

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 absorption spectrometry is good technique for measuring of total concentration of zinc after digestion of the serum with concentrated nitric acid and concentrated perchloric acid, 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 (10 male + 10 female) and their ages are between (35-70) years. This study aims to find relation between the concentration of bound zinc in human blood serum and ischemic heart disease.

Methods: blood samples were collected from clinically suspected patients who attend teaching hospital in Salah-al-Din Governorate with signs and symptoms of ischemic heart disease (proven by specialist physician). Blood samples were centrifuged 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 spectrophotometry, the absorbance was measured at wavelength 213 nm and the zinc label as total zinc. The bound zinc was mathematically calculated, which is higher in patients than control groups.

Conclusion: according to the study data, it was concluded that the level of total zinc is 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 associated 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 used 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 human 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 its trace concentration is essential for maintenance for normal growth and development, while its 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 zinc in plasma is loosely bound to the serum albumin, the remainder being more firmly attached to α -globulins and with minor fractions complexed in histidine and cysteine⁽⁷⁾. In clinical analysis, the total concentration of zinc in serum is generally used as convenient parameters for medical diagnosis of health and disease⁽⁸⁾. However, the biological activity or toxicities of trace elements 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 interest 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 ultrafiltration have been employed for the separation of zinc binding species in serum⁽¹¹⁾. Spectrophotometric methods for determination of zinc were found to be more sensitive and selective, they offer advantages such as reliability and reproducibility in addition to its simplicity and less interference⁽¹²⁾. Zinc reacts with benzildithiosemicarbazone to form yellow colour compound (1:1) which has been used for spectrophotometric determination of zinc in human serum. Visible

spectrophotometer technique is one of the most powerful tools in chemical analysis. Zinc ion reacts with chromophore 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 spectrophotometric methods for trace determination of zinc. The method possesses distinct advantages over existing 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⁾. In general 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 hollow 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 for measurement of ionic zinc concentration. Py-Unicam (England) atomic absorption spectrophotometer was used for measurement of total zinc concentration.

Blood samples were collected from control groups (5 male + 5 female) and patients (29 male + 21 female) with range ages (35-70) years who admitted to Tikrit teaching hospital proven by specialist physician suffering from IHD. The blood after withdrawing 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 °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 otherwise stated. Bio Systems kit was used for determination of ionic Zinc in serum which contains buffer solution pH=8.2, analytical reagent grade (Benzildithiosmicarbazone) and standard solution of zinc (2 µg/ml). 1,3,4,5 and 6 mls were taken from the above standard 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 1.2 µg /ml respectively. The same procedure was used as reported by Nagarjuna Reddy. 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 °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 standard 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 (ZnSO₄·7H₂O) was dissolved in (100) ml

of double distilled water, then (1) ml from the standard 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.

Standard 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 HNO₃ was added to 1ml of serum of patient and control, then 3 drops of conc. Perchloric acid, heat at 50-60 °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 determine 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 451.45 µg /ml comparing with its concentration 628 µg /ml in control 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 29.5 µg/ml and 75.74 µg/ml respectively which 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 histogrametically 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 ($\mu\text{g/ml}$) in Sera of Control and Patients

| Control | 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 released 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 in blood serum sample can be removed by chelating resin batch treatment⁽¹⁹⁾. In this research zinc ion reacted with bezildithiosemicarbazone 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 determined by visible spectrophotometer. The analytical results are summarized in Table (1) which shows good accuracy (recovery 95-101%) and precision (standard deviation 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 heart disease effects on the concentration of ionic zinc which agree with other workers⁽¹⁹⁾. The analytical parameters for determination of total zinc showing in Table (3) is good enough (recovery 103-110%) and standard deviation 3.6-4.2) to use for the determination using regression line obtained.

Table 4. Concentration of Total zinc ($\mu\text{g/ml}$) 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. Speciation 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 Spectrophotometer |
| 75.74 (14.35%) | 29.50(4.47%) | Bound | Mathematical Calculation |

Table 6. Speciation of Zinc($\mu\text{g/ml}$) 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 |

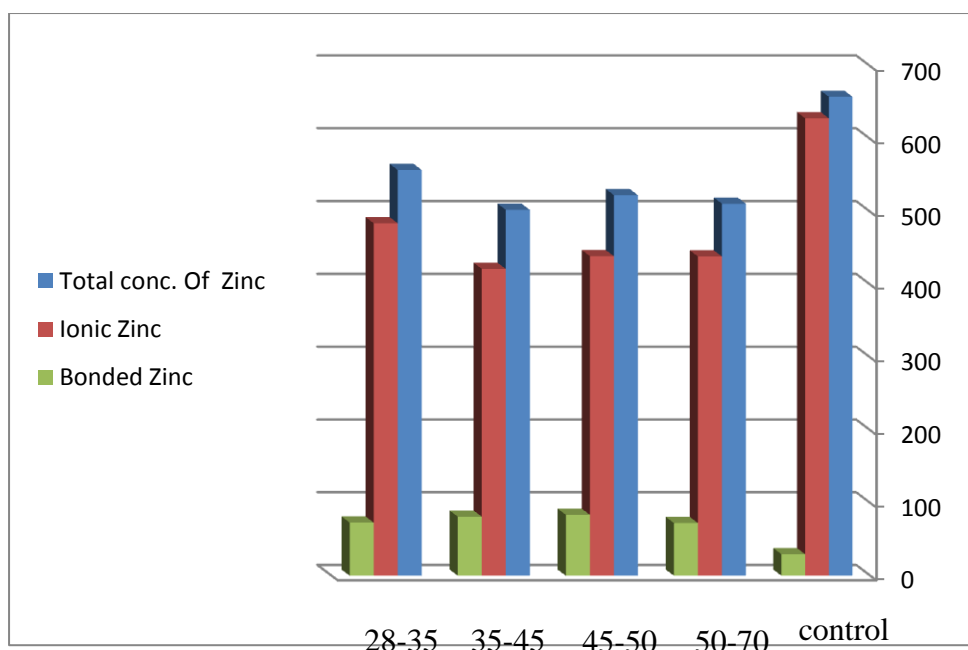


Figure 1. Histogram of Zinc Speciation in terms of Ages

The results in Table (4) and are illustrated that the concentration of total zinc in control is higher than its concentration in patient groups, again the concentration 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 zinc 85.6%, bound zinc 14.3% and total zinc 99.9% in patient groups suffering from IHD, while its 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 subtraction of ionic concentration from total zinc. The concentration of bound zinc is very useful 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 its 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 in terms 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 in human 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

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

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

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