

Determination of some trace elements Zn,Cu and Fe in the blood serum for patients with thyroid disease in middle and south of Iraq using atomic absorption spectrophotometer.

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Abstract

This investigation included the determination of some trace elements Zn, Cu, and Fe in the blood serum by flame-atomic absorption spectrometry after finding the optimum conditions for the estimation which involve the wave length, spectral band, lamp current, the burner width and the burner height. The concentration of zinc in the blood serum of the patients with hyperthyroidism was in the range of (0.596-0.681mg.l⁻¹) which was lower than that of control concentration range (0.801-0.850mg.l⁻¹) whereas for the patients with hypothyroidism was (0.601-0.748mg.l⁻¹). The concentration of copper in the patients with hyperthyroidism was higher range (0.93-1.12mg.l⁻¹) than that of average concentrations in control (0.85-0.86mg.l⁻¹) but decreased in the patients with hypothyroidism in the range of (0.77-0.83mg.l⁻¹). The concentration of iron in the serum was increased in the patients with hyperthyroidism (0.97-1.03mg.l⁻¹) and in the patients with Hypothyroidism (0.80-0.91mg.l⁻¹) comparing with control concentrations (0.90-0.95mg.l⁻¹). The concentration linear range was (4-0.5) mg.l⁻¹ and correlation coefficient (r) was not less than 0.984. The statistical and other analytical results show that the relative standard deviation RSD% for trace elements (Zn, Cu, Fe) were (2.4, 1.5, 2.3) and detection limits were (0.0063, 0.0087, 0.0093)mg.l⁻¹ respectively with percent recovery ranged between (95.0-100)%. The results obtained by this research give a good explanation for the spread of thyroid disease in middle and south of Iraq.

Introduction

It will be known that the decrease in Zn amounts in human serum leads to a growth stopping (Perrone et al, 1999). Also, zinc has a direct role in the formation of thyroid gland hormones and some enzymes and the decrease of this element will cause troubles in the function of these hormones and other enzymes (Karlik et al, 1996). On the other hand, it has been suggested that the increase of Zn concentration will cause troubles in the defensive systems, the function of antioxidant in the thyroid cells and leads to changing in red blood cells characteristics (Shopsis, 1994). Zinc is regarded as an essential element in testosterone hormone formation which is necessary for women more than men especially in the polluted regions with

heavy metals (Shimada et al,1997). Furthermore, Zn enters as an active site in the work of super oxide dimustase enzyme which is acting as an anti free radical enzyme converting the free radicals to hydrogen peroxide and oxygen which protect the sensitive tissues from the destruction by oxidation (Hawk et al, 1998). As for Iron element, it is indispensable for the normal function of hemoglobin in the red cells, myoglobin in the heart muscle and other function in calactase and peroxidase enzyme(Seymen et al, 1997). Also, Fe affect the activity of both glutathion peroxidase and super oxide dimustase enzymes (Zimmermann et al,2000). The scarcity of iron concentration makes the exposure to thyroid deficiency most possible(Rosenzweig et al, 1999), in addition to physiological changes in the body(Beard et al, 1998). There is a relationship between the patients with iron deficiency and the decrease of (T_3, T_4) hormones(Iwase et al,1993). Copper has an essential role in the growth and multiplication processes in addition to its role in the protection of thyroid tissues from free radical, also enters in the components of many enzymes as a catalyst especially in antioxidant enzymes such as super oxide dimustase and terosetase(Esipenko and Marsakova 1990). Moreover, it has an essential role in thyroid gland function(Karlik et al,1996), in which the studies indicate that the concentration of thyroid hormone in the serum and the activity of T_3 hormone were affected with the decrease of this element ratio (Oliver, 1975)(Alar et al,2000)and this will lead to thyroid deficiency disease(Kajil et al, 1992). Also, Cu affect the system work and reduce its activity while the increase of this element will lead to troubles in this system efficiency(1976, أنيس). Kajil and his colleagues indicated in a study that the presence of cupper in the cells may has a defensive role in protection the body cells from the poisoning risks with high concentration of heavy metals such as Cd(Brian, 1983) . In addition to what was mentioned above, cupper enters as a catalyst in red blood cells building and its scarcity is leading to anemia and shortening the life of red blood cells(Perrone et al, 1999). There are a number of studies and researches concern with the determination of (Fe, Zn, Cu) in the biological fluids. In these studies, Fe, Cu and Zn have been determined in the blood serum of patients with nerves diseases(Eberk et al,1999) using solution of 10% Trichloroacetic acid. Brian have determined(Patricia et al, 1999) Zn and Cu in the hair using HNO_3 as a digester material through the dipping of hair in the acid for 24 hour. In the human bone zinc was determined through the

incineration of the bone at $48C^0$ for 24 hour then determine it by the flame atomic absorption spectroscopy(Zaichich et al, 1995).

Moreover, there are another studies to determine Fe, Zn and Cu in the thyroid cells and its effect on the antioxidant enzymes in these cells especially in the patients animals with thyroid deficiency(Brunetto et al, 1999). Also, these studies have concerned with the effect of Fe, Cu and Zn on thyroid gland cancer by determination of these elements in the blood serum of the patients with this disease(Kelson & Shamberger, 1978). In addition, these elements have been determined in the blood serum of children(Bialy et al,1980) and their effects on the children. Our study has focused on determination of these trace elements in the serum of the patients with thyroid disease (hyper or hypo) and their effect on the function of thyroid hormones especially in south and middle of Iraq.

Experimental part

1-Apparatus & instruments

A)Flame atomic absorption spectrophotometer. Type: Shimadzu-680-(P/N206-16150).

B)Ultrasonic bath. Type:Karl kolb-scientific technical supplies D6072-Germany.

C)pH-meter.Type:Coring equipped with pole.

D)Shaker water bath.Type:TECHA.

H)Centerfuge.Type:Shimadzu.

F)Sensitive electronic balance.Type:Mettler(M.30).

2-Chemicals & Reagents

The chemicals which were used in this study were at high purity and equipped from the International companies as shown in table(1).

Table (1):The chemicals purity and its sources.

Chemicals	Chemical formula	%	Company
Trichloro acetic acid	Cl_3CCOOH	99.5	Randox
Hydrochloric acid	HCl	36.5	BDH
Nitric acid	HNO_3	70	Fluka
Cupric nitrate pentahydrate	$Cu(NO_3)_2.5H_2O$	99.0	Gainland
Zinc oxide	ZnO	98.0	Gainland
Ferric oxide	Fe_2O_3	99.0	Gainland

Reagents:

Preparation of the standard solutions

A) Preparation of zinc standard solution (1000mg.l⁻¹).

It was prepared by dissolving of 1.2446gm from zinc oxide (ZnO) in an amount of deionized water, followed by addition of (5M HCl) to complete the dissolving and completing the volume to (1000ml) by ion free water. Diluted concentrations (0.5, 1, 1.5, 2, 2.5) were prepared from zinc standard solution by diluting with deionized water.

B) Preparation of copper standard solution (1000mg.l⁻¹).

It was prepared by dissolving (4.3700)gm from hydrous Copper nitrate $\text{Cu}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ in a few amount of deionized water then completing the volume to(1000ml) by deionized water. Diluted concentrations (0.5, 1, 1.5,2, 2.5) were prepared from the Cu standard solution by diluting with deionized water.

C) Preparation of Iron standard solution (1000mg.l⁻¹).

It was prepared by dissolving (1.4297)gm from ferric oxide Fe_2O_3 in 33ml of concentrated hydrochloric acid (HCl) then heating the solution for 10 minutes and completing the volume to (1000ml) with deionized water. Diluted concentrations (0.5, 1, 1.5, 2, 2.5) were prepared from the Fe standard solution by diluting with deionized water.

3-Optimum experimental conditions

Zn, Cu and Fe were determined after fixing the optimum experimental conditions using flame atomic absorption which its details were shown in instrument manual.

4-General procedure for the flame atomic absorption

A) Determination of Zn in the blood serum(Oliver et al, 1987).

Zn element content in the blood serum was determined by diluting(0.5ml) of the serum sample with eight volumes of 1% V/V HNO_3 .After that, the atomic absorption signal of the resulted solution was registered under the optimum conditions as shown in table(2).

B) Determination of Cu in the blood serum(Ohta et al, 1988).

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 as shown in table(2).

C) Determination of Fe in the blood serum(Ohta et al, 1988).

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.

Results and discussions

1-Effect of Zn on hyperthyroidism and hypothyroidism disease

The results have shown that the Zn blood serum content for the normal men was (0.850mg.l⁻¹) while for the patients men with hyperthyroidism was varying between (0.613-0.681mg.l⁻¹). As for the women, the results have shown that Zn blood serum content for the normal women was (0.80mg.l⁻¹) while for the patients women was (0.596-0.657mg.l⁻¹) as shown in the table (3-1). It is clear from the results mentioned above that 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 and this means the increase of the active thyroid hormone T₃(Iwase,K et al 1993) and the decrease of Zn will lead to oxidation risks for the Methalouonien protein (MT) which is acting as a carrier for Zn and other trace elements.

Table(2-1): The average concentration of Zn in the blood serum for both control group and patients groups.

(A)For patients men with Hyperthyroidism

Group	Averag (mg.l ⁻¹)	S.D
The control from 15-40year	0.850	0.0522
From 15-25	0.681	0.1484
From 25-40 year	0.664	0.1066
From 40 year and above	0.613	0.0838

* average of 20 samples

(B)For patients women with Hyperthyroidism

Group	Averag (mg.l ⁻¹)	S.D
The control from 15-40year	0.801	0.0510
From 15-25	0.657	0.0940
From 25-40 year	0.634	0.0691
From 40 year and above	0.596	0.0536

* average of 20 samples

Concerning the patients with hypothyroidism, the results have shown that the Zn blood serum content ratio for the normal men was (0.850mg.l⁻¹) while for the patients men was varying between (0.665-0.748 mg.l⁻¹). On the other hand, for the normal women Zn blood serum content ratio was (0.801 mg.l⁻¹) while for the patients women was varying between (0.601-0.715mg.l⁻¹) as shown in the table (3-2). The results have shown that the decrease of Zn concentration in the blood serum will lead to decrease the concentration of glutathion peroxidase enzyme and reduce the concentration of T₃,T₄ in the blood serum therefore the activity of (heptatic-1,5-deiodenase) will be reduced by 67% which means that the conversion of T₄ to the active T₃ will be decreased. For the patients with hyperthyroidism, it was found that if there is a scarcity in Zn in their body then its concentration will be increased in the urine while for the patients with hypothyroidism the concentration of Zn in the urine will be decreased(Muller et al, 1996).

Table(2-2): The average concentration of Zn in the blood serum for both control group and patients groups.

(A)For patients men with hypothyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.850	0.0522
From 15-25	0.748	0.0526
From 25-40 year	0.684	0.0887
From 40 year and above	0.665	0.0510

* average of 20 samples

(B)For patients women with hypothyroidism

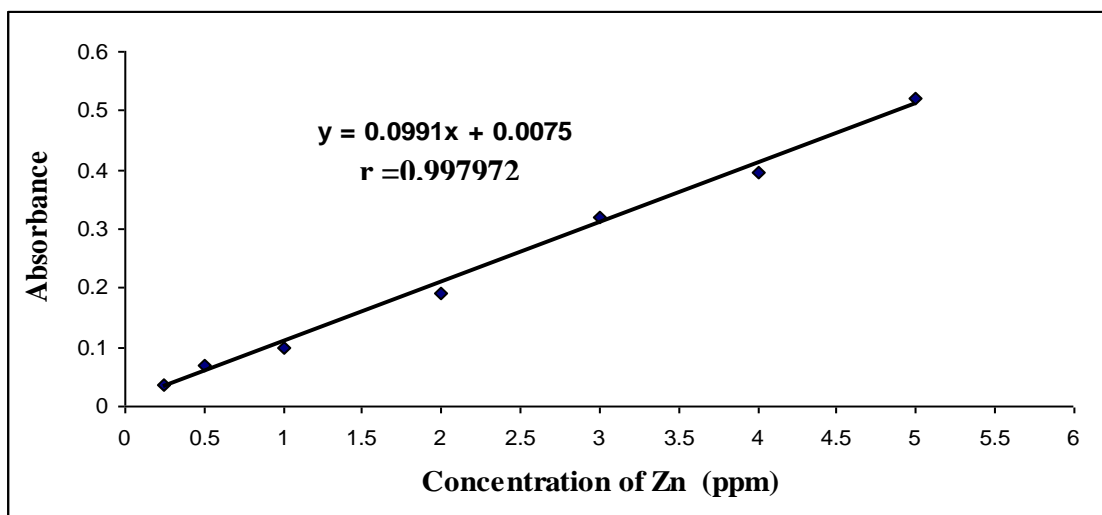
Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.801	0.0511
From 15-25	0.715	0.0649
From 25-40 year	0.642	0.0526
From 40 year and above	0.601	0.0745

* average of 20 samples

Calibration graph of Zn determination:

After fixing the optimum conditions, the calibration graph was drawn up. A linear relationship has arisen for concentrations to range from 1 to 4 mg.l⁻¹ and correlation coefficient 0.9896 which are shown in fig(1). In addition, the accuracy of the flame atomic absorption method was measured using standard solutions from Zn solution then the recovery

percentage and the relative standard deviation (RSD%) were calculated as shown in table(4). The method has shown a high recovery percentage and detection limit of (0.0063 mg.l⁻¹) with high accuracy.



Fig(1): Calibration graph for Zn determination

Table(3): Accuracy & precision for Zn determination.

Zn added (mg.l ⁻¹)	Zn found (mg.l ⁻¹)	Recovery%	RSD%
0.362	0.372	97.31	2.4
0.394	0.400	98.50	3.5
0.420	0.430	97.67	4.7

Zn lower detection limit = 0.0063 mg.L⁻¹ or 6.3μ.gL⁻¹=6.3ppb

2-Effect of Cu on hyperthyroidism and hypothyroidism disease

The results have shown that the Cu blood serum content for the normal men was (0.86 mg.l⁻¹) while for the patient men with Hyperthyroidism was to range (0.93-1.03 mg.l⁻¹). As for the normal women, the Cu blood serum content was (0.85 mg.l⁻¹) while for the patient women with hyperthyroidism was to range (0.95-1.12 mg.l⁻¹). The results of analysis gave a differences in Cu blood serum content for the normal and patients as shown in table (5-1). In view of Cu importance in the body where it has a role in the formation of many enzymes especially super oxide dimustase enzyme 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(Karlik et al,1996). 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 \cdot). Moreover, the researches indicate that the increase of Cu level in the patients with hyperthyroidism leads to increase in T₃ concentration which affect the metabolism of thyroid hormones(Alar et al, 2000), consequently, the inhibition of glutathion peroxidase activity in its work as an antioxidant(Brian, 1983).

Table(4-1):The average concentration of Cu in the blood serum for both control group and patients groups.

(A)For patients men with hyperthyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.86	0.1510
From 15-25	0.93	0.1041
From 25-40 year	0.97	0.8540
From 40 year and above	1.03	0.1269

* average of 20 samples

(B)For patients women with hyperthyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.85	0.1640
From 15-25	0.95	0.2164
From 25-40 year	0.99	0.2022
From 40 year and above	1.12	0.1945

* average of 20 samples

Concerning the patients with Hypothyroidism, the results have shown that the Cu blood serum content for the normal men was (0.86 mg.l⁻¹) while for the patient men with Hypothyroidism was to range (0.79-0.83mg.l⁻¹). As for the normal women, the Cu blood serum content was (0.85 mg.l⁻¹) while for the patients women was to range (0.77-0.81 mg.l⁻¹). The results showed differences in Cu blood serum content in the normal men and women as shown in table (5-2). It was found from the results that the decrease in Cu concentration in the case of Hypothyroidism is leading to decrease in Glutathion peroxidase concentration then a drop in super oxide dismutase concentration consequently. In this case, the risks of cells destruction will be increased, the formation of free radicals(Muller,M et al 1996)and the exposure to Hypothyroidism(Kajil et al, 1992).

Table(4-2): The average concentration of Cu in the blood serum for both control group and patients groups.

(A)For patients men with hypothyroidism

Group	Average (mg.l ⁻¹)	S.D
The control from 15-40year	0.86	0.1510
From 15-25	0.83	0.0731
From 25-40 year	0.81	0.1145
From 40 year and above	0.79	0.1571

* average of 20 samples

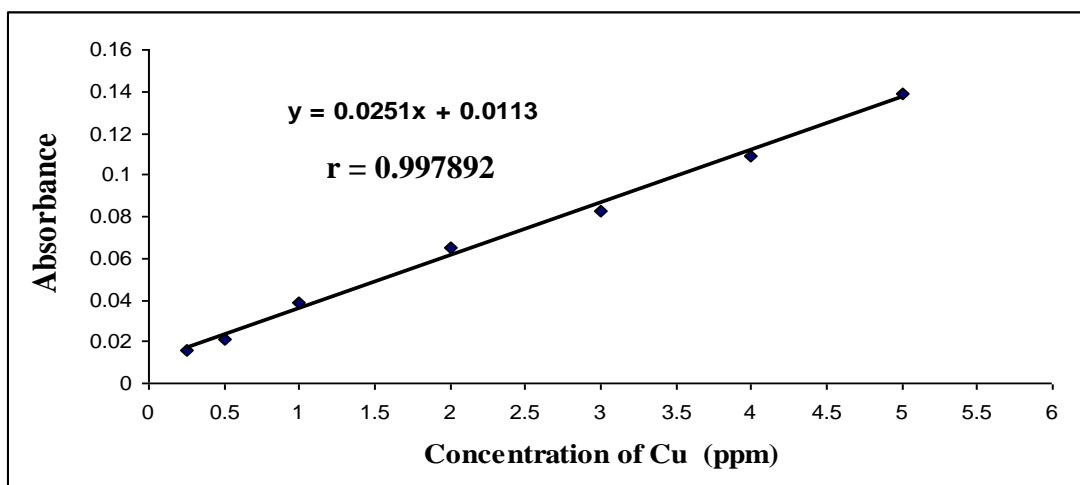
(B)For patients women with hypothyroidism

Group	Average (mg.l ⁻¹)	S.D
The control from 15-40year	0.85	0.1640
From 15-25	0.81	0.1070
From 25-40 year	0.78	0.1286
From 40 year and above	0.77	0.1380

* average of 20 samples

Calibration graph of Cu determination:

After fixing the optimum conditions, the calibration graph was drawn up. A linear relationship has arisen for concentrations to range from 0.5 to 4 mg.l⁻¹ and correlation coefficient 0.9962 which are shown in fig(2). Also, the accuracy of the flame atomic absorption method was measured using standard solutions from Cu solution then the recovery percentage and the relative standard deviation (RSD%) were calculated as shown in table (6). The method has shown a high recovery percentage and low detection limit with high accuracy.



Fig(2):Calibration graph for Cu determination

Table(5): Accuracy & precision for Cu determination.

Cu added (mg.l ⁻¹)	Cu found (mg.l ⁻¹)	Recovery%	RSD%
0.68	0.71	95.7	5.1
1.25	1.27	98.4	3.3
2.14	2.14	100.0	1.5

Cu lower detection limit = 0.0087 mg.L⁻¹ = 8.7µg.L⁻¹ = 8.7 ppb

3- Effect of Fe on hyperthyroidism and hypothyroidism disease

The results have shown that the Fe blood serum content for the normal men was (0.95 mg.l⁻¹) while for the patients men with hyperthyroidism was varying between (0.97-1.08 mg.l⁻¹). As for the normal women, the Fe blood serum content was (0.90 mg.l⁻¹) while for the patients women with hyperthyroidism was varying between (0.92-1.03 mg.l⁻¹) as shown in table (7-1). From these results, it is clear that there is an increase in Fe concentration range in the blood serum for the pathological cases in comparison with the control values. The increase of Fe ion concentration in the blood leads to reduce the activity of (glutathion), (glutathion 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(Seymen et al, 1997). The researches emphasize that the Fe-stored level (Ferritin) increase in the patients with hyperthyroidism(Zimmermann et al, 2000).

Table(6-1): The average concentration of Fe in the blood serum for both control group and patients groups.

(A)For patients men with hyperthyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.95	0.0513
From 15-25	0.97	0.1359
From 25-40 year	0.99	0.1107
From 40 year and above	1.08	0.0918

* average of 20 samples

(B)For patients women with hyperthyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.90	0.1078
From 15-25	0.92	0.1492
From 25-40 year	0.95	0.1143
From 40 year and above	1.03	0.0951

* average of 20 samples

Concerning the patients with Hypothyroidism, The results have shown that the Fe blood serum content for the normal men was (0.95 mg.l⁻¹) while for the patients men was varying between (0.84-0.91 mg.l⁻¹). On the other hand, for the normal women the Fe blood serum content was (0.90 mg.l⁻¹) while for the patients women with hypothyroidism was varying between (0.80-0.87 mg.l⁻¹). Generally, it is clear that there is a decrease in Fe level in the patients with hypothyroidism in comparison with the control values as shown in table (7-2). Fe is regarded as an essential component for some antioxidant enzymes such as (superdome dissimulates) and (Peroxidase) that is, in the case of Fe scarcity their work as an antioxidant will be reduced in the process of extermination the free radicals. Moreover, the scarcity of Fe affect the work of thyroid hormone in conversion (T₄) to (T₃). Furthermore, for the patients with Hypothyroidism, if Fe is increased in their nutrition then this will lead to improvement in the thyroid tumor and this is matching with research(Seymen et al, 1997) and formation of Ferrite will not be high in this disease as in the case for the patients with hyperthyroidism(Dehpanda & Nadkarni, 1992).

Table(6-2): The average concentration of Fe in the blood serum for both control group and patients groups.

(A)For patients men with Hypothyroidism

Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.95	0.0513
From 15-25	0.91	0.0817
From 25-40 year	0.87	0.1949
From 40 year and above	0.84	0.2095

* average of 20 samples

(B)For patients women with Hypothyroidism

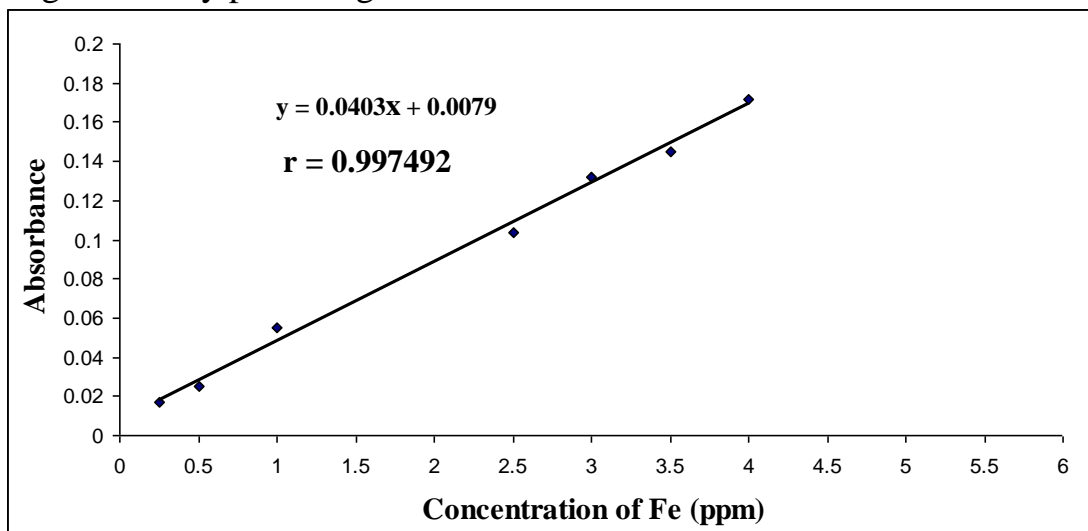
Group	Averag(mg.l ⁻¹)	S.D
The control from 15-40year	0.90	0.1043
From 15-25	0.87	0.1705
From 25-40 year	0.84	0.1908
From 40 year and above	0.80	0.3790

* average of 20 samples

Calibration graph of Fe determination:

After fixing the optimum conditions, the calibration graph was drawn up. A linear relationship has arisen for concentrations to range from 0.5 to 4 mg.l⁻¹ and correlation coefficient 0.9978as shown in fig(3). Also, the

accuracy of the used method was measured through the preparation of standard solutions from Fe solution and then the recovery percentage and the relative standard deviation (RSD%) were calculated as shown in table (8). The method was characterized with high accuracy, low detection limit and high recovery percentage.



Fig(3):Calibration graph for Fe determination.

Table(7): Accuracy & precision for Fe determination.

Fe added (mg.l ⁻¹)	Fe found (mg.l ⁻¹)	Recovery%	RSD%
0.85	0.85	100.0	2.3
0.87	0.89	97.7	5.7
0.90	0.91	98.9	4.1

Fe lower detection limit = 0.0093 mg.L⁻¹ =9.3µg.L⁻¹ =9.3ppb

Conclusion

The results have shown that the trace elements which are studied have an essential role in the exposure to the thyroid gland disease. It was found that the concentration of Zn decrease in both (hyper and hypothyroidism).The concentration of Cu and Fe decrease in the patients with hypothyroidism but increase in the patients with hyperthyroidism.

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تقدير بعض العناصر النزرة (Fe,Cu,Zn) في مصل الدم للأشخاص المصابين بمرض الغدة الدرقية في وسط وجنوب العراق باستخدام مطيافية الامتصاص الذري

عبد المجيد خورشيد احمد ، مصطفى راجي اياد و كامران شكر حسين
كلية العلوم – جامعة كركوك

الخلاصة

تضمن هذا البحث تقدير بعض العناصر النزرة (الخاصين، النحاس، الحديد) في مصل الدم باستخدام تقنية الامتصاص الذري اللهبى بعد إيجاد الظروف المثلى للتقدير مثل الطول الموجي وحزمة الطيف وتيار المصباح (مصباح الكاثود المجوف) وكذلك عرض وإرتفاع المشعل.

كان معدل تراكيز الخاصين في مصل دم المصابين بمرض الزيادة في إفراز الغدة الدرقية يتراوح من (0.596-0.681 ملغم.لتر⁻¹) وهو أقل من معدل التراكيز الطبيعي للأصحاء والذي كان يتراوح من (0.801-0.850 ملغم.لتر⁻¹) في حين كان معدل هذا العنصر لدى المصابين بنقص إفراز الدرقية يتراوح من (0.601-0.748 ملغم.لتر⁻¹). أما بالنسبة لمعدل تراكيز النحاس في مصل دم المصابين بمرض الزيادة في إفراز الغدة الدرقية فقد كان أعلى (0.93-1.12 ملغم.لتر⁻¹) من معدل تراكيز الأصحاء والذي كان يتراوح من (0.85-0.86 ملغم.لتر⁻¹) في حين أنه إنخفض لدى المصابين بنقص إفراز الغدة الدرقية (0.77-0.83 ملغم.لتر⁻¹). أما معدل تراكيز الحديد في مصل الدم للأشخاص المصابين بزيادة إفراز الغدة الدرقية فقد كان مرتفعا بين (0.97-1.03 ملغم.لتر⁻¹) ولدى المصابين بنقص إفراز الغدة الدرقية من (0.80-0.91 ملغم.لتر⁻¹) مقارنة مع التراكيز لدى الأصحاء والتي كانت تتراوح من (0.90-0.95 ملغم.لتر⁻¹). وكان المدى الخطي للتراكيز لهذه العناصر يتراوح بين (4-0.5) جزء من المليون وبمعامل ارتباط (r) لا يقل عن 0.984. والنتائج الإحصائية التحليلية بينت أن معدل الإنحراف القياسي النسبي %RSD للعناصر النزرة (الخاصين، النحاس، الحديد) هو (2.4, 1.5, 2.3) وحد الكشف (0.0093, 0.0087, 0.0063) جزء من المليون على التوالي وبنسبة إسترجاع (95.0-100)%. هذه الدراسة تعطي فكرة جيدة عن إنتشار أمراض الغدة الدرقية في مناطق وسط وجنوب العراق.