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RESEARCH ARTICLE

Autoimmune, antibodies (TPOA), (TgAb) and (TSH), (T_4), (T_3), in hypothyroid patients in two locations Sudan (Elobied and Khartoum state)

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Abstract

The aim of this study was investigated the hypothyroid patients in two locations in Sudan, first group (86 patients) was collected from Kordfan State and second group (114 patients) was from Khartoum State. A questionnaire was filled by the 200 patients and 50 healthy individuals as control group. TSH, T_3, T_4, TPOAB and Tg AB ranged determined. TSH for first group varied 0.12 - 561.32 (µg/dl) and for second group varied 2.06-2599 (µg/dl). T_3 for first group ranged 0.1- 0.8(ng/ml) and for second group varied 0.3-7.1(ng/ml). T_4 for first group ranged 1.6-6.3(µg/dl) and for second group varied 2.3- 6.2(µg/dl). TPOAB for first and second group ranged 12.2-9854 and 14.2- 3001.5(u/ml) , respectively. Whereas Tg AB for first and second group varied 13.7-2999 and 371.7-9628.6(iu/ml), respectively. Patients were also subdivided according to the duration of the diseases from 0-48 months and greater than 48 months, the results revealed that T_3 and T_4 values are almost the same in both groups (in all patients), but TSH is higher in second group, TPOAB and Tg AB values were higher in first group. The conclusion indicated the cause of hypothyroidism is related with low levels of T_3 and T_4.

1.0 Introduction

The thyroid is an endocrine gland, butterfly - shaped that manufactures certain hormones (chemical substances) which released into the blood stream and act as messengers to affect the cells and issues in distant parts in the body (Davis and Davis, 1996). It is regulate the heart rate, blood pressure, body temperature and the rate at which food is converted into energy. The principle hormones of the thyroid gland are T_3 (Thyroxine) and T_4 (Triiodothroxine) and their concentration are 93% and 7%, respectively. Normal total plasma T_4 level is 8µgldl (103 ηmol//l) and plasma T_3 is 0.15 µg/dl (230 ηmol//l) (Zahoor et al., 2009). Plasma proteins that bind thyroid hormone (1%) are albumin formerly called thyroxin-binding prealbumin (TBPA) and now called transthyretin, globulin and thyroxin-binding globulin (TBG) in which the albumin has largest capacity to bind T_4 and TBG the smallest (AACC, 2001 - 2013). Normally 99.98% of T4 in plasma is bound; the free T4 is about 2 µg/dl. The free T_4 in plasma is physiological active causing the inhibition of the TSH secretion. The free T_4 in plasma is important in the metabolic control of human body and the free T_4 believed to be direct indicator of thyroid status in individual. Free T_3 like free T_4 measurement also reflects the thyroid status of individual accurately (Glinoer et al., 1993). Thyroid hormones are essential for the function of every cell in the body. They help regulate growth and the rate of chemical reactions (metabolism) in the body. Thyroid hormone also helps children grow and develop (Oppenheimer et al., 1987). Hypothyroidism describes an under active thyroid gland that is producing depressed levels of thyroids hormone. The two major active hormones produced by the thyroid are T_3 and T_4. These are synthesized from tyrosine

residues in thymoglobulin in a series of discrete steps. Iodine is the most important element in the biosynthesis of the thyroid hormone. This iodine is oxidized by the thyroid peroxides enzyme (TPO) and linked to tyrosine molecule in thymoglobulin to form monoiodotyrosines (MIT) and diiodytyrosines (DII) neither of which are metabolically active (Oppenheimer et al., 1987). The activity of the thyroid hormone is control by pituitary glycoprotein hormone, thyroid stimulating hormone (TSH) which regulated by thyrotropin releasing hormone (TRH) and the feedback effects of circulating thyroid hormones at the hypothalamic and pituitary levels (Glinoer et al., 1993). Causes of hypothyroidism include idiopathic atrophy of thyroid gland or defect in thyroid controlling hormone due to defect in pituitary or hypothalamic gland, or thyroid hormone resistance, goitrogens or drugs- induced or infiltration in the thyroid by thyroid damaging immune cells leading to damage of thyroid (Hashimotoes thyroiditis) (Michael and Michael, 1993). Thyroglobulin (TgAb) a heavily glycocylated, iodinated protein is a major autoantigen in autoimmune thyroditis (Goronzy and Weyand, 2007). Age, gender, race and area, all have appreciable effect on the level of T_3, T_4, TSH (Zahoor et al., 2009). An autoimmune disorder is a condition that occurs when the immune system mistakenly attacks and destroys healthy body tissue (Koenig et al., 1989). Thyroid autoantibody appear mostly with the presence of lymphocytes in the targeted organ ((Dayan *et al*., 1996 and Weetman et al., 1983). There are three types of antibodies: Thyroid Peroxidase Antibodies (TPOAb), Thyroglobulin Antibodies (TgAb), and Thyroid Stimulating Hormone Receptor Antibodies (TRAb) (AACC, 2001 - 2013), these antibodies affect different targets in the thyroid gland. The three main targets are as follows: Thyroglobulin, a protein specified in housing the thyroid hormones T_3, T_4 and TSH; the Thyroid Microsomal Antigen also known as thyroid peroxidase, an enzyme in charge of regulating how much hormone the gland should produce; and the thyrotropin receptors (Dayan *et al*., 1996). A certain percentage of patients who are healthy may be positive for one or more thyroid antibodies." Doctors who attend to such patients will most likely do routine follow-ups on the patient's health since, even though it is highly unlikely that they will present any thyroid problems, there is still a chance that they will develop some type of dysfunction with time (AACC, 2001 - 2013).

The study was basically designed to see level of thyroid and thyroid stimulation hormones and antibodies (TPOA and (TgAb) in hypothyroid patients, in two locations (Kordfan and Khartoum State and also investigate the effect of age on T_3, T_4 and TSH.

2.0 Material and Methods

Study population: study was carried out on a sample selected from different sex and age, they are grouped from days to 82 years during the period from January to June 2009 in Elobied State and Khartoum State (Table 1), these patients were came from different towns and villages in Sudan.

2.1 Collection of blood samples

Blood was collected from 200 goiterous under aseptic conditions. Five ml of venous blood were taken by disposable syring and left to clot inside the syring. Centrifugation was done after complete clot formation had taken place. Serum was separated in a plane container and stored at 20 °C.

2.2 Data collection: the survey questionnaire was designed containing personal data: Name, Sex, Age, Tribe and Residence, finally history including consanguinity and goiter in parents.

3.0 Method

Principle of method: enzyme linked immune assay micro plate test (Bulter, 1993).

Procedure: First the reagents were warmed at room temperature, then room temperature was measure with thermometer and all this process was done at 28^{o}c. Plate opened and labeled to perform the determination in duplicate, as two wells for each at six points off the standard curve (S_o-S_s), two wells for each sample diluents and 100µl sample was pipette, standard added to each labeled well and incubated for 30 minutes at room temperature, washing plate was performed by using tecan washer, with 300µl of diluted wash solution. Washing producer was repeated ten times. 100µl conjugate added to each wells, incubate for 15 minutes at room temperature. Plate was washed with 300µl of diluted wash solution, pipette 100ml of stop solution into all wells incubated for 5 minutes at room temperature. State fax 2600 reader was used for reading of optical density (OD) of each well at 450 nm the blank well for zero adjustment, concentration was calculated. The color is stable for at least 30 minutes

Calculation: concentration of sample direct performed from the concentration of antibodies present in the sample was calculated by interpolating the absorbance in the calibration curve (Bulter, 1993).

3.1 Measurement of TSH:

Principle of TSH immnuometric assay: The labeled antigen binds to immobilized antibody and was measure after the labeled antibody was added. Producer of TSH**:** samples were loaded in bar-coded sample cup holders and placed onto a loud plate form. Run was pressed and test unit are converted into the analyzer moved onto the main incubation carousel. The pipette added samples and alkaline phosphates labeled reagent. Test units were incubated on the main carousel at 37°c for 30 minutes. The units were shuttled to spin/wash station, where bound and free label were separated.

Substantiate was added and the test units are transferred to the luminometer chain to 10 minutes incubation at 37°c that cause the signal to reach maximum limits. The photocounts were measured with a photomultiplier tube (PMT) count per second (CPS) are converted to analyzer curves (Bulter, 1993).

3.2 Measurement of T_4

Principle of T_4: direct competitive analog immune assay. Procedure was same as in TSH.

Material supplied**:** plastic test cups containing lyophilized twelve magnetic beads with anti-T_4 rabbit polyclonal antibody, 140 ml of T_4 conjugated to bovine alkaline phosphate and ANS (8 anilino-I-naphthalene sulfuric acid) with sodium azides as a preservative (Bulter, 1993).

3.3 Measurement of T_3

T_3: material supplied: plastic test cups containing lyophilized magnetic beads with anti-T_3 sheep mono clonal antibody, 125 ml of T_3 conjugated to bovine alkaline phosphate and ANS (8-anilino-I napjthalene sulfuric acid) with sodium azide as a preservative. Procedure of T_3 was same as same in T_4.

4.0 Results and Discussion

There are great differences in the value of TSH in different age groups, maximum value of TSH (2599 µg /dl) was clearly observed in sub-group 41-60 years in Khartoum location (Table 4), while the minimum value of TSH(0.12 µg /dl) was observed in sub-group 0-20 years in Kordfan location (Table 6). These are consistency of T_3 value in the different age groups except in the elderly above 80 years (Table 6), but there is marked increase in the T_3 level in sub-group 21-40 years Table 3). For T_4 value in Kordfan group the results were almost the same except in sub-group 0-20 years, it's a bit lower (Table 2). T_4 Values in Khartoum group, there were also in the same range except in sub- group 21-40 years, it is a bit higher (Table 3). There is great discrepancy in the values of TPOAB in the different age groups, but it is ranged 1210 - 9654 u/ml except that in sub-group above 80 years, it is only 122 u/ml. There is a wide range in the values of Tg Ab which is between 137 to 2999. The antibodies TPOAB and Tg AB were found to be higher in all patients than the control group. These results indicated that the high values of antibodies may be associated with autoimmune disease and also T_3 and T_4 value was found to be low in all patients than the control group.

Table (1) shows valid patients (200) that selected from Kordfan and Khartoum State, which grouping according to age

Age (Years)	Patients from Kordfan (Group I)	Patients from Khartoum (Group II)
0-20	7 patients	16 patients
21-40	55 patients	28 patients
41-60	20 patients	44 patients
61-80	3 patients	22 patients
> 80	1 patient	4 patients
Total	86 patients	114 patients

Table (2) shows TSH, T3, T4, TBOAB and Tab of sub-group 0-20 years

	TSH µg /dl	**T_3 mg/ml**	**T_4 µg/ml**	**TBOAB u/ml**	**Tg Ab iu/ml**
Kordfan (Group I)	22.9	0.7	16	9839	123
Khartoum (Group II)	31.17	0.8	2.8	114	661
Control	0.5	0.9	5.1	65	132

Table (3) shows TSH, T3, T4, TBOAB and Tab of sub-group 21-40 years

	TSH µg /dl	**T_3 ng/ml**	**T_4 µg/ml**	**TBOAB u/ml**	**Tg Ab iu/ml**
Kordfan (Group I)	103	0.6	6.8	9854	2999
Khartoum (Group II)	816	7.1	6.2	1961	9628
Control	3.2	1.1	6.3	55	120

Table (4) shows TSH, T3, T4, TBOAB and Tab of sub-group 41-60 years

	TSH µg /dl	**T_3 ng/ml**	**T_4 µg/ml**	**TBOAB u/ml**	**TgAb iu/ml**
Kordfan (Group I)	561	0.7	3.2	1210	7793.4
Khartoum (Group II)	2599	0.6	4	3001	5815.8
Control	2.5	0.9	7	60	142

Table (5) shows TSH, T3, T4, TBOAB and Tab of sub-group 61-80 years

	TSH µg /dl	**T_3 ng/ml**	**T_4 µg/ml**	**TBOAB u/ml**	**TgAb iu/ml**
Kordfan (Group I)	0.7	0.7	2.8	50.5	110.7
Khartoum (Group II)	211	0.3	2.3	748	195
Control	**0.7**	**1.0**	**4.9**	**58**	**110**

Table (6) shows TSH, T3, T4, TBOAB and Tab of sub-group >80 years

	TSH µg/dl	**T_3 ng/ml**	**T_4 µg /ml**	**TBOAB u/ml**	**TgAb iu/ml**
Kordfan (Group I)	0.12	0.1	3.1	12	137
Khartoum (Group II)	2.06	0.6	4	12.2	371
Control	**0.6**	**1.4**	**7.8**	**67**	**130**

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