 2.23 ± 0.08, 2.00 ± 0.35 and 1.68 ± 0.05%, respectively. Fractions of molecular weights 44, 35, 34, 11 and 10 kDa did not vary appreciably following hypothyroidism.

Six new fractions with molecular weights 55, 50, 40, 31, 17 and 12 kDa covering the average areas of 2.10 ± 0.00, 3.53 ± 0.16, 2.50 ± 0.14, 1.68 ± 0.06, 3.20 ± 0.00 and 2.50 ± 0.00%, respectively, were detected in subjects with hypothyroidism. On the other hand, a 26 kDa protein fraction, covering the percent area of 3.95 ± 0.04%, in normal subjects, could not be detected after hypothyroidism.

DISCUSSION

The transport and metabolism of the thyroid hormones occupy an important place in clinical and experimental thyroidology. The effects of thyroid hormones on intermediary metabolism are clinically evident in patients with hypothyroidism (Ingbar, 1985). In the present study, most of the high and low molecular weight protein fractions are found to be markedly enhanced in hypothyroid compared to healthy group which may be due to increased anabolism and decreased catabolism of proteins in response to hypothyroidism.

Both the synthesis and degradation of proteins are decreased, the latter especially so, with the result that nitrogen balance is usually slightly positive. The decrease in protein synthesis is reflected in retardation of growth and a lessened effectiveness of growth hormone. Permeability of capillaries to protein is increased accounting for the high concentration of proteins in effusions and perhaps in the spinal fluid. In addition, the total exchangeable albumin pool is increased as a result of the relatively greater decrease in albumin degradation than in albumin synthesis. A greater than normal proportion and quantity of exchangeable albumin is localized in the extravascular space. The total concentration of serum proteins, as a result, may be increased (Ingbar, 1985).

Changes in plasma and other extracellular fluid proteins are prominent in human hypothyroidism. Plasma low density lipoprotein levels increase nearly three fold, reflecting a decrease in catabolism and turnover out of proportion to effects on synthesis

(Walton *et al.*, 1965). Net production of albumin also increases and despite reduction in both synthesis and degradation there is a rise in total body miscible albumin pool. The latter increase in total exchangeable albumin is attributable entirely to an estimated 20-70% increase in extravascular pool and is accompanied by increased capillary permeability to albumin (Schwartz, 1955; Lewallen *et al.*, 1959). Most pronounced impact of thyroid hormone deficiency, in the present investigation also, has been observed on albumin (66 kDa) and proalbumin (58 kDa) fractions, indicating appreciable enhan ements of 174% and 125%, respectively, in hypothyroid compared to healthy subjects. Enhanced capillary permeability may also account for a proportion of the approximate doubling in average protein content of the cerebrospinal fluid in hypothyroidism (Bronsky *et al.*, 1958) as well as for some of the protein both in interstitial fluid and in various serious effusions associated with this condition (Loeb, 1978). In hyperthyroidism, on the other hand, there is increased anabolism of proteins resulting in positive nitrogen balance and high serum albumin concentration (Varley *et al.*, 1980).

Conclusively, in hypothyroidism, synthesis of secreted, functional and structural proteins in many tissues is impaired, most obviously in children. Protein catabolism is also impaired. For several proteins, such as lipoproteins and albumin, degradation is impaired more than synthesis and thus increased quantities are found (Parving *et al.*, 1979). All these functions, in hypothyroid patients, are restored to normal by replacement doses of thyroid hormone (Ingbar, 1985).

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