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Estimation of Serum Trace metals and Thyroid Hormones in Hypothyroidism and Hyperthyroidism Patients

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Abstract

In this work, the serum content of the trace elements; (Fe, Cu and Zn) and thyroid hormones (T3, T4 and TSH) in hypothyroidism and hyperthyroidism patients were determined and compared to those of normal persons. The results included the statistical values of the thyroid hormones TSH, T4, T3, iron, copper and zinc in serum patients and healthy people between the ages of (15-60) years, as was studied by age and gender. Sixty patients and thirty normal healthy control persons participated in this study. The results were discussed in terms of: i- Thyroid disorder**; (**hypothyroidism or hyperthyroidism), ii- sex (male or female) and iii- ages (15-30, 31-45 or 46-60 years). The results of this work as discussed indicate, the role of the trace elements in many metabolic processes either as essential nutrients or as cofactors for different enzymes contributed directly or indirectly to the hypothyroidism. Consequently, the results suggest that the metabolism of iron, copper and zinc is abnormal in hyperthyroidism disease.

Keywords: hypothyroidism, hyperthyroidism, trace metal, thyroid hormones.
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1. Introduction

The maintenance of optimal health requires an adequate supply of carbohydrates, proteins, lipids, macronutrients, micronutrients, and trace elements [1]. Many trace elements play an essential role in a number of biological processes through their action as activators or inhibitors of enzymatic reactions, by competing with other elements and proteins for binding sites, by influencing the permeability of cell membranes, or through other mechanisms. Trace elements are known to influence hormones at levels of action, including hormone secretion and activity and binding to target tissue. Conversely, hormones influence trace metals metabolism at several levels of action, including excretion and transport of trace metals [2- 5]. Hence, trace elements assay in biological fluids can be used as diagnostic or prognostic aid in patients with different hormonal disturbances alongside with other biochemical parameters. Thyroid hormones play an important role in human body metabolism. After binding with a specific nuclear receptor, T3/T4 induces transcription of genetic code via mRNA and regulates proteosynthesis in most tissues. Thyroid hormones regulate the rate of metabolic processes and consequent development of organism [6]. Decreased thyroid hormone synthesis and low levels of circulating thyroid hormones result in biochemical and/or clinical hyperthyroidism. This condition occurs more frequently in women; the overall incidences are about 3% of the general population [7]. hyperthyroidism probably is initiated by autoimmunity against the thyroid gland in addition to different other causes [8]. The thyroid glands of most of these patients first have autoimmune "Thyroiditis ", which means thyroid inflammation. This causes progressive deterioration and finally fibrosis of the gland, with resultant diminished or absent secretion of thyroid hormone. Several other types of hyperthyroidism also occur, often associated with development of enlarged thyroid glands called thyroid goiter [9]. Deficiency of thyroid hormones causes many metabolic processes to slow down. Symptoms of hyperthyroidism include enlargement of thyroid gland-or goiter, impairment of cognition slowing of mental and physical performance, increased risk of coronary heart diseases many and different other symptoms [10]. The status of different trace elements in hypothyroidism is not well established. Furthermore, serum manganese in hypothyroidism patients is not studied previously. The thyroid gland makes and releases thyroid hormones (T4, T3 and TSH) to help regulate body growth and metabolism. The function of thyroid hormones (T4, T3) are qualitatively the same. But very in intensity and speed of action [11]. The half-life of T3 is almost 1 day, while the half-life of T4 is 7day.thyroid hormones work to regulate growth, development and metabolism. Thyroid hormones are also particularly important during pregnancy and early childhood [12]. Thyroid disorders are commonly separated in to two major categories; Hyperthyroidism (characterized by increased activity of the thyroid gland) and hyperthyroidism (causes gland failure, which is caused by the presence of antithyroid antibodies) [13]. These objects

attack the thyroid tissue and its recipients and then destroy them [14]. However, under normal conditions, thyroid hormones are regulated by feedback, which inhibits thyroid hormones from the production of Thyrotropin Releasing Hormone (TRH) from the hypothalamus gland and production of TSH from the front of the pituitary gland [15]. Low levels of thyroid hormones cause a slowdown in metabolic processes in general [16,17]. In this work, the serum content of the trace elements; (Fe, Cu and Zn) and thyroid hormones (T3, T4 and TSH) in hypothyroidism and hyperthyroidism patients were determined and compared to those of normal persons. The results are statistically treated in terms of both age and gender.

2. Experimental

All chemicals used in the present work were of highest purity (BDH, Fluka or Sainland) and were used without further purification.

Instruments: the following apparatus were used throughout the work: i) flame atomic absorption spectrophotometer (type: analytik jena nov AA-350), distillation apparatus type: Raypa, pH-meter type: Coring, (iv) centrifuge, type: TDL-60B, (v) water bath, (TECHA) and (vi) sensitive electronic balance type: Sartorius.

Reagents:

I- Preparation of Fe standard solutions

1000mg/l Fe was prepared by dissolving (1.4297) g of ferric oxide Fe_2O_3 in 33ml of concentrated hydrochloric acid (HCl) then heating the solution for 10 minutes and completing the volume to (1000 ml) with deionized water. Diluted concentration was prepared from the Fe standard solution by diluting with deionized water.

ii- Preparation of zinc standard solutions.

1000mg/l Zn was prepared by dissolving 1.2446 g of zinc oxide (ZnO)in an amount of deionized water, followed by addition of (5M HCl) to complete the dissolving and completing the volume to (1000 ml) by ion free water. Diluted concentrations were prepared from Zn standard solution by diluting with deionized water.

iii- Preparation of cupper standard solution

1000 mg/l Cu was prepared by dissolving (4.3700) g from hydrous Cupper nitrate Cu $(NO_3)_2.5H_2O$ in a few amounts of deionized water then completing the volume to (1000ml) by deionized water. Diluted concentrations were prepared from the Cu standard solution by diluting with deionized water.

iv- Sample collection

Blood samples were taken after an overnight fast period from the arm vein with a disposable syring, then allowed to clot within a one hour at room temperature. The serum was separated from clotted blood by Centrifuge at about 3000 revolution/min for a period of 5 to 10 min. Serum portions in the tubes were kept closed to prevent contamination and loss of serum by evaporation. Re centrifugation was done to spin down the residual erythrocytes, then the serum was transferred to a polyethylene container and stored at freezing temperature for preparation of analysis. Fe, Zn, and Cu were determined after fixing the optimum experimental conditions using flame atomic absorption.

General procedure for the flame atomic absorption

Determination of Fe in the blood serum

Fe content in the blood serum was determined by mixing (5ml) from the serum sample with (25ml) from HCl and keeping the mixture in an incubator for 30 minutes then adding (250ml) from Trichloroacetic acid to the mixture. After that, (50ml) from the resulted solution will be injected to the atomic absorption spectrophotometer to register the signal of atomic absorption under the optimum conditions.

Determination of Zn in the blood serum

Zn element content in the blood serum was determined by diluting(0.5ml) of the serum sample with eight volumes of (1% V/V HNO3). After that, the atomic absorption signal of the resulted solution was registered under the optimum conditions.

Determination of Cu in the blood serum.

Cu content in the blood serum was determined by diluting (5ml) from the serum sample with(5ml) from (W/V 0.03%polyvinylalcohol). After that, the atomic absorption signal of the resulted solution was registered under the optimum conditions

Samples of study:

This study was conducted at the Azadi Teaching Hospital in Kirkuk from the beginning of July 2018 until the end of November 2018. The study included 60 cases of patients with hyperthyroidism or hypothyroidism as well as the health group of 30 people, divided into two groups:

Control group:

The current study included health natural persons as a control group, the number was (30) people, including (15) males and (15) females. As if they were referred to the blood bank unit, and their ages ranged from (15-60) years.

Patients group:

Blood samples of people with thyroid disease were obtained from Azadi Hospital and a small part of the laboratories at Doctors Street in Kirkuk, sixty of them were (30) male and (30) females, aged (15-60) years, were confirmed to be infected with the disease after laboratory tests, and diagnosis by specialized doctors.

Collection of Blood Samples:

The samples were collected by drawing blood from the vein from the front attachment package with a(5)ml plastic injection .The models were taken and the blood volume ranged from approximately (3-5)ml of the infected persons and the students, The blood samples were placed in test tubes.

Serum preparation:

The serum was obtained from blood drawn in a plastic tube free of anticoagulation ,Leaving the blood at room temperature (25^0c) until it coagulates ,And placed in the centrifuge for (15)minutes to obtain serum ,and then withdraw the serum free of red blood cells by Micro pipette , and put the serum in clean and sterile tubes ,and kept in

freezing at $^{-}25^{0}$c until the use of serum for the tests of the chemical under study.

Measurement the level of TSH in the serum:

Thyroid hormones were measured by following the steps indicated in the hormone system (Ichroma^{TM}).

Procedure

1- Transfer (150 mL) (Human serum/plasma/control) of sample using a transfer pipette to a sample mixing tube.

2- Add (75mL) detection buffer to the sample mixing tube containing sample(serum/plasma/control).

3- Close the lid of the sample mixing tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately).

4- Pipette out 75 Ml of a sample mixture and load it into the sample well on the cartridge.

5- Leave the sample-loaded cartridge at room temperature for 12 minutes.

6- To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma test. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.

7- Press 'Select' button on the instrument for ichroma tests to start the scanning process.

8- Instrument for i chroma tests will start scanning the sample-loaded cartridge immediately.

9- Read the test result on the display screen of the instrument for ichroma tests.

Calculation

After measuring the absorption of the sample and the standard solution and control solution, the results are automatically calculated by computer.

Measurement the level of T4 and T3 in the serum:

Thyroid hormones were measured by following the steps indicated in the hormone system (Ichroma^{TM}).

Procedure

1- Transfer (75 mL) (Human serum/plasma/control) of sample using a transfer pipette to a sample mixing tube.

2- Mix well by pipetting 10 times.

3- Add (75mL) of solution B using a transfer pipette with new tip to the tube containing the solution A and sample mixture.

4- Close the lid of the solution A tube and mix the sample thoroughly by shaking it about 10 times.

5- Incubate the solution A+ Solution B+ sample mixture at room temperature for 8 minutes.

6- Pipette out 75 Ml of a sample mixture and load it into the sample well on the cartridge.

7- Insert the sample-loaded test cartridge into the slot of the i-chamber or an incubator (25c).

8- Leave the sample-loaded test cartridge in the i-chamber or an incubator for 8 minutes.

9- To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma test. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.

10- Press 'Select' button on the instrument for ichroma tests to start the scanning process.

11- Instrument for ichroma tests will start scanning the sample-loaded cartridge immediately.

12- Read the test result on the display screen of the instrument for ichroma tests.

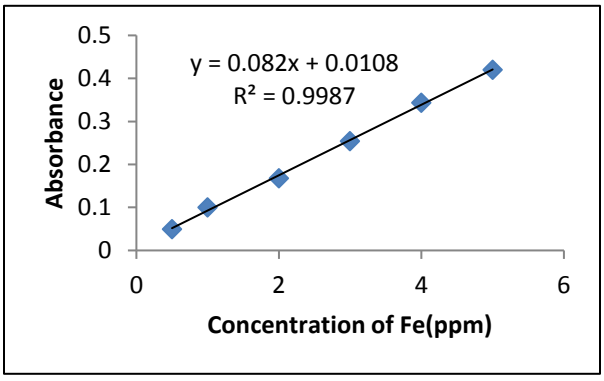
Calculation

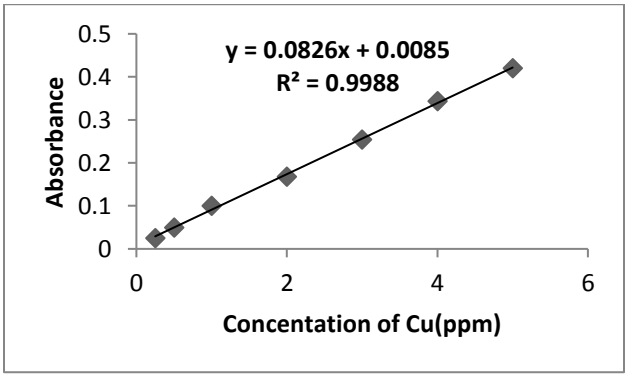
After measuring the absorption of the sample and the standard solution and control solution, the results are automatically calculated by computer.

3. Results and discussion

1- Determination of trace metals in blood serum

The trace metals Fe, Cu and Zn in blood serum were determined using atomic absorption technique. The calibration graphs are shown in Fig. (1) and the statistical analytical data are cited in Tables (1-3).





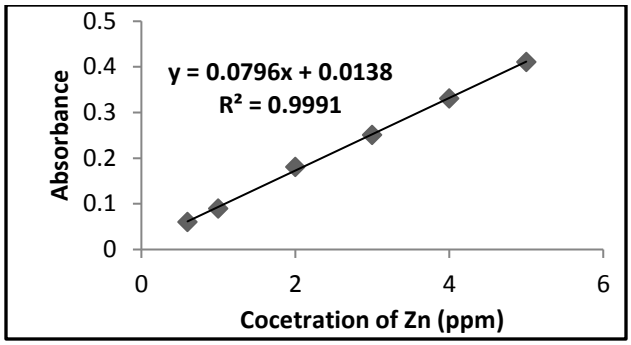


Fig. (1): Calibration graphs of Fe, Cu and Zn

Table (1): Statistical data for Fe determination

Fe added(mg/l)	Fe found(mg/l)	Recovery%	Ere%	S.D	RSD%
0.82	0.82	100.0	0	0.0176	2.9
0.84	0.86	97.6	2.38	0.0214	5.3
0.91	0.92	98.9	1.09	0.016	4.4

Fe lower detection limit = 0.0091 mg/l.

Table (2): Statistical data for Cu determination

Cu added(mg/l)	Cu found(mg/l)	Recovery%	Ere%	S.D	RSD%
0.412	0.419	98.32	1.69	0.0156	3.72
0.463	0.465	99.56	0.43	0.0158	3.41
0.501	0.503	99.60	0.39	0.0214	4.26

Cu lower detection limit = 0.0086 mg/l

Table (3): Statistical data for zn determination

Zn added(mg/l)	Zn found(mg/l)	Recovery%	Ere%	S.D	RSD%
0.330	0.338	97.63	2.42	0.015	4.64
0.359	0.362	99.17	0.83	0.016	4.44
0.403	0.405	99.50	0.49	0.021	5.35

Zn lower detection limit = 0.0064 mg/L

Measure the level of trace elements

Table (4) and Fig. (2) show the values of Fe, Zn and Cu in serum of patients compared to those of healthy peoples as control. The cited data indicate that there is a significant increase in both the concentration of Fe and Cu in patients with hyperthyroidism, while slightly decrease is observed in case of patients with hypothyroidism. On the other hand, decrease in Zn concentration is observed in case of hyperthyroidism and hypothyroidism disorder.

The increase of Fe ion concentration in the blood leads to reduce the activity of (glutathione),(glutathione peroxidase) and (super oxide dimustase) in the patients with hyperthyroidism because of the increase of the oxidation level and radicals which cause the destruction of the cell wall then the troubles in the elements equilibrium and troubles in the resulted metabolism from this disease will occur consequently [18]. It was reported that the Fe- stored level (Ferritin) increase in the patients with hyperthyroidism [19].

Cu ion has an important role in the formation of many enzymes especially super oxide dimustase in addition to its role in Fe metabolism in the body. Therefore, in the case of hyperthyroidism the oxidation of lipids and fatty acids will be increased [20]. Also, there is a probability for destruction of the cell wall leading to troubles in the ion distribution in addition to destruction the proteins which is connecting with Cu then the increase in free Cu concentration will occurs in the cells leading to generation of free radicals such as (OH^-). Moreover, it was reported that the increase of Cu level in the patients with hyperthyroidism leads to increase in T3 concentration which affect the metabolism of thyroid hormones [21].

The decrease of Zn level in the blood serum leads to increase the activity (deiodination) through the increase of (Heptatic-5-deionase) enzyme activity which convert T4 to T3 leading to the increase of the active thyroid hormone T3 [22]. And the decrease of Zn will lead to oxidation risks for the Methalouonien protein which is acting as a carrier for Zn and other trace elements.

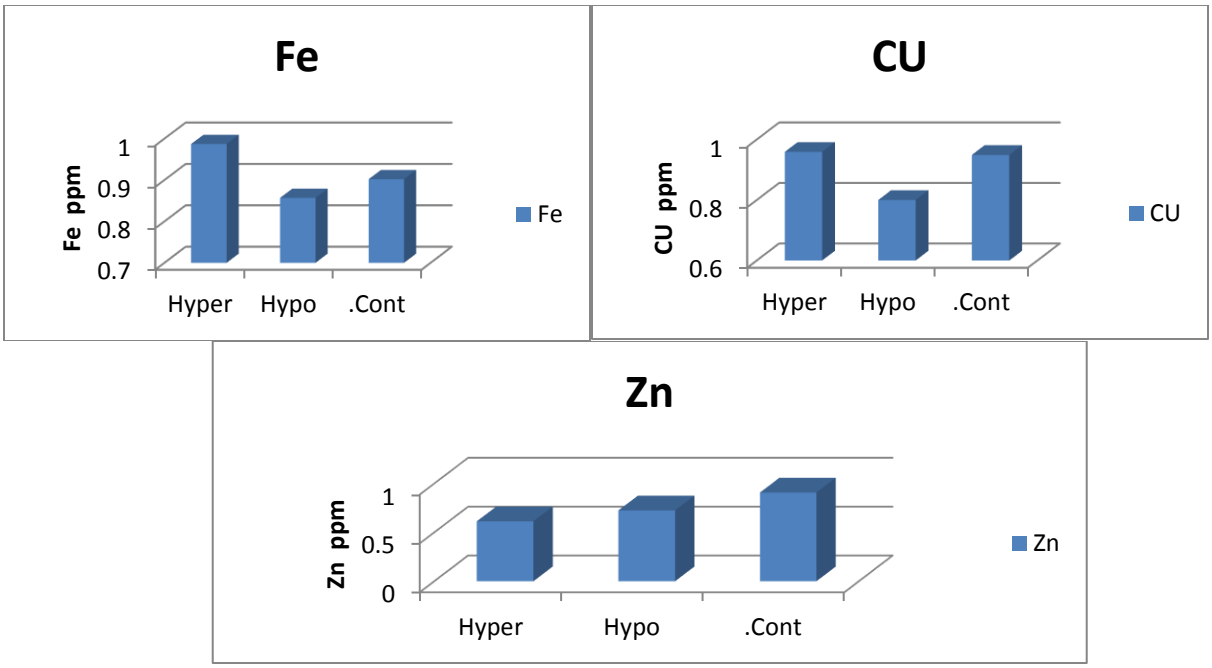


Fig. (2): Variation of Fe, Cu and Zn in the serum of patients compared to healthy people as control

Table (4): The values of Fe, Zn and Cu in serum of patients and control.

Metal / group	Cu	Zn	Fe
Hyperthyroid	0.959 (a)	0.613 (c)	0.987 (a)
Hypothyroid	0.800 (b)	0.725 (b)	0.857 (b)
Control	0.949 (a)	0.908 (a)	0.9022 (a)
P- VALUE	0.0004	0.0003	0.0006

The same letters (A or B) means no differences between them under 0.05 significantly levels according Dancun test

The values of trace elements (Fe, Cu and Zn) in Hyperthyroid and Hypothyroid paints were studied in relation to both ages and gender, the results are summarized numerically in Tables 5 and 6. Inspection of the results obtained shows that:

i- Iron content is, more or less, increases with the increase of patient ages, and the age group of (46-60) years is more prone to hypothyroidism. This is consistent with previous researcher [23].

ii- there is an increase in the concentration of Cu with age progression between hypothyroidism and control. This is consistent with that obtained early [23].

iii- There is a slight decrease in zinc concentration with age progression.

iv- Although the trace metal concentrations show slight differences according to gender, but it can be noticed that female patients are more prone than male patients.

Table (5): The values of Fe, Zn and Cu in serum of patients and control according to age.

	Hyperthyroid			Hypothyroid			Control		
Age	Fe	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn
15-30	0.955 a	0.9240 Ab	0.61100 C	0.8500 B	0.8110 bc	0.7530 b	0.8914 b	1.0350 A	0.9264 A
31-45	0.986 A	0.9667 ab	0.60933 C	0.8482 B	0.7864 c	0.7164 b	0.9700 a	0.8475 C	0.9275 A
46-60	0.9913 a	0.9943 ab	0.62429 C	0.8727 B	0.8045 bc	0.7091 b	0.8630 b	0.9100 ab	0.8660 A
Mean	0.9913 a	0.9616 a	0.8569 b	0.8569 B	0.8006 b	0.7261 b	0.9081 b	0.9308 A	0.9066 A

*The same letters mean no significant differences between them according to dancun test.

Table (6): The values of Fe, Zn and Cu in serum of patients and control according to gender.

	Hyperthyroid			Hypothyroid			Control		
	Fe	Cu	Zn	Fe	Cu	Zn	Fe	Cu	Zn
Male	0.9712	0.9818	0.6094	0.8587	0.8000	0.7713	0.8580	0.9040	0.8710
Female	1.0040	0.9340	0.6173	0.8556	0.8006	0.6793	0.9223	0.9695	0.9245
Mean	0.9876	0.9579	0.6133	0.857	0.8003	0.7253	0.890	0.9367	0.8977

*The same letters mean no significant differences between them according to dancun test.

2- Determination of thyroid hormones in blood serum

Table (7) and Fig. (3) show the values of the thyroid hormones T3, T4 and TSH in serum of patients compared to those of healthy peoples as control. The cited data shows that there is an increase in the concentration of TSH in patients with hypothyroidism compared to healthy people, while there is a significant decrease in the concentration of T4, T3 in patients with hypothyroidism compared to healthy people. The rise in TSH level and the reduction in the level of T4, T3 in patients with hypothyroidism may be due to Hoshimotoes disease which is a self-immune thyroid inflammation caused by the presence of antibodies produced and present in the blood of these patients. These antibodies inhibit immunoglobulin, attack and destroy the tissue of thyroid and thus reduce the effectiveness of TSH in stimulating the growth of the thyroid gland and the production of its hormone [24]. The results of patients with hypothyroidism in this study agreed with previous work [25] in terms of low levels of T4, T3 and high level TSH.

Table (7) the values of TSH, T4and T3 in serum of patients and healthy people

Hormone	T S H	T4	T3
Hyperthyroid	0.087	179.72	4.79
Hypothyroid	12.89	14.11	0.56
Control	2.04	99.67	1.38
P- VALUE	0.0008	0.0009	0.00005

The same letters mean no differences between them under 0.05 significantly levels according Dancun test

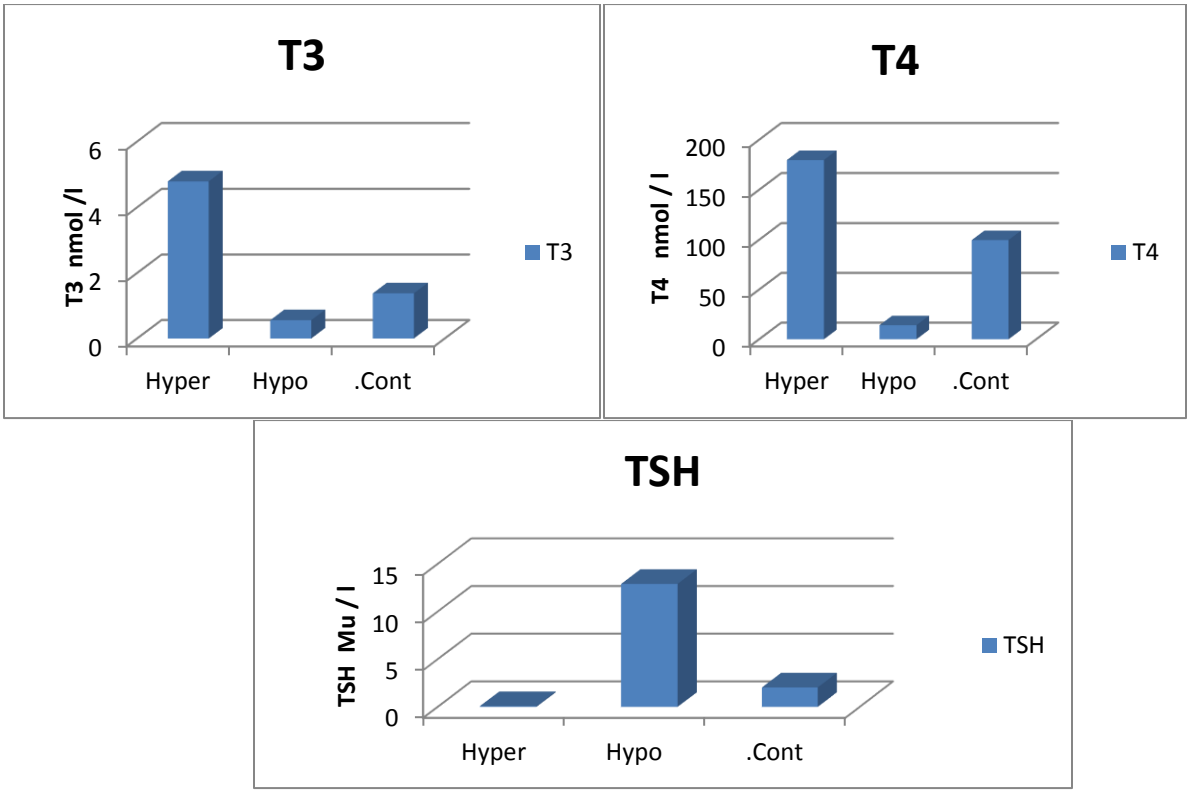


Fig. (3): Variation of T3, T4 and TSH in the serum of patients compared to healthy people as control

The values of thyroid hormones (TSH, T4 and T3) in Hyperthyroid and Hypothyroid paints were studied in relation to both ages and gender, the results are summarized numerically in Tables 8 and 9. Inspection of the results obtained shows that:

i- There is an increase in the concentration of TSH hormone for the (31-45) year-old group in patients with hypothyroidism compared to the age group (15-30) year and the age group of (46-60) year.

ii- There is a significant decrease in the concentration of T4, T3 in patients with hypothyroidism compared with healthy people.

iii- There is an increase in TSH concentration in patients with hypothyroidism in males with a concentration of (22.55nmol/l a) compared to healthy males (2.125 nmol/l c), while the concentration of female infected (11.97 nmol/l b) compared to healthy females (1.84 nmol/l c). Thus, it can be noted that there is an increase in TSH values for both females and males compared to healthy people.

Table (8): The values of TSH, T3 and T4 in serum of patients and control according to age.

	Hyperthyroid			Hypothyroid			Control		
Age	TSH	T4	T3	TSH	T4	T3	TSH	T4	T3
15-30	0.11	194.2	4.890	11.38	13.63	0.5600	1.776	98.99	1.3500
31-45	0.079	171.3	4.707	29.61	15.22	0.5545	2.425	96.78	1.4250
46-60	0.700	177.1	4.843	10.261	13.44	0.5727	2.09	102.93	1.3900
Mean	0.296	177.5	4.813	17.083	14.09	0.5624	2.079	99.56	1.3883

*The same letters mean no significant differences between them according to dancun test.

Table (9): The values of Fe, Zn and Cu in serum of patients and control according to gender.

	Hyperthyroid			Hypothyroid			Control		
	TSH	T4	T3	TSH	T4	T3	TSH	T4	T3
	0.0965	182.3	4.647	11.97	11.744	0.6000	1.840	106.48	1.290
	0.076	176.8	4.960	22.55	16.475	0.5250	2.125	96.57	1.4227
Mean	0.0863	179.55	4.803	17.260	14.109	0.5625	1.9825	101.52	1.356

*The same letters mean no significant differences between them according to dancun test.

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