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CORRELATION BETWEEN STRESS AND THYROID FUNCTION IN PATIENTS SUFFERING WITH HYPOTHYROIDISM

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ABSTRACT

Hypothyroidism is an endocrine syndrome due to which the metabolic rate of the body system is affected. The disorder and the changes are due to the imbalance in the hormone secretion which is responsible for thyroiditis. Other disorders like hypertension, diabetes caused due to hormonal changes are all linked, directly or indirectly to the syndrome. The association between the thyroid hormones and the stress hormones is less understood. We investigated a total 50 subjects of which 25 were eligible for further study. The level of TSH, T3, T4, Cortisol and prolactin were determined using ELISA following standard protocol. The study reveled that level of TSH (80%), T3 (70%) and T4 (60%) are comparatively higher from their normal values. Cortisol and prolactin were in normal range. Our findings suggests that excess thyroid hormones may induce tissue injury secondary to production of other hormones in excess thus leading to many other disorders and majorly affecting the metabolism in vertebrates.

Keywords: *hypothyroidism, hormones, ELISA, stress*

INTRODUCTION

Endocrine diseases are amongst the common and abundant disorders affecting a large population worldwide increasing autoimmune dysfunctions**.** Hypothyroidism is a common endocrine disorder, result of impaired production and secretion of the thyroid hormone**.** The thyroid disease, the thyroiditis, is due to the reduced secretion of the two thyroid hormones the T3 and T4, secreted by the stimulation of a hormone produced by the pituitary gland at the base of the brain in response to signals from the hypothalamus gland in the brain the thyroid stimulating hormone (TSH) [1]. These hormones control the metabolic rate of all cells throughout the body. Metabolism refers to all the chemical reactions of the body and, also, how energy is made available. Therefore, the thyroid influences every system, organ and muscle in the body. They help in regulation of growth and the rate of chemical reactions, metabolism in the body[2]. Variety of the malfunction in body systems, the central nervous systems, reproductive, gastrointestinal, muscular and the endocrine system are all the signs of the disorder. Problems with an under-functioning thyroid gland, known as hypothyroidism, can be the cause of many recurring symptoms thus imparting numerous other disorders and fatigue[3]. The thyroid hormones affect synthesis, mobilization and degradation of lipids, although degradation is influenced more than synthesis. Thyroid dysfunction in particular hypothyroidism is associated with dyslipidemia which increase the risk of other endothelial dysfunctions, hypertension and cardiovascular diseases. Overt hypothyroidism associated with hypercholesterolemia and a marked increase in circulating concentrations of total LDL-Cholesterol[4].

Several experimental evidences showed that hypothyroidism leads to a depression of humoral and cell-mediated immune responses, effects that

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were reversed by restoration of the euthyroid state. Stress induces changes in the secretion of several hormones, which affect immune function by either increasing or decreasing immune activity**.** The thyroid hormones are essential for the maintenance of neurotransmitters associated with stress, and have also a significant impact on the immune response[5]. It has been shown that hypothyroidism is associated with thymic growth depression and with a decrease in circulating lymphocyte numbers[6]. The changes occurring both in stress and thyroid hormones, prolactin, cortisol, thyroid stimulating

Table 1: Concentrations of the hormones

S.No.	TSH (µIU/ml)	T3 (ng/ml)	T4 (nmol/l)	Cortisol (nmol/l)	Prolactin (mIU/l)
Referance range	**0.5-5**	**0.56-1.88**	**59-153**	**63-600**	**74-745, 45-320**
01.	1.4	2.09	71	28	150
02.	19.0	3.02	72	107	270
03.	19.7	5.50	197	33	80
04.	9.7	3.05	63	42	30
05.	6.9	4.09	78	24	90
06.	8.3	4.50	88	62	30
07.	12.0	3.08	72	02	20
08.	19.3	4.56	96	15	60
09.	18.4	1.05	117	45	190
10.	6.8	6.04	101	38	40
11.	17.9	0.50	130	28	80
12.	8.0	0.06	84	103	250
13.	13.7	1.16	191	56	460
14.	1.3	1.01	95	116	430
15.	6.7	1.06	84	60	600
16.	9.7	1.23	97	45	1100
17.	8.5	2.24	76	72	670
18.	7.7	3.34	65	43	570
19.	4.2	1.21	93	49	420
20.	0.7	1.09	52	46	410
21.	3.8	0.82	55	57	560
22.	5.5	1.50	101	90	490
23.	14.9	1.25	139	43	660
24.	5.1	1.13	96	37	430
25.	1.5	0.79	65	91	520

hormone (TSH), triiodothyronine (T3), and thyroxine (T4) are the major cause for such changes in the body system. Study has been done to analyze these changes in the hormone level and the difference with the euthyroid subjects.

MATERIALS AND METHODS

Study participants

Prospectively 50 patients, age ranging from 20-60 years from OPD of Hospitals and Medical colleges associated with Barkatullah University and other Autonomous organizations of Bhopal were included. Clinical examinations including height, body weight and body mass index (BMI) was calculated. Blood pressure was taken after 10 min in a resting position. Complete medical histories, including history of bleeding and smoking habits, heart disease, diabetes, stroke or other neurological disorders or depression; significant medication, use of beta-blockers, inhaled beta agonists, hormonal contraceptives, corticosteroid use within prior three months, psychotropic medication use within prior eight weeks; psychiatric hospitalization within past year; was confirmed at the beginning of the study session. On the basis of the specified conditions, out of 50, 25 participants were eligible for the study.

Blood collection and sample preparation

10 ml of venous blood was withdrawn from patients of hypothyroidism and normal healthy subjects after overnight fasting with dry disposable syringe and needle by vene puncture under all aseptic conditions. Then the serum was separated after 30 minutes of blood collection by centrifuging at 3000 rpm for 10 minutes. This serum sample was used for hormonal assays.

Hormonal Analysis

All the tests were performed using commercially available enzyme immunoassay kits. The level of the hormones in serum sample of the subjects was determined using ELISA technique.

Serum Thyroid stimulating hormone (TSH)

The TSH enzyme linked immunosorbent assay (ELISA) applies quantitative sandwich immunoassay. The microtiter plate was pre-coated with a monoclonal antibody specific for TSH. Standards, samples and control (50uL in each) were added to the microtiter plate wells and TSH (50µL) if present binds to the antibody pre-coated wells. In order to quantitatively determine the amount of TSH present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for TSH was added to each well to sandwich the TSH immobilized on the plate. The microtiter plate was incubated, and then the wells were thoroughly washed to remove all unbound components. TMB (3, 3', 5, 5' tetramethyl-benzidine) substrate solution (100µL) was then added to each well. The enzyme (HRP) and substrate were allowed to react for a short incubation period. Only those wells that contain TSH and enzyme-conjugated antibody exhibit a change in color. The enzyme substrate reaction is terminated by the addition of a sulphuric acid solution (150µL) the stopping reagent and the color change was measured by the ELISA reader at a wavelength of 450 nm.

Serum Triiodothyronine (T3)

The T3 immunoassay was performed to determine the presence of total thyroxine in human serum using competitive microplate enzyme immunoassay. Plates were coated with anti-T3 antibodies. Serum reference, patient specimens and control (25µL) was first added to the microplate well. Enzyme-T3 conjugate (150µL) was added. A competition reaction results between the enzyme-triiodothyronine conjugate and serum containing the native total triiodothyronine for antibody combining sites immobilized on the well. Unbound conjugate was removed by washing 5 times. The enzyme activity in the antibody-bound fraction is inversely proportional to the native cortisol concentration. The enzyme activity was revealed by a color change in TMB-Substrate (100µL) solution and by taking the absorbance by the ELISA reader at 450 nm.

Serum Thyroxine (T4)

The T4 immunoassay was performed to determine the presence of total thyroxine in human serum using competitive microplate enzyme immunoassay. Plates were coated with anti-T4 antibodies. Serum reference, patient specimens and control (25µL) was first added to the microplate well. Enzyme-T4 conjugate was added (200µL). Thyroxine present in the sample competes with conjugate to bind with anti-T4 to form an antigen antibody complex. Unbound conjugate was removed by washing 5 times. The enzyme activity in the antibody-bound fraction is inversely proportional to the native cortisol concentration. The enzyme activity was revealed by a color change in TMB-Substrate (100µL) solution and by taking the absorbance by the ELISA reader 450 nm

Serum Cortisol

The cortisol immunoassay was performed using competitive microplate enzyme immunoassay. Plate coated with anti-cortisol antibodies was used. Serum reference, patient specimens and control (25µL) was first added to the microplate well. Enzyme-Cortisol conjugate (100µL) was added. Enzyme-Cortisol conjugate binds with anti-cortisol coated microplate to form an antigen-antibody complex. Unbound conjugate was removed by washing 5 times. The enzyme activity in the antibody-bound fraction is inversely proportional to the native cortisol concentration. The enzyme activity was revealed by a color change in TMB-Substrate solution (100µL) and by taking the absorbance by the ELISA reader[7].

Serum Prolactin

The prolactin immunoassay is based on principle of sandwich method. Specific wells were selected for the standard serum, patient specimens and control (25µl). The test samples were allowed (25µL) to react simultaneously with the two antibodies, resulting in the PRL molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells were washed with washing solution to remove unbound labeled antibodies. A solution of TMB-Substrate (100µL) was added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of stopping reagent (150µL), changing the color to yellow. The enzyme activity was revealed by a color change in TMB-Substrate solution and by taking the absorbance by an ELISA reader[8].

RESULTS AND DISCUSSIONS

The study was conducted on the 50 patients out of which 25 patients were found to be suffering with hypothyroidism which fulfilled the conditions required for the study. Out of 25 patients 80% of the patients have given the positive result for TSH (0.5-5µIU/ml), 20% of the patients were suffering with hyperthyroidism and 10% were in normal condition. T3 (0.56-1.88ng/ml) and T4 (59-153nmol/l) also gave higher values in 70% and 60% of the patients. However the level of cortisol (63-600nmol/l) was lower in 85% of the patients and 15% was under the normal range. The level of prolactin (74-745mIU/l) however was under normal range and only 20% of the patients had low prolactin level. The concentrations of all the hormones are mentioned in Table 1.

We have found that patients having high TSH value showing hypothyroidism have shown a bit rise in serum Prolactin level and low serum Cortisol values suggesting a slightly increased level of stress among the recent onset of the disease, following this pilot study now we have started the screening of the patients' stress level by the set prescribed questionnaire and hope in future definitely we will find direct correlations when serum cytokines level, thyroid and stress hormone and stress factors will be analyzed at large sample size.

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