Biochemical study of the association between Zn, Cu, and Se with hormones of thyroid gland in patients with thyroid disorder.

A study Submitted to the College of Science, Karbala University in partial fulfillment of the Requirement for the Degree of High Diploma Degree in Clinical and Pharmaceutical analysis

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2010 **1431**

بسم الله الرحمن الرحيم

﴿ وَقُلِ اعْمَلُواْ فَسَيَرَى اللّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ ﴾

التوبة الآية ١٠٥

Dedication

To
My parents …for their love and encouragement
To
My wife… for her love and help

To
My children:
Refqaa
Marwaa
Demaa

Hassan

Acknowledgements

In the name of Allah, the first who deserves all thanks and appreciation for granting me will, strength and help with which this research has been accomplished.

It is great pleasure to express my gratitude to a number of persons whose guidance and help have been of immense value in completing this study.

My great dept goes to my supervisors, Prof. Dr**.** Sahib Ali Mehdi and Prof. Dr**.** Alaa Frak Husain who took the task of supervising this thesis, advising and cooperation during my work.

I would like to thank the department of chemistry, college of Science University of Karbala.

Special thanks to the department of biochemistry, college of pharmacy in University of Karbala, and Laboratory Unit in AL-Hussein (General Hospital).

I present my respect and gratitude to Dr. Thamer. K AL-Janabi for his comments in the statistical analysis of the data.

This work has been accomplished with the direct and indirect help of many people. I wish to express my deep thanks, especially to:

- Sincere thanks and deep appreciation to the candles that brighten my long way in life (my family) especially to my wife for encouragement and help ; to my father and Mather, for their support during and after distress .
- I thank everybody whose effort has put a stone in building up this work.

Certification

We certify that this research work reported in this thesis has been carried under our supervision at the department of chemistry of the college of science in the University of Karbala, in partial fulfillment of the Requirement for the High Diploma Degree in Clinical and Pharmaceutical analysis.

Signature:

Name: *Prof.Dr*. Sahib Ali Mehdi

Date:

Signature:

Name: *Prof.Dr*. Alaa Frak Husain

Date :

In review of available recommendations, I forward this study for debate by the examining committee.

Signature:

Prof.Dr. Alaa Frak Husain

Head of the Chemistry Department

Date:

Summary:

This study was conducted in AL-Hussein General Hospital and out patients clinic in Karbala city ,to determine the value of thyroid hormone (T3 triiodothyronine, T4 tetraiodothyrionine and thyroid stimulating hormone and the concentration of the Zinc, Copper and Selenium in patients with thyroid disorder (hyperthyroidism 31) and hypothyroidism21), and evaluate the association between them. All patients were (females) the range of is age from 23to 57 years, and Control Groups Were 40,They were collected from the medical staff and patients relatives who were free from signs and symptoms of thyroid disorder all of these group are females .

The study shows the following results:-

1- The concentration of serum Zinc in patient with hyperthyroidism was higher than control ($p<0.001$).

2- The concentration of serum Copper in patient with hyperthyroidism was higher than control ($p<0.001$).

3- The concentration of serum Selenium in patient with hyperthyroidism was lower than control ($p<0.001$).

4- The concentration of serum Zinc in patient with hypothyroidism was lower than control ($p<0.001$).

5- The concentration of serum Copper in patient with hypothyroidism was lower than control ($p<0.001$).

6- The concentration of serum Selenium in patient with hypothyroidism was lower than control ($p<0.001$).

7- Determination of serum Copper concentration in atomic absorption and with spectrophtometric method, and then compared.

8- The result of atomic absorption method more economic, more precise and easier than spectrophtometric method.

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Abbreviations

T3	Triiodothyronine
T4	Titraiodothyrionine
TSH	Thyroid stimulating hormone
TRF	Thyrotropin-releasing factor
TRH	Thyroid releasing hormone
TMB	tetramethylbenzidine
Cu	Copper
Se	Selenium
Zn	Zinc
µL	Microleter
µg	microgram
nm	Nanometer
dL	Deciliter
µIU/ml	Microinternational Unite
ml	Milliliter
FAAS	Flame atomic absorption spectroscopy
SOD	Superoxide dismutase
SePP	Selenoprotein
ng	Monogram
s	Standard Deviation

Chapter one Introduction &Literature Review

Introduction

1.1 Thyroid gland:-

Thyroid gland located immediately below the larynx on each side of and anterior to the trachea is one of the largest endocrine glands , normally weighing 15-20 grams in adults, and is increased in pregnancy [1]. Measuring about (5 centimeters) as show in figure (1-1) [2].

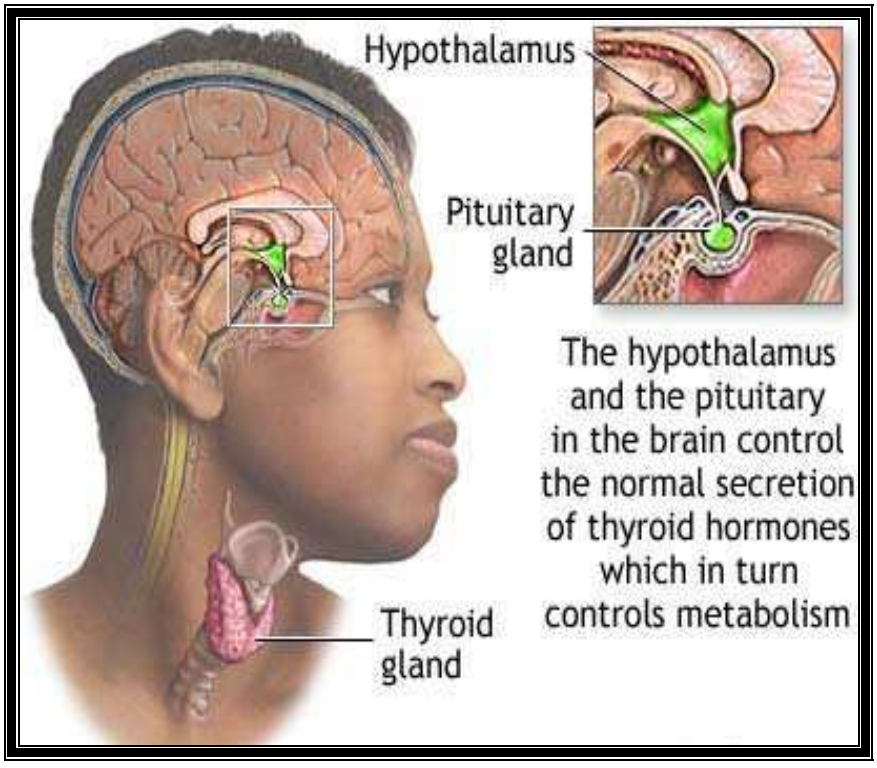
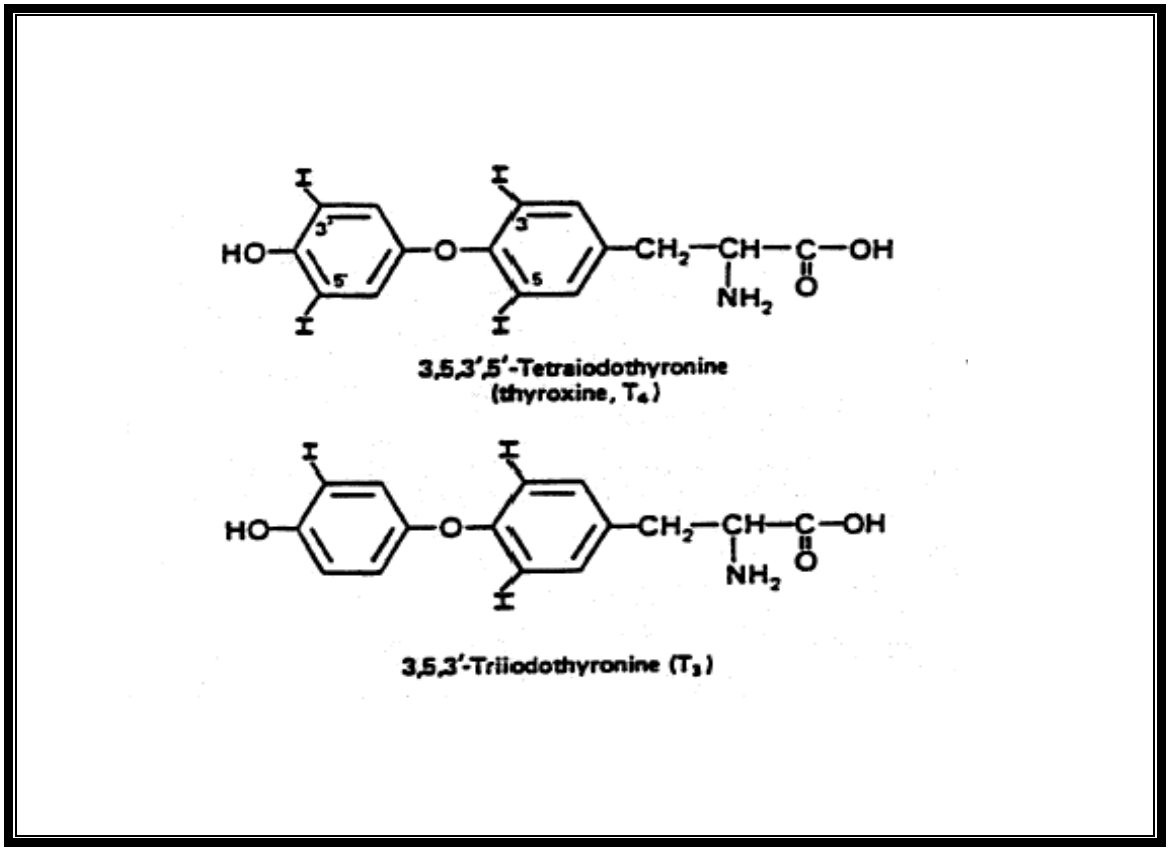


Figure (1-1) the thyroid gland.

The thyroid gland is essential to normal body growth in infancy and childhood. It is also regulate the metabolic rate. It secreted the hormone thyroxin directly into the blood by absorbing iodine from the diet because the iodine an essential component of the hormone [3]. The Chemical structure of thyroxin (T4) and triiodothyronine (T3)



Figure(1-2) the Chemical structure of thyroxin (T4) and triiodothyronine (T3)

1.2 Biosynthesis of thyroid hormone.

The primary function of thyroid gland is production of thyroxin (3, 5, 3^-, 5^- tetraiodothyrionine) T_4 and (3, 5, 3^- triiodthyrionine) T_3.and calcitonin a hormone concerned with calcium homostasis [3].

The formation of thyroid hormone s involves the following complex sequence of events:

1- Active uptake of iodide by follicular cells.

2-oxidation of iodide and formation of iodotyrosyl residues of thyroglobulin

3-formation of iodothyronines from idotyrosines.

4- Proteolysis of thyroglobulin and release of T_4 and T_3 into blood

5- Conversion of T_4 to T_3 [4, 5 and 6].

This process summarized in figure (3-1) .

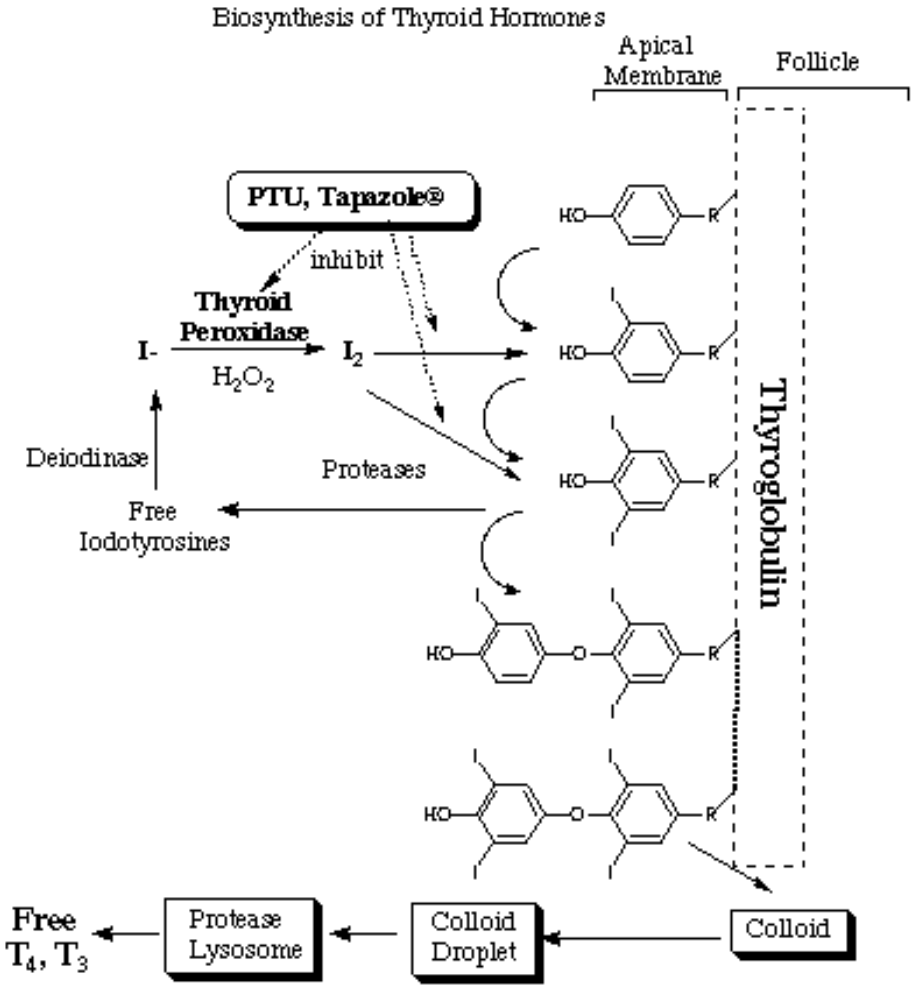


Figure (1-3) Biosynthesis of thyroid hormone.

1.3 Thyroid –Stimulating hormone TSH:

Thyrotropin or [thyroid-stimulating hormone (TSH)], is a glycoprotein hormone released by the anterior pituitary gland that stimulates the thyroid gland to release thyroxine and T3. The release of thyrotropin is triggered by the action of thyrotropin-releasing factor (TRF), a substance found in the hypothalamus of the brain. TRF, once released from the hypothalamus, travels in the blood stream to the anterior pituitary, where it causes the release of thyrotropin. This latter substance, a glycoprotein is carried to the thyroid gland by the blood,

where it stimulates the uptake of iodine, the conversion of diiodotyrosine to thyroxine, and the secretion of thyroid hormones into the bloodstream. Thyroxine inhibits the further release of thyrotropin by interfering with the action of TRF; thus the levels of thyroid hormones are regulated. If not enough iodine is available in the diet, then not enough thyroxine will be made to shut off the release of thyrotropin [7].

1.4 regulation of thyroid gland

The synthesis of thyroid hormones is controlled by feedback regulation. T_3 appears to be more actively involved than T4 in the regulation process. The production of (TSH) by pituitary and (TRH) by hypothalamus are inhibited by T_3 and, to a lesser degree by T_4. The increased of TSH and TRH occurs in response to decreased circulatory levels of T_3 and T_4. , the body has sufficient stores of hormones to last for several weeks. Hence it takes some months to observe thyroid functional deficiency [8].

The recent studies indicate that this process is physiologically regulated. Changes in pituitary conversion of T4 to T3 are often the opposite of those that accrue in the liver and kidney under similar circumstances [7]. As show in figure (1-4) [9].

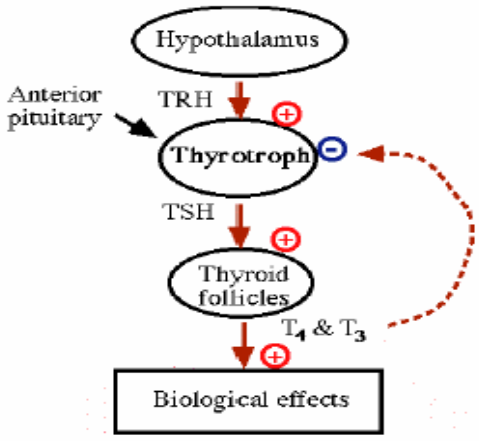


Figure (1-4) regulation of thyroid gland

1.5 Disorder of thyroid gland.

It is general term representing several different diseases involving thyroid hormone and thyroid gland. Thyroid disorders are commonly separated into two major categories:

1- Hyperthyroidism.

- Graves's disease.
- Hashimotos disorder.
- Thyroid cancer.
- Generalized.

2- Hypothyroidism

- Goiter.
- Cretinism.
- Myxedema**.**

1.5.2 Hyperthyroidism (thyrotoxicosis).

Hyperthyroidism is condition in which there is overproduction of thyroid hormone by the thyroid gland ,causing the levels of thyroid hormone in the blood to be too high ,people who have it are often said to have a “overactive thyroid” [10,11] .

The signs and symptoms of hyperthyroidism are Attributable to the effects of excess thyroid hormone in the circulation. The severity of signs and symptoms may be related to the duration of the illness, the magnitude of the hormone excess, and the age of the patient. The following list illustrates the spectrum of possible Signs and symptoms associated with the various causes Hyperthyroidism as show in table (1-1).

Common Causes
1- Graves disease 2- Toxic multinodular 3- Solitary toxic
Clinical feature
• Nervousness and irritability • Palpitations and tachycardia • Heat intolerance or increased sweating • Tremor • Weight loss or gain • Alterations in appetite • Frequent bowel movements or diarrhea • Dependent lower-extremity edema • Sudden paralysis • Exertional intolerance and dyspnea • Menstrual disturbance (decreased flow) • Impaired fertility • Mental disturbances • Sleep disturbances (including insomnia) • Changes in vision, photophobia, eye irritation, diplopia, Or exophthalmos • Fatigue and muscle weakness • Thyroid enlargement (depending on cause) • Pretibial myxedema (in patients with Graves’ disease)

These symptoms [13], [12].as show in figure (1-5).

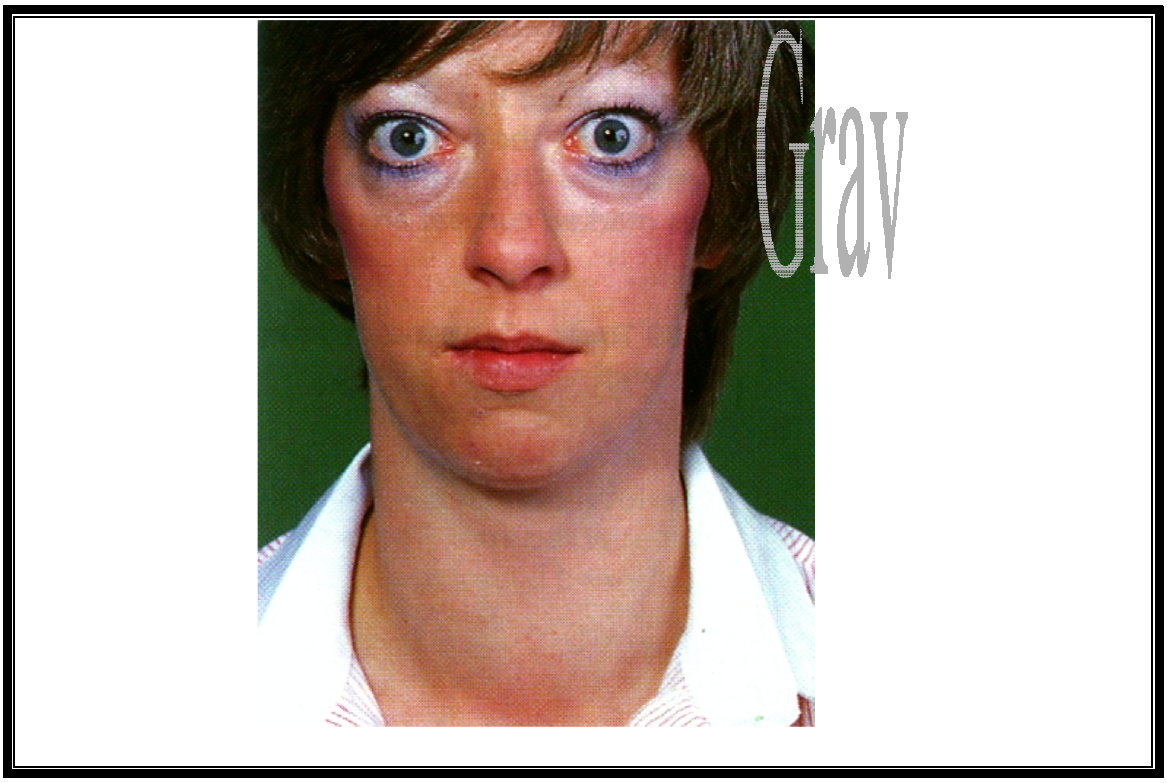


Figure (1-5) patient with hyperthyroidism disease

1.5.3 Hypothyroidism:

Hypothyroidism is the clinical picture that one sees when the thyroid is unable to produce enough thyroid hormones, triiodothyronine (T_3) and thyroxin (T_4), to keep blood levels normal and to satisfy the needs of peripheral tissues. Most patients have primary hypothyroidism, a result of disease in the thyroid that destroys its ability to produce adequate thyroid hormones. Hypothyroidism is occasionally secondary, caused by disease in the pituitary gland or hypothalamus resulting in inadequate production of thyroid-stimulating hormone (TSH). The clinical presentation of hypothyroidism is variable, but often includes symptoms such as cold intolerance, fatigue, constipation, hair loss, dry skin, and other symptoms of a sluggish metabolism. As previously discussed, these symptoms are nonspecific and the diagnosis is often missed for months or even years. The clinical picture varies from mild symptoms early in the disease to

very severe symptoms, such as confusion, lethargy, and even coma in the later stages. Physical findings may include goiter, slow speech and thinking, very dry skin, and slow relaxation of deep tendon reflexes [12].

1.5.1 Goiter.

The term non toxic Goiter refers to the enlargement of the thyroid which is not associated with overproduction of thyroid hormone or malignancy. The thyroid can become very large so that it can easily be seen as a mass in the neck .this picture depicts the outline of a normal size thyroid in black and the grater enlarged in pink. There are a number of factors which may cause the thyroid to become enlarged. A diet deficient in Iodine a Goiter but this is rarely the cause because of the readily available iodine or diets [13].

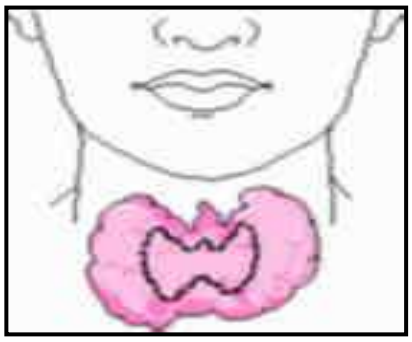


Figure (1-6) The Goiter.

1-6 Trace elements:-

Trace elements are present in the body in very low amount ,usually less than 1 microgram per gram of tissue .They can be subdivided into four major groupings based on their physiological function [13] .

1.6.1 Classification.

1.6.1.1

for which recommended daily allowance has been established .these element have been shown to be essential for normal growth, development, and maintenance and specific biological role has been identified .The elements in this group that are considered are Iron, Zinc ,Iodine, and Selenium .

1.6.1.2-

Trace elements for which there is definite evidence of an essential role in human metabolism but for which (RDA) has not yet been established .This includes the transition metals chromium, cobalt, and molybdenum.

1.6.1.3.

Trace element that are consistently found in tissues or biological fluids in "ultra trace" amounts but that have not yet been shown to be either essential or detrimental at these levels of concentration .these include lithium ,Nickel, tin, silicon and vanadium

1.6.1.4.

Trace metals that have no known biological function in humans but that, if present at relatively low levels cause pathological changes. These toxic trace elements include

Aluminum, cadmium, mercury, lead and arsenic [13].

1.6.2. Zinc:-

The total content of zinc in adult body is about 2g and mainly an intracellular element. The biochemical function of the Zn is an essential component of the several enzymes such as carbonic anhydrase, alcohol dehydrogenase, and alkaline phosphates. And the storage and secretion of the insulin from the pancreas requires Zn and it is necessary to maintain

the normal levels of vitamin A. The body of the adult requirement 10-15 mg/day and the rich source of Zn are meat, fish, milk, eggs. The concentration of the Zn in serum is about 100 µg/dl. Zinc deficiency is associated with growth retardatiorr, poor wound healing, anemia. The zinc toxicity is often observed in welder's duo to inhalation zinc oxide fumes [3].

1.6.2.1 Zinc and thyroid function.

Zinc is crucial in both the production of T-4 thyroid hormone (thyroxin) in the thyroid gland, as well as in the conversion of T-4 to T-3 thyroid hormone, the active form (thyronine). Zinc is necessary for the TRH hypothalamus hormone to stimulate the pituitary gland, which signals the thyroid gland to produce thyroid hormone.Moreover, zinc is needed at the intracellular level to help the thyroid nuclear receptors attach and drive the reading of the DNA genetic code. Keep in mind that the main function of thyroid hormone is to help put the genetic code into action [29]

1.6.3- Copper.

The body contains about 100mg copper distributed in different organs. The biochemical functions of Cu that is an essential constituent of several enzymes such as cytochrome oxidase, catalase, ascorbic acid oxidase and uricase.Due to its presence in a wide variety of enzyme, copper is involved in many metabolic reaction .also Cu is necessary for the synthesis of hemoglobin and necessary cross-linking these structural protein and involved in the conversion of iron from Fe^{2+} to Fe^{3+}in which form iron (transferring) is transported in plasma, Cu also necessary for the syntheses phospholipids and development of bone and nervous system require Cu.The body of adults requires 2-3mg/day copper and the sources of it liver, kidney, meat, egg yolk and green leafy vegetable

The copper concentration on plasma is about 100-200 mg/dL .The sever deficiency of Cu causes demineralization of bones, anemia and myocardial fibrosis, graying of hair and the defect in the intestinal absorption of Cu causes (make's disease) and abnormal copper metabolism causes Wilson's disease [3].

1.6.3.1 Copper and thyroid function.

Copper plays an important role in maintaining a healthy thyroid gland and preventing thyroid disease and other problems. Copper has separate role in providing thyroid support, yet, Copper plays an important role in thyroid metabolism, especially in hormone production and absorption. Copper stimulates the production.of the thyroxin hormone (T4), and prevents over-absorption of T4 in the blood cells by controlling the body's calcium levels. Besides this, copper is also required for the synthesis of phospholipids, (a class Of fats) that are found in the myelin sheaths that insulates nerves to protect them. Phospholipids are required for the stimulation of TSH (Thyroid Stimulating Hormone). Therefore correct levels are needed to prevent thyroid problems, and can be used in the treatment of thyroid disease [14].

1.6.4. Selenium.

Selenium is a member of the same group oxygen and sulfur. It is known that in plants selenium is present predominantly as selenomethionine, whereas in animals selenocysteine is the major form. Four selenium atoms are covalently bound to cysteine residues in the enzyme glutathione peroxidase, which has strong antioxidant properties and, in animal models, acts synergistically with Vitamin E.Selenium deficiency has been demonstrated in Keshan syndrome (this name from city in china) where soil selenium content is very low.Se toxicity is

characterized by dermatitis, loose hair, and diseased nails [13]. A daily intake of Selenium of 50-200 ug of Se has been recommended for adult's .the good source of Se are organ meats (liver, Kidney) and sea foods [3].

1.6.4.1 Selenium and thyroid gland:-

The thyroid contains more Se per gram of tissue than any other organ . And Se, like iodine, is essential for normal thyroid function and thyroid hormone homeostasis .Four selenium atoms are covalently bound to cysteine residues in the enzyme glutathione peroxidase, which has strong antioxidant properties and, in animal models, acts synergistically with Vitamin E. glutathione peroxidase is present in the cytoplasm and mitochondria of tissues it is also found in erythrocytes, platelets, and plasma. A second enzyme, type 1 iodothyronine deiodinase, has been identified, and it contains one Se atom per molecule. This Selenium-metalloenzyme plays a role in the conversion of T_4 to T_3 [15] [16].

1.7 Aims of the study.

The aims of this study are:

1-measuring the levels of the thyroid hormone (T3, T4, and TSH) in thyroid disorder patients (hypothyroidism, hyperthyroidism)

2- Evaluating the concentration of the Zinc, Copper and Selenium in Corresponding patients (by atomic absorption)

3- Correlating between trace element and thyroid hormone in patients with thyroid disorder.

4- Evaluating of concentration of the Copper Corresponding patients (by spectrophotometer).

5- Comparing between two techniques (atomic absorption & spectrophotometer).

Chapter two
Material & Method

MATERIAL& METHODS

2-1. Chemicals:-

All common laboratory chemicals and reagent are of the high available purity and specific chemicals used in this study and they are obtained from the following companies.

Chemicals	**Company**
Triiodothyronine (T3)	Biocheck foster city
Tetraiodothyrionine (T4)	Biocheck foster city
Thyroid stimulating hormone (TSH)	Biocheck foster city
Standard solution of Copper (1000 P.P.M)	With atomic Absorption instrumental
Standard solution of Zinc (1000 P.P.M)	With atomic Absorption instrumental
Selenium Standard solution of	With atomic Absorption instrumental
Nitric acid (HNO_3)	Fluka
Dithiozone	Fluka
Copper salt $Cu(SO_4)_2$	Fluka
Carbon tetrachloride CCL4	Fluka

2.2. Instrumentals:-

The instrumental used in this study and their suppliers are shown below .

Instrumental	**Company**
Mine ELISA spectrophotometer	BPA 160 (chain)
Atomic absorption	Hitachi (Japan)
Centrifuge test tube	Hitesh (German)
Spectrophotometer	Appile (Japan)
Different grade of atomic micropipettes	Humane (Human)
Shakier	Humane (Human)

2.3. Patients and Control

Two groups of thyroid dysfunction patients (all females and not pregnant) were included in this study. All samples were collected from laboratory unit in Al-Hussein General Hospital in Karbala and out patients clinic. The patients were classified into three groups.

1- Groups I: - consisted of 31 patients with hyperthyroidism (mean age 41±9.7 yr)

2- Groups II: consisted 0f 26 patients with hypothyroidism (mean age 41±11yr).

3- Control groups: - These groups consisted of 40 people (females and not pregnant) and free symptoms of thyroid disease (hyperthyroidism and hypothyroidism) (mean of age 40.68±11.1yr).

2.4. Specimen Collection:

The samples were collected from Patients who were admitted for treatment in AL-Hussein General Hospital in Karbala and out patients clinic, Five milliliter of venous blood were drawn from patients and control. Slow aspiration of the venous blood sample via the needle of syringe to prevent the hyemolysis with tourniquet applied 15 cm above the anterior. All the samples that were grossly hemolysed were neglected and other new samples were taken.

The samples were dropped into clean disposables tubes, left at room temperature for 30 min. for clotting formation and then centrifuged for 20 min. at 5000run per min.

2.5. Methods:-

Each serum sample was analyzed for T3, T4 TSH, Zn, Cu, and Se. All assays were obtained by running duplicates for test, Control and Standard.

2.5.1 Evaluation of thyroid hormone (T3, T4, TSH)

2.5.1.1 Evaluation (Triiodothyrionine) T3:

2.5.1.1. A Principle of the test.

To determine the value of T3 Used an enzyme linked immunosorbant assay specific antibody is coated on micro titer wells .A measured amount of patient serum, a certain amount of monoclonal anti-T3antibody, and constant amount of T3 conjugated with horseradish peroxidase are added to the micro titer wells. During incubation the anti –T3antibody is bound to the second antibody on the wells and T3 and conjugated T3 compete for the limited binding sites on the anti – T3antibody. After 60 min. incubation at room temperature the wells are washed 5 times by water to removed unbound T3 conjugated. A solution of TMB reagent is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotmtrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T3 standard assayed in the same way, the concentration of T3in the unknown sample is then calculated the normal rang of T3 is 0.6-1.85µ g/ml [١٧-19] .

2.5.1.1. B Procedure.

1- Added 50 µL of standard, samples into appropriate wells

2- Added 50 µL of the antibody reagent into each well. Mix thoroughly for 30 min.

3-Added 100ul of working conjugate reagent (add 0.1ml of T3-HRPOconjugate Concentration to 1.0 ml of T3 conjugate diluents (1:10 dilution)), and mix well. Into each well. Mix thoroughly 30 seconds .it is important to have a complete mixing in this step.

4- Incubated at room temperature for 60 min.

5- To remove the incubation mixture flicking content in to a waste container.

6- Rinsed and flicked the micro titer wells 5 times with distilled water

7- Then Strike the wells sharply onto absorbent paper to remove residual water droplets

8- Added 100 µl of TMB Reagent into each well gently mix for 10 seconds

9- Incubated the mixture at room temperature in the dark for 20 minutes without shaking

10- To stop the reaction added 100ul of stop solution to each well.

11- Then gently mix for 30 seconds.

12- The absorption was read at λ 450 nm.

13-To calculate the absorbance value for each sample and standard and construct standard curve by plotting the absorbance of each standard against the concentrations of each standard in µg/ml (by Excel program) ,the absorbance in the (y) axis and concentration on the (x) axis

Then using the absorbance value of each sample and determined the concentration of the sample by application of the equation of standard curve [20], [21].

2.5.1.1. C Calculation

a- To calculate the absorption value (at λ 450 nm) for each of the set of standard (ready in the Kit) 0, 0.75, 1.5, 3.0, 6.0 and 10 µg/ml

b- Constructed a standard curve by plotting the absorbance obtained for each standard against its Concentration in µg/ml on linear graph paper (or by excel) ,with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis .

C- To obtain the absorbance value for each sample; determine the corresponding concentration of T3 in µg/ml from the standard curve.

2.5.1.2 Evaluation of T4 (Tetraiodothyrionine)

2.5.1.2 A principle.

To determine the value of T4 Used an enzyme linked immunosorbant assay. A Certain amount of anti-T4 antibody is coated on micro titer wells .A measured amount of patient serum, and constant amount of T4 conjugated with horseradish peroxidase are added to the micro titer wells. During incubation, T4 and conjugated compete for the limited binding sites on the anti-T4antibody. After 60 min. incubation at room temperature, the wells are washed 5 times by water to removed unbound T4 conjugated. A solution of TMB reagent is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotmtrically at 450 nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T4 standard assayed in the same way, the concentration of T4in the unknown sample is then calculated by plotting the standard curve. The normal rang of T4 is 4.8to 12.0 µg/dl [22], [23].

2.5.1.2. B Procedure.

1- Added 25 µL of standard, samples into appropriate wells

2-Added 100 µL of working conjugate reagent (add 0.1ml of T3-HRPOconjugate Concentration to 1.0 ml of T3 conjugate diluents (1:10 dilution), and mix well. In to each well. Mix thoroughly 30 seconds .it is important to have a complete mixing in this step.

3- Incubated the mixture at room temperature for 60 min.

4- Removed the incubation mixture by flicking content in to a waste container.

5- Rinsed and flicked the micro titer wells 5 times with distilled water

6- Then Strike the wells sharply onto absorbent paper to remove residual water droplets

7- Added 100 µL of TMB Reagent into each well gently mix for 10 seconds

8- Incubated at room temperature in the dark place for 20 minutes without shaking

9- Stop the reaction by adding 100ul of stop solution to each well.

10- Gently mix for 30 seconds.

11- Read the absorption at 450 nm

12-calculate the absorbance value for each sample and standard and construct standard curve by plotting the absorbance of each standard against the concentrations of each standard in ng/ml (by Excel program), the absorbance in the (y) axis and concentration on the (x) axi Then using the absorbance value of each sample and determined the concentration of the sample by application of the equation of standard curve [24], [25].

2.5.1.2 C Calculation.

a- To calculate the absorption value (at 450) for each of the set of standard (ready in the Kit) 0, 2, 5, 10, 15, and 25 µg/dl ready to use

b- Constructed a standard curve by plotting the absorbance obtained for each standard against its Concentration in ng/ml on linear graph paper (or by excel) ,with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis .

C- To obtain the absorbance value for each sample; determine the corresponding concentration of T4 in µg/dl from the standard curve

2.5.1.3 Evaluation TSH (thyroid stimulating hormone)

2.5.1.3.A Principle of the test:-

To determin the Value thyroid stimulating hormone (thyrtropin). Used an enzyme linked immunosorbant assay

The assay system utilizes a unique monoclonal antibody directed against a distinct determinant on the intact TSH molecules. Monoclonal antibody is used for solid phase immobilization (on the micro titer wells). Antibody-enzyme (horseradish peroxides) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 2 hour incubation at room temperature, the wells is washed with distill water to remove unbound labeled antibody. Solutions of TMB reagent is added and incubates for 20 min. resulting in the development of a blue color. The color development is stopped with addition of stop solution changing the color to yellow .the concentration of TSH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm [26], [27].

2.5.1.3 B procedure.

1-Added 100 µL of standard, samples into appropriate wells.

2-Added 100µL of Enzyme conjugate reagent into each well.

3- Thoroughly mix for 30 seconds. It very important to mix them completely.

4- Incubated at room temperature for 120 min. with shaking.

5- Removed the incubation mixture by flicking content in to a waste container

6- Rinsed and flicked the micro titer wells 5 times with distilled water.

7- Then Strike the wells sharply onto absorbent paper to remove residual water droplets.

8- Added 100 µL of TMB Reagent into each well gently mix for 10 seconds.

9- Incubated at room temperature for 20 minutes without shaking.

10- To stop the reaction added 100ul of stop solution to each well.

11- Gently mix for 30 seconds.

12- Read the absorption at 450 nm

13-calculate the absorbance value for each sample and standard and construct standard curve by plotting the absorbance of each standard against the concentrations of each standard in ng/ml (by Excel program), the absorbance in the (y) axis and concentration on the (x) axis

Then using the absorbance value of each sample and determined the concentration of the sample by application of the equation of standard curve [28], [29].

5.1.3 C **Calculation**

a- To calculate the absorption value (at 450) for each of the set of standard (ready in the Kit) 0, 0.1, 0.5, 2, 5, and 10 µIU/ml

b- Constructed a standard curve by plotting the absorbance obtained for each standard against its Concentration in ng/ml on linear graph paper (

or by excel) ,with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis .

C- To obtain the absorbance value for each sample; determine the corresponding concentration of TSH in µIU/ml from the standard curve.

2.6 Evaluation of trace element.

Flame atomic absorption is a very common technique for detecting metals and metalloids in environmental samples. It is very reliable and simple to use. The technique is based on the fact that ground state metals absorb light at specific wavelengths. Metal ions in a solution are converted to atomic state by means of a flame. Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve [30]

2.6.1 Principle:-

The technique of flame atomic absorption spectroscopy (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 ^{o}C.

During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths, as shown in figure (2-1).

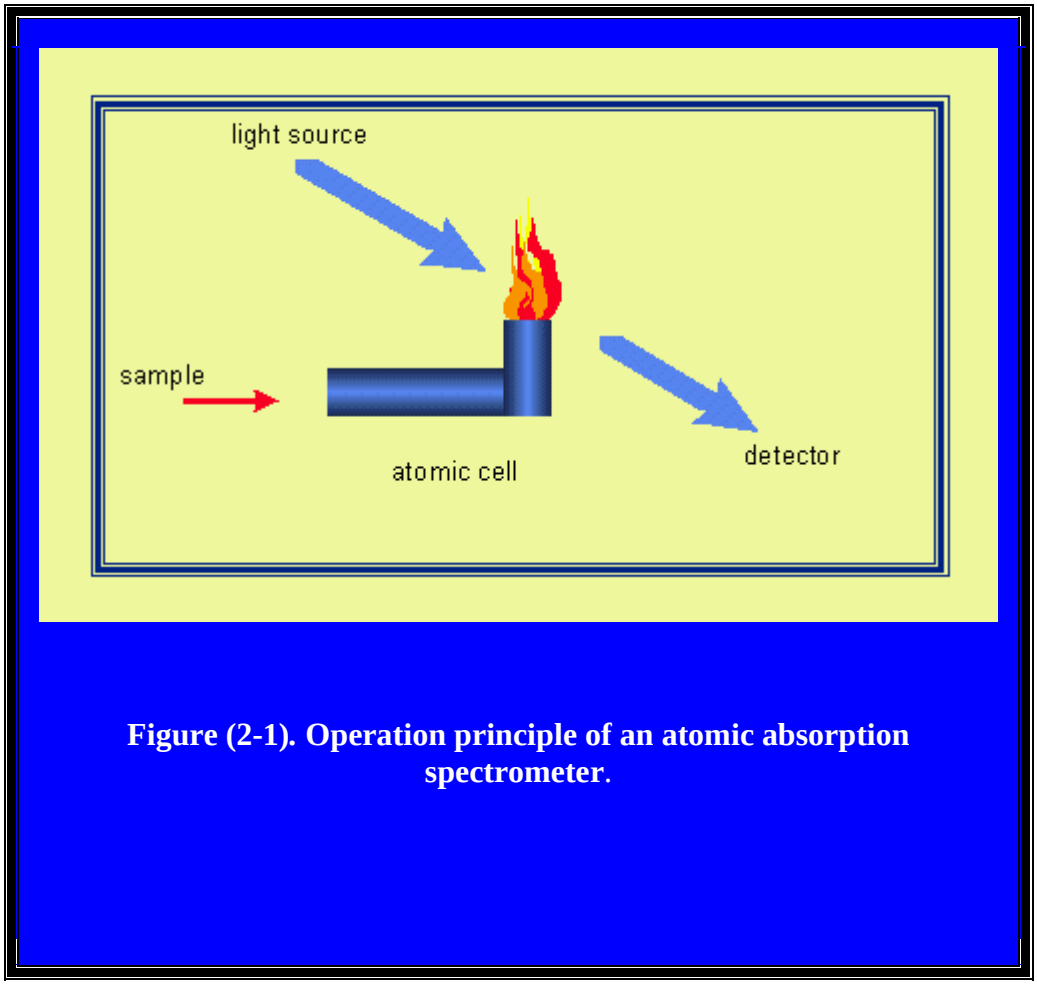


Figure (2-1). Operation principle of an atomic absorption spectrometer.

The characteristic wavelengths are element specific and accurate to 0.01-0.1nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as photomultiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample [39].

2.7. Zinc.

A Hitachi hollow-cathode Zinc lamp was used as the source of a current of 10 am the spectrophotometer was operated at 213.8 nm .the standards and sample were prepared by dilution with deionizer distilled

water, the ratio of sample dilution is 1/100 Zinc concentration calculated by liner regression lines.

2.7.1 Preparation of standards solutions of Zinc.

From 1000 PPM (solution) prepare standards solution (25, 50,100,125,150) µg/dL.

Drawed 25µL from standard solution (1000 PPM) and dilute it to 5mL Deionizer water

And in the same method prepare (50, 75, 100, 1205, 150) ug/dL. Contract a standard curve by plotting the absorbance obtained for each standard against its concentration in µg/dL with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis, and obtained the concentration from the equation of the striate line.

2.7.2 b- Determination of Zinc in the serum:

Serum sample were prepared by dilution with deionzed distilled water in a dilution of 1/100 (50uL of serum in 5mL of deionzed distilled water) Then determine the concentration of Zinc equation of the strait line of standard curve of the Zinc.

2.8. Copper.

A Hitachi hollow-cathode copper lamp was used as the source of a current of 10 am the atomic absorption was operated at 324.8 nm .the standards and sample were prepared by dilution with deionized distilled water, the ratio of sample dilution is 1/10 with 0.1 N HNO_3 ,Copper concentration was Calculated by liner regression lines.

2.8.1 Preparation of standards of Copper

From 1000 PPM (solution) prepare standards solution (50,100,150,200,250,300) µg/dL.

Then Draw 50uL from standard solution (1000 PPM) and dilute it to 3mL 0f (0.1 HNO_3)

And in the same method prepare (100,150,200,250,300) µg/dL.

Contract a standard curve by plotting the absorbance obtained for each standard against its concentration in µg/dL with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis, and obtained the concentration from the equation of the striate line.

2.8.2 Determination of copper in the serum:

Serum sample were prepared by dilution with a dilution of 1/10 (300uL of serum in 3 ML of 0.1N HNO_3) Then determine the concentration of Copper in the equation of the striate line of standard curve of the Copper.

2.9 Spectrophotometric determination of copper ion of patients with thyroid disease.

2.9.1 Preparation of solution:-

The solution were prepared in this technique is dithiozone and standard solution of Copper (Copper sulfate) 0.001%.

2.9.1.1 Preparation of Dithiozone (diphenylthiocarbazone H2D2):-

To Prepare 0.001% (dissolve 0.0005g of Dithiozone by CCL4 then complete it to 50 ML)

2.9.1.2 Preparation of standard solution

1- To Prepare stock solution (0.1g/100mL) by dissolved 0.245 of Copper salt by distilled water and complete to 100 (0.1g/100mL) (stock 1)

2- From (stock1) draw 1mL and diluted to 100 ML to prepare (1mg/100mL) this concentration covalent to (1000µg/1oomL) (stock 2)

3- from Step 2 prepare standard solution

2.9.1.3. Procedure.

Added 2ML of Dithiozone solution to the standards and samples then adjusted the PH =1 , then transfer the mixture to the separation funnel and mix to 3-5 min then separate the organic phase and

non organic phase and determined the absorption of organic phase at λ= 550 nm. Then calculate the absorbance value for each sample and standard and construct standard curve by plotting the absorbance of each standard against the concentrations of each standard in µg/ml), the absorbance in the (y) axis and concentration on the (x) axis

Then using the absorbance value of each sample and determined the concentration of the sample by application of the equation of standard curve.

2.10. Statistical **Analysis.**

All results were presented as mean ± standard deviation (M± SD) Statistical analyses were preformed using SAS taking ($P<0.05$) as lowest limit significances. All the statistical analyses were done through the SAS program.

Chapter three
Results & discussion

RESULTS&DISCUSSION

3.1 Result:

A total of (97) patients were studied; all of these patients were female and with age ranging between 22-55 years. A mean age of patients with hypothyroidism **(41±11.1)** years as show in table ( ), the mean age of patients with hyperthyroidism (41±**97**) and the mean age of control group (40.68±11.1) as showN in table (3-1), the serums samples used in this study.

3.2 Biochemical parameters

3.2.1 Serum TSH, T3 and T4 levels

Serum TSH, T3 and T4 were determined in hyperthyroidism, hypothyroidism and normal control (immunoassay). The mean concentration of serum TSH level in patient with hyperthyroidism (0.198±0.15) are significantly lower than the mean of normal control (4.48+1.79**)** as shown in table (3-2)**,** and The Mean concentration of serum TSH level in patients with hypothyroidism (14.5±1.84) higher than the mean of normal control (4.48+1.79) as shown in table (3-3) this result obtained from standard curve of TSH as shown in figure (3-1). The mean concentration of serum T3 level in patients with hyperthyroidism (3.78±0.9) are significantly higher than the mean of normal control (1.11±0.317**)** as shown in table (3-2) and the mean concentration of T3 level in patients with hypothyroidism (0.35±0.105) are significantly lower than the mean of normal control (1.11±0.317**)** as shown in table (3-2) this result obtained from standard curve of T3 as show in figure (3-2).

The mean concentration of serum T4 level in patient with hyperthyroidism (23±3.94) are significantly higher than the mean of normal control (7.94±2.14 **)** as shown in table (3-2) and the mean concentration of T٤ level in patient with hypothyroidism (3.4±0.48) are significantly lower than the mean of normal control (7.94±2.14**)** as show in table (3-3)**.** This result was obtained from standard curve of T4 as show in figure (3-3).

3.2.2 Serum Zinc.

Concentrations of serum Zn in patients with thyroid disease are summarized in table (3-1). Serum Zn was determined in patients with hyperthyroidism, hypothyroidism and normal control (atomic absorption). The mean concentration of serum Zinc in patients with hyperthyroidism was significantly higher than control (91.92p<0.001). The mean concentration of Zn in patients with hypothyroidism was significantly lower than control (52.58 p<0.001). There was a negative correlation between Zn and TSH (-0.65470 p<0.001), and positive correlation with T3 (0.7185 p<0.001) and positive correlation with T4 (0.75313 p<0.001) and positive correlation with Cu (0.77540 P<0.001) and negative correlation with Se .The result of Zn concentration obtained from the standard curve of Zn as shown in figure (3-4).

3.2.٣ Serum Copper.

Concentration**s** of serum Cu in patient with thyroid disease are summarized in table (3-1). Serum Cu was determined in patients with hyperthyroidism, hypothyroidism and normal control (atomic absorption). The mean concentration of serum Copper in patients with hyperthyroidism was significantly higher than control (177.27p<0.001). The mean concentration of Cu in patients with hypothyroidism was significantly lower than control (87.19 p<0.001) and there was a negative

correlation with TSH (-0.71234 p<0.001), and positive correlation with T3 (0.80899p<0.001) and positive correlation with T4 (0.86240 p<0.001) and positive correlation with Zn (0.77540) and negative correlation (-0.37242) .The result of Cu concentration obtained from the standard curve of Cu as show in figure (3-5).

3.2.4 Serum Selenium.

Concentration**s** of serum Se in patient with thyroid disease are summarized in table (3-1)**.**Serum Se was determined in patient with hyperthyroidism, hypothyroidism and normal control (atomic absorption). The mean concentration of serum Selenium in patients with hyperthyroidism was significantly lower than control (0.34p<0.001). The mean concentration of Se in patients with hypothyroidism was significantly lower than control (0.53p<0.001) and there was a negative correlation with TSH (-0.00081 p<0.001), and negative correlation with T3 (-0.24359 p<0.001) and negative correlation with T4 (-0.34735 p<0.001) and negative correlation (-0.348) with Zn and negative correlation (- 0.372) with Cu.

Table (3-1) the Correlation Coefficients between T3, T4, TSH, Zn, Cu and Se

	T3	T4	TSH	Zn	Cu	Se
T3	r 1.00000	r 0.91891 <0.0001	r-0.80201 <0.0001	r0.74185 <0.0001	r0.80899 <0.0001	r -0.243 0.0265
T4	r 0.91891 <0.0001	r 1.00000	r -.077174 <0.0001	r 0.75313 <0.0001	r 0.86240 <0.0001	r-0.3473 0.0013
TSH	r -0.80201 <0.0001	r -.077174 <0.0001	r 1.00000	r -0.65470 <0.0001	r -0.71234 <0.0001	r - 0.00081 0.9942
Zn	r 0.7185 <0.0001	r 0.75313 <0.0001	r -0.65470 <0.0001	r 1.00000	r 0.77540 1.00000	r - 0.348 0.0012
Cu	r 0.80899 <0.0001	r 0.86240 <0.0001	r -0.71234 <0.0001	r 0.77540 <0.0001	r 1.00000	r - 0.372 0.005
Se	r -0.24359 <0.0265	r -0.34735 0.0013	r -0.00081 0.9942	r -0.34844 0.0012	r -0.37242 0.0005	r 1.000

3.3 Standard curves.

3.3.1 Standard curve of TSH.

To determine the thyroid stimulating hormone used in this study the immunoassay technique , plot the standard curve between the absorption (Y axis's) and the concentration of TSH hormone (X axis's) in µIU/mL, obtained the strata line with equation(Y=0.1542x+0.1136) and R^2 =0.9998 as shown in figure (3-1) .

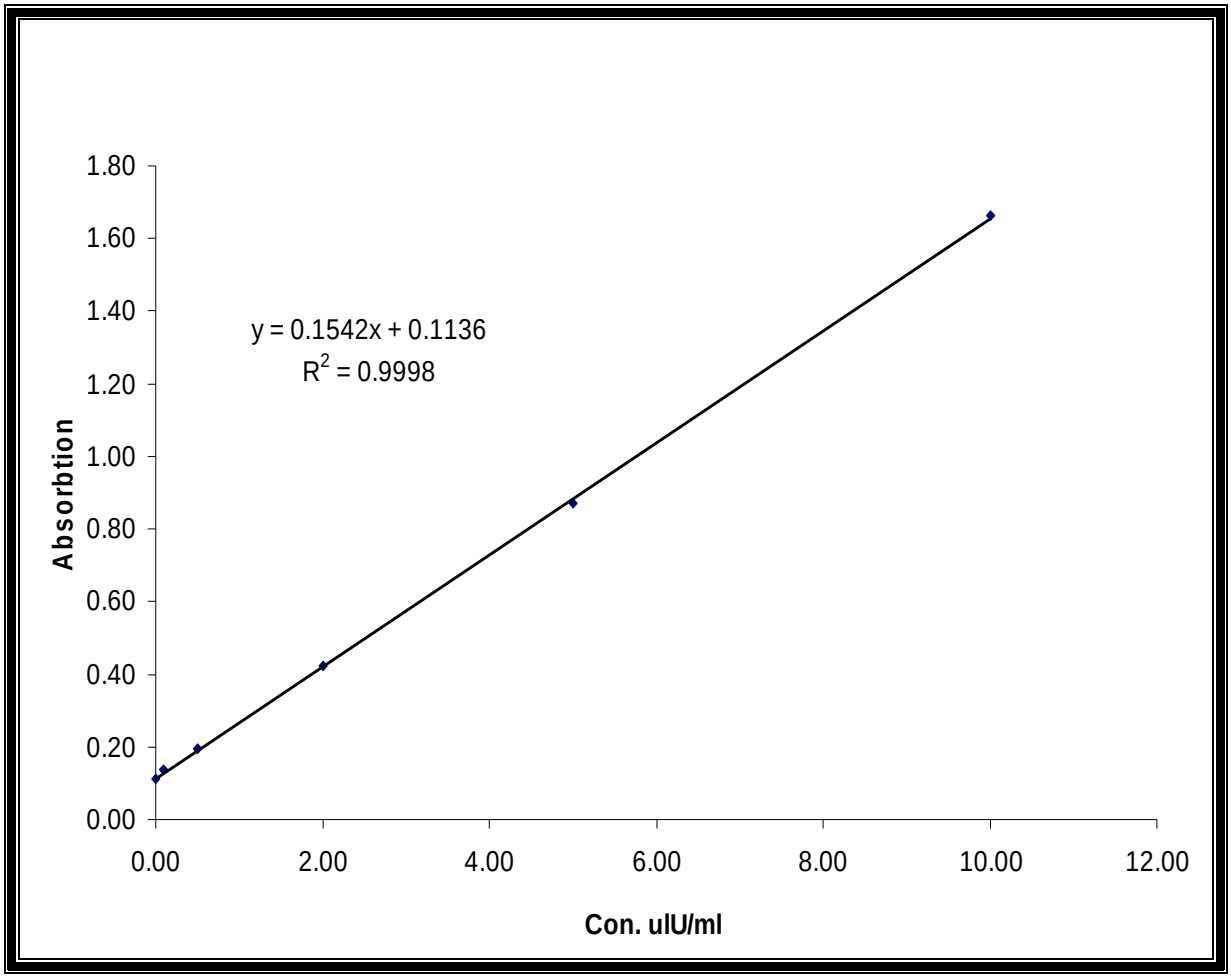


Figure (3-1) standard curve of TSH.

3.3.2 Standard curve of T3.

To determine the triiodothyronine hormone used in this study the immunoassay technique, plot the standard between the absorption (Y axis's) and the concentration of T3 hormone ((X axis's) in ng/mL, obtained the curve with equation(Y=-0.1404x+1.8657) and R^2 =0.9851 as show in figure (3-2).

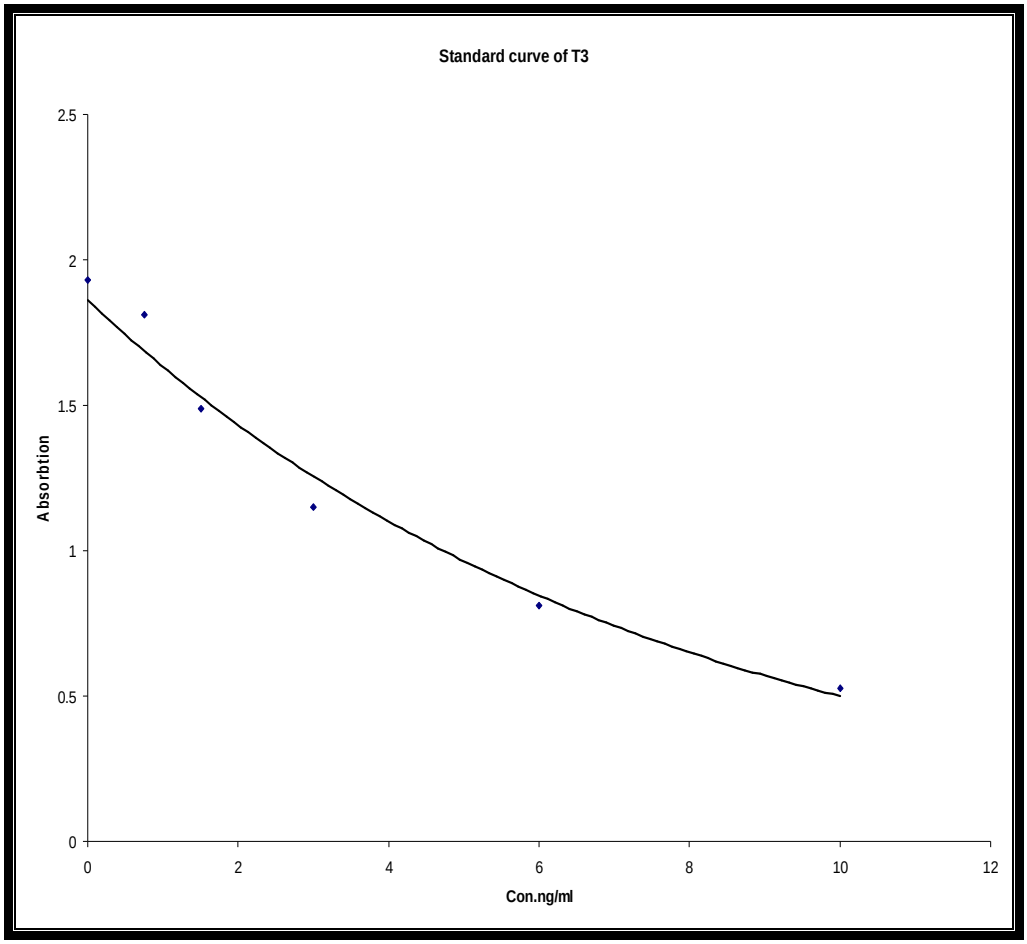


Figure (3-2) Standard curve of T_3.

3.3.٣ Standard curve of T4.

To determine the Tetraiodothyrionine hormone used in this study the immunoassay technique, plot the standard between the absorption (Y axis's) and the concentration of T4 hormone in µg/dL, obtained the curve with equation(Y=-0.05x+1.41) and R^2 =0.6908 as show in figure (3-3).

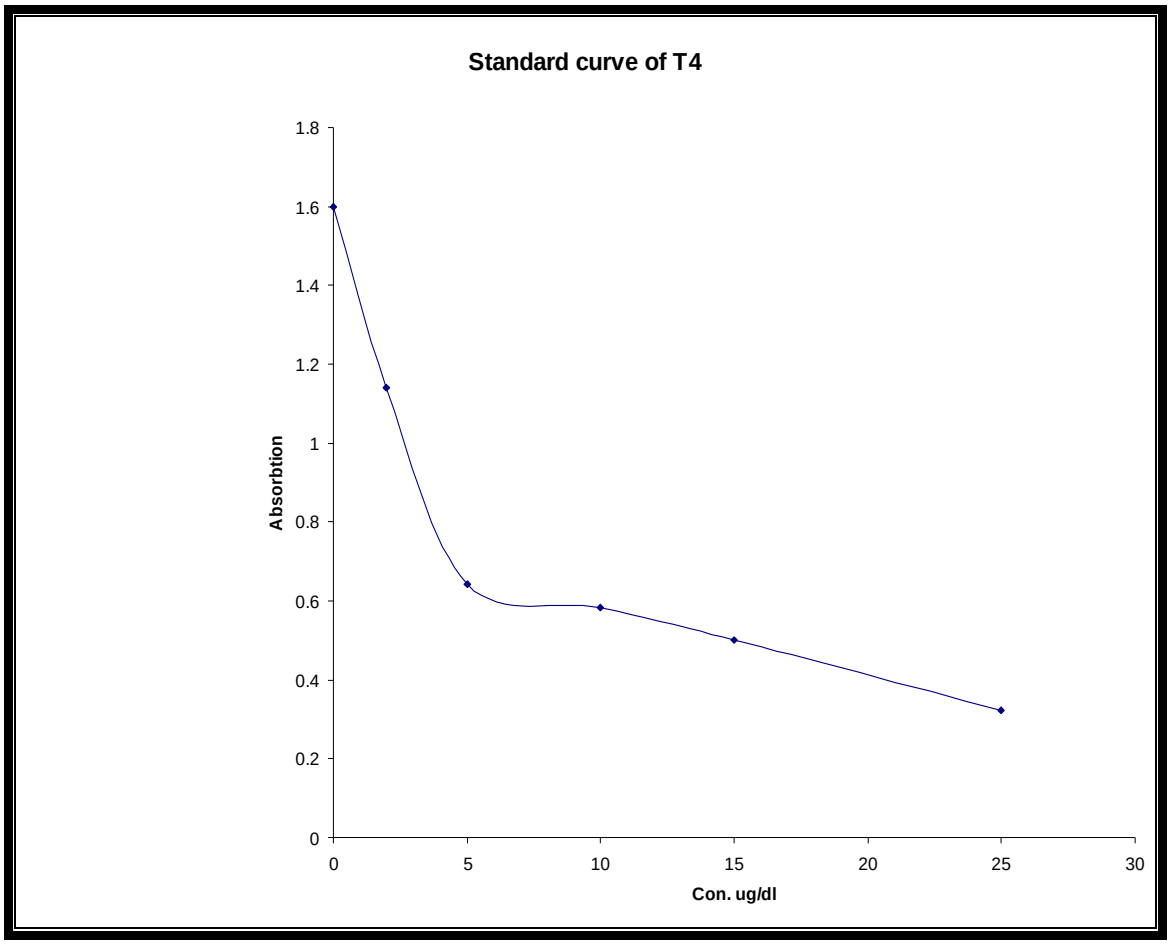


Figure (3-3) Standard curve of T4

3.3.٤ Standard curve of Zinc.

To determine the concentration of Zn in patients with thyroid dieses, evaluated the concentration of a series standard solution of Zn (atomic absorption), then plot the standard curve between the absorption (Y axis's) and the concentration of standard solution of Zinc in mg/dL , obtained the a standard curve (strait line) with equation (Y= 0.0001x+0.000006) and R^2= 0.9962 as show in figure (3-4) .

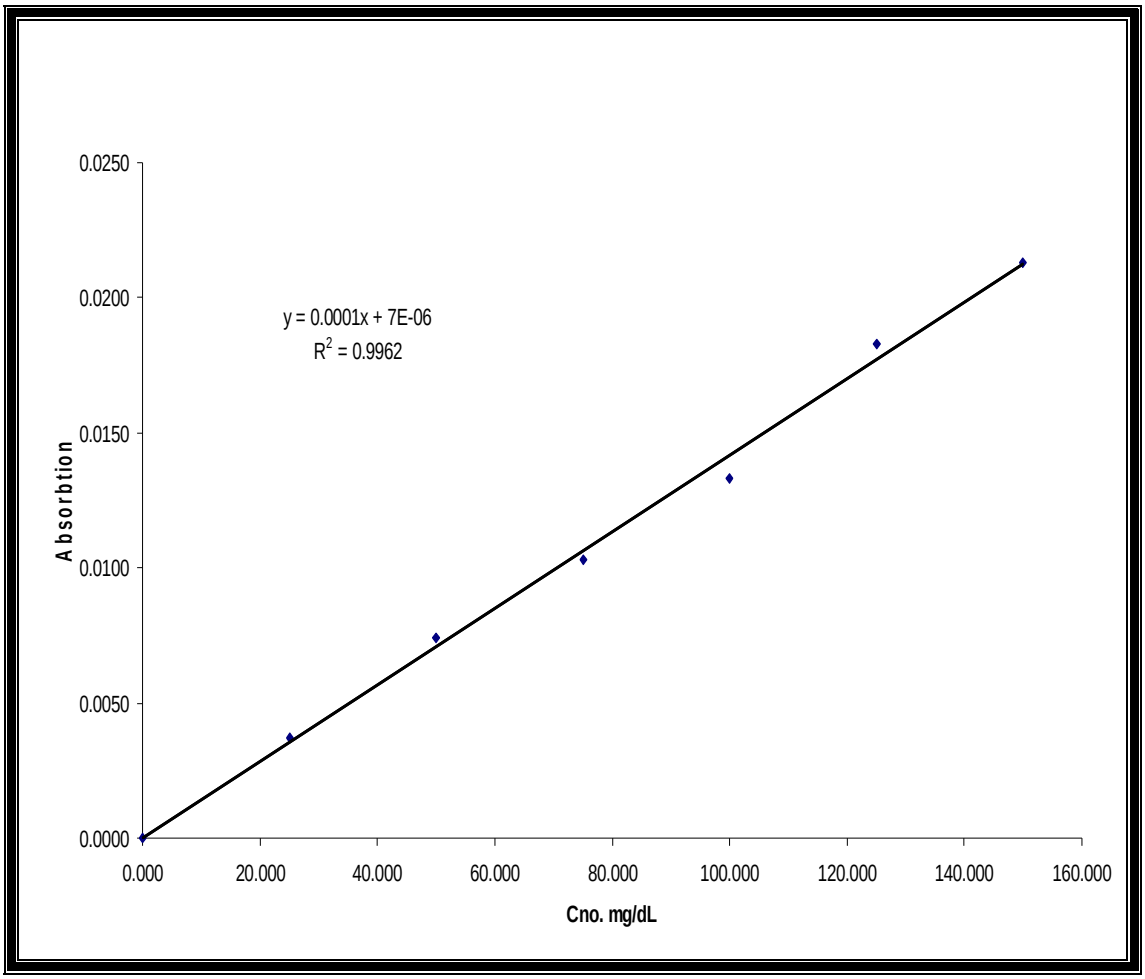


Figure (3-4) Standard curve of Zinc

3.3.5 Standard curve of Copper.

To determine the concentration in patients with thyroid dieses, evaluate the concentration of a series standard solution of Cu (atomic absorption), then plot the standard curve between the absorption (Y axis's) and the concentration of standard solution of Zinc in mg/dL, obtained the a standard curve (strait line) with equation (Y= 0.0001x+0.0003) and R^2= 0.9989 as show in figure (3-5).

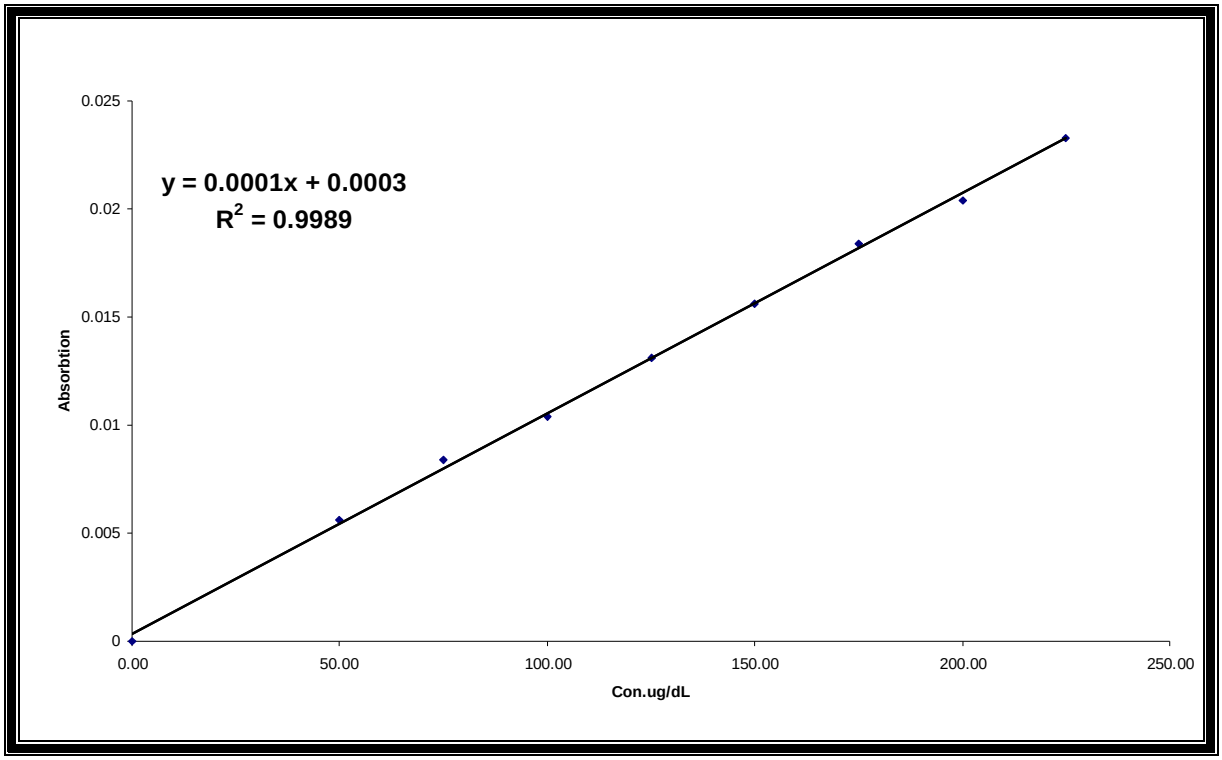


Figure (3-5) standard curve of Copper

3.4. Comparison between hyperthyroidism and control Groups.

The comparisons between the concentration of Copper, Zinc and selenium in patients with hyperthyroidism are summarized in table ( ), Zn and Cu increase in patients with hyperthyroidism but the concentration of Selenium decrease in patients with hyperthyroidism.

Table (3-2) comparison between patients with hyperthyroidism and control group.

		Hyperthyroidism Group	Control Group
Number of patient	**(n)**	**31**	**40**
Mean of age	**(year)**	**41±9.7**	**40.68±11.1**
Total T3	**(ng/mL)**	**3.78±0.9**	**1.11±0.317**
Total T4	**ug/dL**	**23±3.94**	**7.94±2.14**
TSH	**uIU/mL**	**0.198±0.15**	**4.48±1.79**
Zn	**Mg/dL**	**92.02±8.2**	**86.2±9.9**
Cu	**Mg/dL**	**179.3±9.1**	**118.1±25**
Se	**Ug/dL**	**0.34±0.33**	**0.89±0.4**

3.5. Comparisons between hypothyroidism and Control group.

The comparisons between the concentration of Copper, Zinc and selenium in patients with hypothyroidism are summarized in table ( ), Zn, Cu and Selenium decrease in patients with hypothyroidism.

Table (3-3) Comparison between patients with hypothyroidism and Control

		Hypothyroidism	Control groups
Number of patients	**(n)**	**26**	**40**
Mean of age	**(year)**	**41±11.1**	**40.68±11.1**
Total T3	**(ng/mL)**	**0.35±0.105**	**1.11±0.317**
Total T4	**(ug/dL)**	**3.4±0.48**	**7.94±2.14**
TSH	**uIU/mL**	**14.5±1.84**	**4.48±1.79**
Zn	**Mg/dL**	**54.4±12**	**86.2±9.9**
Cu	**Mg/dL**	**82±16.5**	**118.1±25**
Se	**ug/dL**	**0.53±0.28**	**0.89±0.33**

3.6. Comparisons between hyperthyroidism and hypothyroidism

The comparisons between the patients with hyperthyroidism and the patients with hypothyroidism are summarized in table ( ). Zn, Cu and Se decrease in hypothyroidism. Zn, Cu increase in hyperthyroidism, Se decrease in hyperthyroidism.

Table (3-4) Comparison between patients with hypothyroidism and hyperthyroidism

		hyperthyroidism	Hypothyroidism
Number of patients	**(n)**	**31**	**26**
Mean of age	**(year)**	**41.97±13.8**	**40.68+11.1**
Total T3	**(ng/mL)**	**3.78±0.9**	**0.35+0.105**
Total T4	**(ug/dL)**	**23±3.94**	**3.4+0.48**
TSH	**(uIU/mL**	**0.198±0.15**	**14.5±1.84**
Zn	**Mg/dL**	**92.02±8.2**	**54.4±12**
Cu	**Mg/dL**	**179.3±9.1**	**82±16.5**
Se	**µg/dL**	**0.34±0.33**	**0.53±0.28**

3.6 Determination of Copper ion in patients with thyroid disease by using spectrophotometric method.

A nother technique to determine the concentration in patients with thyroid disease, evaluated the concentration of a series standard of solution of Cu was spectrophotometric method**.** Then plot the standard curve between the absorption (Y axis's) and the concentration of standard solution of Zinc in ug/10ml, obtained the standard curve (strait line) with equation (Y= 0.0004x+0.0078) and R^2= 0.9728 as show in figure (3-5).
In this part of this study is measured the concentration of Copper ion to three patients with hyperthyroidism and three patents with hypothyroidism and compared the result of this technique with the result of atomic absorption technique. The value of concentration of copper ion in patients with hyperthyroidism and hypothyroidism in this technique summarized in table (3-5) and table (3-6) respectively.

Table (3-5) The value of Copper in patient with hyperthyroidism (spectrophotometer).

	T3	T4	TSH	Cu
1-	4.1	26	0.09	191
2-	5.2	29	0.05	184
3-	4.4	26.6	0.08	193

Table (3-6) the value of Copper in patient with hypothyroidism (spectrophotometer).

	T3	T4	TSH	Cu
1-	0.19	2.1	16.3	102
2-	0.22	2.0	18	87
3-	0.36	2.7	13.2	81

3.6.1 Compression of serum Copper concentration between the atomic absorption and spectrophotometric method in patients with hyperthyroidism and hypothyroidism.

The comparison between atomic absorption and spectrophotometric method was summarized in table ( ).the concentration of Cu in atomic absorption lower than in spectrophotometric method.

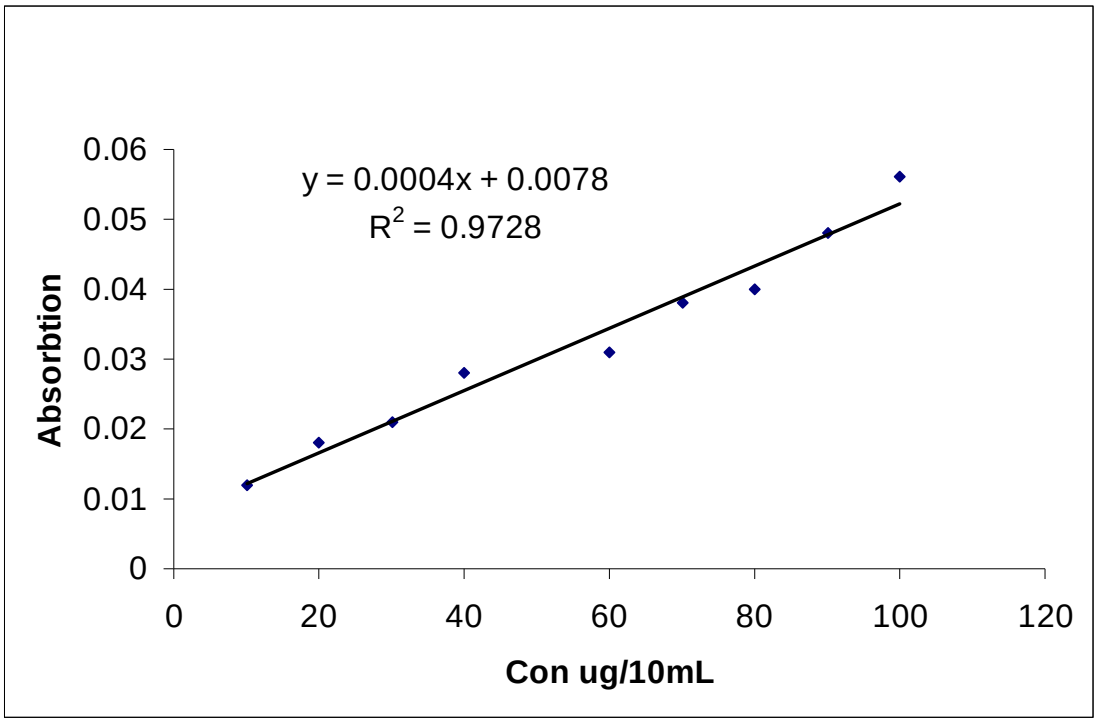
Table (3-7) Compression of Copper concentration between the atomic absorption and spectrophotometer in patients with hypothyroidism and hyperthyroidism.

Hypothyroidism	Atomic absorption	Spectrophotometer
1-	٩٧	102
2-	81	87
3-	٧٥	81
hyperthyroidism		
1-	187	191
2-	175	184
3-	183	193

3.٦.2 Serum Copper concentration in spectrophotometric method.

When compare the atomic absorption method with spectrophotometer Technique the first one is more economic than the second method because the spectrophotometer Technique required more Chemicals (Reagent) and it needs more preparation than atomic

absorption, and the result in atomic absorption is more precise . The Standard curve of the Copper in this technique is shown in figure (3-6).



Figure(3-6) Standard curve of copper (spectrophotometric method).

3.7 Serum Zinc

Zinc works in the body as an important antioxidant .Deficiency of Zinc causes a deficiency of the antioxidant enzyme Superoxide dismutase (SOD), and leads to oxidative stress and ant oxidative response in the patients with hyperthyroidism.

Zinc is thought to have a role in the synthesis and action of many hormones, via Zinc transcription factors. Zinc depletion is associated with low circulating concentration of T4 and testosterone [31].

Therefore Zinc deficiency was shown to impair the metabolism of thyroid hormone.

Yoshikazu Nishi et al (Hiroshima University School of medicine) reported the mean Concentration of plasma Zinc in hypothyroid and hyperthyroidism patients was lower than that control, and (Wollf,1956)

reported Higher Zinc serum in hyperthyroidism and (Bremner and Fell 1977) showed that Plasma Zinc concentration in hyperthyroidism or in hypothyroidism were not different from those of healthy subject [32] . Katsuaki Aihara MD, et all reported no statistically significant was found in plasma zinc between patients with thyroid disease and healthy control [33].

3.8 Serum Copper

Both intracellular and extracellular (SOD) are copper-and Zinc-containing enzymes, able to convert superoxide radical to hydrogen peroxide, which can be subsequently removed by catalase and other antioxidant defenses [34].

Copper plays an important role in maintaining a healthy thyroid gland and prevens thyroid disease and other problems .Copper separated role in providing thyroid support, Copper plays an important role in thyroid metabolism, especially in hormone production and absorption .Cu stimulates the production of thyroxin hormone (T4), and prevent over absorption of T4 in the blood cell by controlling the body calcium levels .Besides this, Copper is also required for the synthesis of phospholipids that required for the stimulating of TSH.

Therefore correct levels are needed to prevent thyroid problem, and can be used in the treatment of thyroid disease.

Gungor Akcay et all (Department of intemal Medicine Turkey) reported that Copper were significantly higher than normal in hyperthyroidism and lower than normal in hypothyroidism [35].

3.9 Serum Selenium

During thyroid hormone synthesis, thyroid tissue is exposed to H2O2 making it imperative that protective systems can prevent damage to the gland. This tissue protection can be achieved by selenium-dependent products, e.g. the glutathione peroxidase. Serum levels of (Se) are considered to depict the adequacy regarding GPx levels and activity. In addition to this, the determination of selenoproteins, e.g. selenoprotein P (SePP), can deliver further information on the adequacy of Se levels.

Katsuaki Aihara, et all reported that plasma Selenium levels were significantly lower than normal in hyperthyroidism [36].

Conclusions

Conclusions

1- The concentration of Serum Copper in patients with hyperthyroidism is higher than the control group ($p<0.001$).

2- The concentration of Serum Zinc in patients with hyperthyroidism is higher than the control group ($p<0.001$).

3- The concentration of Serum Selenium in patients with hyperthyroidism is lower than the control group ($p<0.001$).

4- The concentration of Serum Copper in patients with Hypothyroidism is lower than the control group.

5- The concentration of Zinc in patients with Hypothyroidism is lower than the control group ($p<0.001$).

6- The concentration of Selenium in patients with Hypothyroidism is lower than the control group ($p<0.001$).

7- Determination of this trace element by atomic absorption was more precession and economic than Spectrophotometric Method.

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دراسة كيموحياتية للعلاقة بين بعض العناصر الأثرية وهرمونات الغدة الدرقية لدى المرضى المصابين باضطراب الغدة الدرقية.

مقدمة إلى قسم الكيمياء ولجنة الدراسات العليا في

كلية العلوم جامعة كربلاء من متطلبات نيل درجة الدبلوم

العالي في (التحليلات الكيميائية والدوائية).

من قبل

حسن حيدر خضر السلامي

بكلوريوس علوم الكيمياء / الجامعة المستنصرية

إشراف

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الخلاصة

تمت هذه الدراسة خلال فترة تشرين الثاني ٢٠٠٩ م ولغاية شباط ٢٠١٠م على (٥٧) مريضاً مصاباً بأمرض الغدة الدرقية (أفراط هرمونات وقلة هرمونات الغدة الدرقية) كانوا جميعهم من الأناث (غير حوامل وغير مدخنات) وقد أجريت على المرضى المراجعين لمستشفى الحسين في كربلاء وكذلك في المختبرات الخاصة . تتراوح أعمارهم بين ٢٠-٥٥ سنة هذا بالإضافة إلى مجموعة سيطرة الأصحاء عدد (٥٠) شخص .

تم إجراء فحوصات هرمونات الغدة الدرقية (TSH, T3, T4) وكذلك تم قياس بعض العناص الأثرية , الزنك والنحاس والسلينيوم .ومن خلال هذه الدراسة وجد أن

١- تركيز الزنك في مصل الدم للمرضى المصابين في فرط هرمنات الغدة الدرقية أعلى من تركيز الزنك في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٢- تركيز النحاس في مصل الدم للمرضى المصابين في فرط هرمونات الغدة الدرقية أعلى من تركيز النحاس في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٣- تركيز السلسنيوم في مصل الدم للمرضى المصابين في فرط هرمونات الغدة الدرقية أقل من تركيز السلسنيوم في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٤- تركيز الزنك في مصل الدم للمرضى المصابين في قلة أفراز هرمنات الغدة الدرقية أقل من تركيز الزنك في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٥ - تركيز النحاس في مصل الدم للمرضى المصابين في قلة أفراز هرمونات الغدة الدرقية أقل من تركيز النحاس في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٦ - تركيز السلسنيوم في مصل الدم للمرضى المصابين في قلة أفراز هرمونات الغدة الدرقية أقل من تركيز السلسنيوم في مصل الدم للمجموعة الأصحاء ($P<0.001$).

٧- تم قياس تركيز النحاس على المطياف الضوئي ومقارنة النتائج مع طريقة الأمتصاص الذري وقرنة النتائج.

٨- نتائج طريقة الأمتصاص الذري أكثر دقة وأكثر سهولة وكذلك أقتصادية من طريقة الطياف الضوئي .