Original paper

Measurement of Zinc Concentration in Human Blood Serum Byspectophotometric Methods

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Abstract

Background: speciation of zinc means the determination of the concentration of chemical formula of zinc species which is more indicated than total concentration of zinc for clinical diagnosis. It was shown that atomic absortion spectrometry is good technique for measuring of total concentration of zinc after digestion of the serum with concentrated nitric acid and concentrated perchloricacid, while ionic concentration of zinc was determined by visible spectrometry.

Study design and objective :this study was conducted control healthy people from the workers in Tikrit teaching hospital,gendar (10male + 10 female) and their ages are between (35-70)years. This study aimes to find relation between the concentration of bound zinc in human blood serum and ischemaic heart disease.

Methods: bloods samples were collected from clincilay suspected patients who attend teaching hospital in salah-aldingovernate with sign and symtoms of ischemic heart disease (proven by specialist physician). Blood samples were centrifugated and serum was separated to examine for speciation of zinc concentration

Results: the zinc was determined by two analytical methods. The first method is visible spectrophotometry, the absorbance was measured at wavelength 578 nm and the zinc label as ionic zinc. The second method is atomic absorption spectrophotometrymetry, the absorbance was measurd at wavelength 213 nm and the zinc label as total zinc. The bound zinc was mathmetical calculated, which is higher in patients than control groups

Conclusion:according to the study data, it was concluded that the level of total zinc higher than ionic zinc in control and patient, and the levels of ionic and total zinc in patient groups are lower than their levels in control groups

List of abbreviation:

FAAS: Flame Atomic Absorption Spectrometry

GFAAS: Graphite Furnace Atomic Absorption Spectrometry

Keywords: Spectrophotometer, Atomic, Bound, Concentration, Total, Ionic.

Introduction

Zinc assoiciated with many enzyme systems such as metallo-enzyme and enzyme activator [(1)]. Zinc and copper were determined in human blood for medical diagnosis [(2)]. In clinical analysis, the total concentration of zinc and copper in serum are generally use for medical diagnosis of health and disease [(3)]. However, the biological activities or toxicities of zinc depend on their chemical forms in blood serum and thus data for their total concentration are not sufficient for medical diagnosis,therefore speciation of analyses of zinc species in humen blood serum

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now receiving great interest not only in medical diagnosis, but also in biological and environmental science [4]. During the last twenty years ago, it has been an increasing interest in environmental concentration by zinc due to it's trace concentration is essential for maintance for normal growth and development,whileit's high concentration is toxic [5].

Zinc plays an important physiological role in human blood distributed 75-85% in erythrocytes (mostly as carbonic anhydrase), 12-22% in plasma and 3% in leukocytes [6].One third of of zinc in plasma is loosely bound to the serum albumin,the reminder being more firmly attached to α-globulins and with minor fractions complexed in histidine and cysteine[7].In clinical analysis,thetotal concentration of zinc in serum is generally used as convenient parameters for medical diagnosis of health and diseas[8].However, the biological activity or toxicities of trace element strongly depend on their chemical forms in blood serum,and thus data for their total concentrations are not sufficient for medical diagnosis[9].

Therefore, speciation analyses of zinc species in human blood serum are now receiving great interst not only in medical diagnosis, but also in biological and environmental sciences[10]. Various techniques such as sucrose density gradient centrifugation, polyethylene glycol precipitation, electrophoresis ,chromatography and ultafiltration have been employed for the separation of zinc binding species in serum[11]. Spectrophotometric metods for determination of zin was found to be more sensitive and selective, they offer advantages such as reliability and reproducibility in addition to its simpilicty and less interference [12]. Zinc reacts with benzildithiosemicarbazone to form yellow colour compound (1:1) which has been paid for spectrophotometric determination of zinc in human serum.Visible spectrophotometer technique is one of the most powerful tools in chemical analysis. Zinic ion reacts with chromophere to produce colour complex. The intensity of colour is directly proportional to the concentration of zinc present in serum [13]. The literature reviewed very sensitive, highly specific spectrophometricmethods for trace determination of zinc. The method possesses distinct advantages over exiting method with respect to sensitivity, selectivity range of determination, simplicity, speed, pH, accuracy, precision, and ease of operation. AAS technique was used by many workers [14] to measure the total concentration of toxic heavy metals such as As, Cu, Cd, Mn, Pb, Se and Zn in human blood serum and urine[15]. Ingeneral GFAA more sensitive than FAAS for determination of zinc level in serum.

The AAS technique is based on conversion of ionic zinc to atomic zinc by flame or furnace, the light absorbed from hallaw cathode lamp of zinc is directly proportional to the concentration of zinc present in sample[16]

Material and method

A shimadzu (Kyoto, Japan) (Model-160) double beam Uv/Visible spectrophotometer was used of measurement of ionic zinc concentration. Py-Unicam (England) atomic absorption spectrphotometer was used for measurement of total zinc concentration.

Blood samples were collected from control groups (5male+5female) and patients (29male+21female) with range ages (35-70)years who admitted to Tikrit teaching hospital proven by specialist physician suffering from IHD. The blood after withdrawning using disposable syringe was put in disposable plain tubes, the serum was separated by centrifuge at 400 rpm for at least 30 minutes, the serum was

stored at 2-4 ^{o}C until the analysis for ionic and total Zinc.

Procedure for Determination of Ionic Zinc concentration

All of the chemicals used were analytical reagent grade unless otherwisestated. Bio Systems kit was used for determination of ionic Zinc in serum which contains buffer solution pH=8.2, analytical reagent grade (Benzildithiosmicarbazone) andstandarad solution of zinc (2µg/ml). 1,3,4,5 and 6 mls were taken from the above standarad solution and put each in 10 ml volumetric flask, then diluted to the mark with double distilled water to get the concentration 0.2, 0.6, 0.8, 1.0 and 1.2µg /ml respectively. The same procedure was used as repoted by NagarjunaReddy. D and others[17]for determination of ionic zinc in serum. The colourimetric reagent was mixed with the buffer solution and stored for two weeks at 2-8 ^{o}C and labelled as working reagent. (50)µl of each standard solution was added to (1) ml of working reagent, mixed and leave for 5 minutes, the absorbance was read at 578 nm and the colour stable at least 30 minutes. The percentage of recovery for spectrophotometric method was calculated showing in Table(1). (50)µl from the serum of each patient and control was added to 1ml of working reagent, shaking the solution and leave for 5 minutes then absorbance was measured at the same minor of preparation of standarad curve of zinc by spectrophotometry, the mean of reading was recorded and fitted to calibration curve to determine concentration of ionic zinc in sample Table (2)

Procedure for determination of Total Zinc Concentration

A 100 ml amount of stock solution of zinc (1000 µg/ml) was prepared by dissolving (440) mg of zinc sulphate heptahydrate ($ZnSO_4 7H_2O$) was dissolved in (100) ml of double distilled water ,then (1) ml from the standarad solution diluted to 100ml in volumetric flask to get (100) µg/ml, then 2,6,10,12 and 14 ml were taken and put in each in 100 ml volumetric flask,then diluted to mark with double distilled water to get the concentration ranges 0.2, 0.6, 1.0, 1.2 and 1.4 µg /ml respectively.

Standarad solutions were aspirated directly to the flame of atomic absorption technique (mixture of air-acetylene), the absorbance was read at 213nm for each concentration of zinc. One drop of Conc HNO_3 was added to 1ml of serum of patient and control, then 3 drops of conc. Perchloric acid, heat at 50-60 ^{o}C and aspirate the sample to flame AAs in the same minor for calibration curve of zinc by AAS, the absorbance was read and fitted to calibration curve to determind total zinc in sample. The results obtained are listed in Table 4)

Results. The accuracy and the precision of the method of analysis are illustrated in Table (1). Of the (50) blood samples from the clinically suspected patients included in this study (29 males and 21 female) show that the mean concentration of ionic zinc 451.45µg /ml comparing with it'sconcentration 628 µg /ml incontrol groups (Table-2-)

The accuracy and the precision of the method of analysis illustrated in Table (3). The mean concentration of total zinc 527.2 µg /ml comparing with its concentration 658 µg /ml in control groups (Table (4) . The bound zinc concentration in control and patient groups was calculated 29.5µg/ml and75.74µg/ml respectivelywhich described in Table (5).The results in Table(6) shows the variation in the concentration of total,ionic and bound zinc according to the ages and illustrated histogrameticaly in Figure (1).

Table 1. Accuracy and precision of Visible Spectrophotometer

Zn added	Zn found	% Recovery	% RSD
0.33	0.32	96	2.8
0.42	0.42	95	3.2
0.60	0.61	101	2.0
0.85	0.82	98	1.8
1.0	0.95	95	2.0

Table 2. Concentration of Ionic zinc (µg/ml) in Sera of Control and Patients

Countrol	**Concentration and Patients Numbers**				
	600(1)	630(2)	670(3)	620(4)	670(5)
	610(6)	690(7)	610(8)	630(9)	640(10)
	600(11)	620(12)	690(13)	630(14)	620(15)
	620(16)	630(17)	610(18)	680(19)	600(20)
Mean	628				
RSD	6.68%				
Patients.					
	410(1)	430(2)	470(3)	423(4)	435(5)
	464(6)	450(7)	500(8)	422(9)	458(10)
	473(11)	468(12)	489(13)	422(14)	470(15)
	466(16)	481(17)	410(18)	416(19)	450(20)
	350(21)	480(22)	470(23)	488(24)	458(25)
	496(26)	430(27)	383(28)	457(29)	427(30)
	448(31)	470(32)	470(33)	416(34)	438(35)
	419(36)	479(37)	437(38)	412(39)	497(40)
	488(41)	496(42)	470(43)	430(44)	490(45)
	470(46)	410(47)	429(48)	429(49)	456(50)
Mean	451				
RSD	7.98%				

Table 3. Accuracy and Precision of AAS

Zn added	Zn found	% Recovery	% RSD
0.33	0.32	103	4.0
0.42	0.44	104	3.8
0.60	0.62	103	3.6
0.85	0.89	104	4.0
1.0	1.10	110	4.2

Discussion

Inagaki .k and others[18] were determined the total concentration of zinc in blood serum sample using inductive couple plasma with mass spectrometry as detector after the serum sample was digested with concentrated nitric acid. In this work concentrated nitric and concentrated perchloric acid were used for digestion of serum sample to ensure that all zinc is relased from serum sample matrix,then determined by flame AAS which is less risk of contamination.

Ionic zinc concentration such as zinc loosely and firmly bind zinc inblood serum sample can removed by chelating resin batch treatment[19].In this resarch zinc ion reacted with bezildthiosemicarbazone to form yellow complex(M:L), then zinc ion

concentration was determined by visible spectrophotometer which indicate that albumin-zinc and α_2-macroglobulin were determind by visible spectrophotometer. The analytical results are summarized in Table (1) which shows good accuracy (recovery 95-101%) and precision (standarad devation 2.0-3.2) and acceptable regression line obtained. As seen in Table (2) that ionic zinc in control is higher than its concentration in patient groups, this indicates that ischemic hrart disease effects on the concentration of ionic zincwhich agree with other workers[19]. The analytical parameters for determination of total zinc showing in Table (3) is good enough (recovery 103-110%) and standaraddevation 3.6-4.2) to use for the the determination using regression line obtained.

Table 4. Concentration of Total zinc (µg/ml) in Sera of Control and Patients

Control.	**Concentration and Patients Numbers**				
	660(1)	650(2)	700(3)	704(4)	700(5)
	640(6)	630(7)	640(8)	650(9)	680(10)
	620(11)	640(12)	630(13)	630(14)	650(15)
	660(16)	650(17)	630(18)	620(19)	630(20)
Mean	658				
RSD	4.82%				
Patients.					
	427(1)	446(2)	432(3)	482(4)	450(5)
	461(6)	446(7)	420(8)	438(9)	426(10)
	495(11)	478(12)	423(13)	464(14)	412(15)
	401(16)	458(17)	498(18)	422(19)	450(20)
	467(21)	460(22)	455(23)	410(24)	458(25)
	496(26)	402(27)	488(28)	498(29)	427(30)
	476(31)	464(32)	490(33)	444(34)	438(35)
	428(36)	426(37)	474(38)	460(39)	410(40)
	478(41)	460(42)	498(43)	456(44)	442(45)
	412(46)	468(47)	424(48)	474(49)	478(50)
Mean	451				
RSD	7.98%				

Table 5. Spciation of Zinc in Serum of Patients

Patients(n=50)	**Control (n=20)**	**Species of Zinc**	**Method of Analysis**
451.45 (85.60%)	**628.5(95.53%)**	**Ionic**	**Visible Spectrophotometer**
527.2 (99.9%)	**658 (100%)**	**Total**	**Atomic Absorption Spectrophotometr**
75.74 (14.35%)	**29.50(4.47%)**	**Bound**	**Mathmetical Calculation**

Table 6. Speciation of Zinc(µg/ml) species in serum of patient group

Age group(year)	**Total conc. Of Zinc**	**Ionic Zinc**	**Percentage of ionic Zinc%**	**Bonded Zinc**	**Percentage of bonded Zinc%**
Control	658	628.5	95.53	29.5	4.47
Patients					
28-35	511.16	438.73	86.15	72.43	13.91
35-45	523.06	439.23	84.26	83.83	15.74
45-50	502.76	421.6	83.47	81.16	14.45
50-70	557.5	484.42	86.52	73.07	14.48

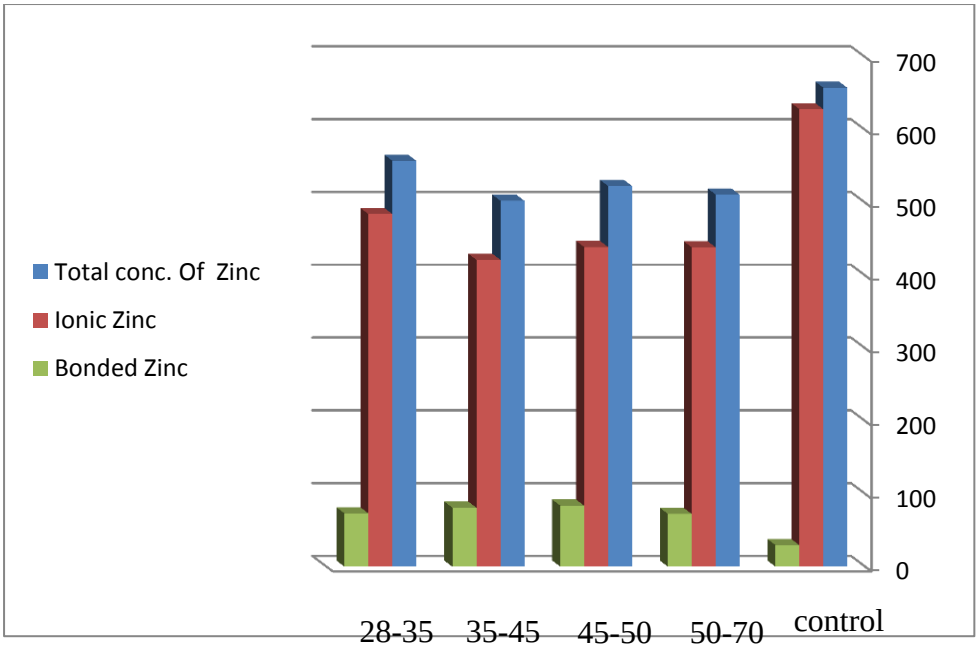

Figure 1. Histogram of Zinc Speciation in terms of Ages

The results in Table (4) and are illustrated that the concentration of total zinc in control is higher than it's concentration in patient groups, again theconcentration of total zinc effects by IHD. Good agreement between the distribution of total, loosely and firmly bound zinc in serum obtained by present method which are summarized in Table (5) with those obtained by other method [20]. The speciation of ionic zinc85.6%,bound zinc 14.3% and total zinc 99.9% in patient groups suffering from IHD, while it's speciation in control groups ionic zinc 95.5%, bound zinc 4,4% and total zinc 99.9%

In the present speciation analysis method involving determination of zinc bound to α_{2}- macroglobulin and zinc-albumin by visible spectrophotometric which are loosely and firmly bound zinc in human blood serum.The bound zinc which is strong binding between zinc and amino acid obtained by substraction of ionic concentration from total zinc. The concentration of bound zinc is very usefuel for clinical analysis and medical diagnosis [21].

It has been observed in this study that the concentration of bound zinc in patient groups 75.74 µg/ml (14.35%) higher than it's concentration in control groups 29.5 µg/ml (4.47%) .

The results in Table (6) show significant difference between control and patient group but no differences between them interms of the range of ages, the present results agree with results obtained by Lynch and others [22].

Conclusions

The results of this work were concluded that the concentration of total zinc inhuman blood serum is not good indicator for diagnosis of ischemic heart disease, the concentration of bound zinc is good index for diagnostic of ischemic heart disease.

Recommendation

Futher studies with large control and patient groups involving larger areas are recommended to predict the exat relation between bound zinc concentration and

IHD. Gelfiteration, electrophoresis techniques are recommended to use for determination exactly the concentration of bound zinc with protein.Copper ion should be addeded for the future studies due to the competition between copper and zinc toward protein.

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