DETERMINATION OF SERUM TRACE ELEMENTS MAGNESIUM, COPPER, ZINC, AND SELENIUM IN ASTHMATIC PATIENTS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

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Abstract

The aim of this study is to evaluate the levels of trace elements Magnesium (Mg), Zinc (Zn), Copper (Cu), and Selenium (Se) in blood sera of asthmatic patients by Atomic Absorption Spectrophotometry (AAS). The concentrations of Mg, Cu, and Zn have been determined by Flame Atomic Absorption spectrophotometry (FAAS), and Se with flameless Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS). The study involves (55) asthmatic patients as study group and (28) subjects as control from both genders.

Serum levels of Mg, Cu, and Se were significantly higher ($p<0.001$ for all) in patients when compared with healthy subjects, while Zn level was relatively significant ($p<0.05$). Our observations confirm the efficacy and applicability of (AAS) in determination of trace elements levels in blood sera of asthmatic patients and the effect of these elements in pathogenesis and treatment of the disease.

Introduction

The analytical chemistry defines a trace element as an element in a sample that has an average concentration of less than 100 microgram per gram. These elements occur in very small quantities in living organisms, some of them are essential for life processes, while others are detrimental, even beneficial elements may be toxic at higher levels (1).

Atomic absorption spectroscopy (AAS) provides a sensitive mean of determining more than 60 elements. The method is well suited for routine measurements by relatively unskilled operators. Although the AAS technique detects only one element at a time, elemental determination is quick and accurate, besides offering good specificity and sensitivity (2).

In the recent studies free oxygen radicals were accused for the pathogenesis of bronchial asthma (3), which is a chronic inflammatory disease of the respiratory tract (4). There are some defense mechanisms to avoid the harmful effect of oxidant radicals. The enzymes responsible for antioxidant defense have trace elements like selenium (Se), zinc (Zn), copper (Cu), and magnesium (Mg) within their structure (5). Many studies pointed to the biological role of these elements in many physiological and pathological conditions as they play an important role in protection the body from free radicals and toxic minerals, and decreased levels of these elements has its effect on antioxidant systems and lead to hyperactivity and inflammation in respiratory system (6,7).

This study was conducted to determine serum levels of some trace elements (Se, Zn, Cu, and Mg), to define the relation between their levels and bronchial asthma by using AAS techniques for their quantitative analysis.

Patients, Materials, and Methods

The study group consisted of 55 asthmatic patients for both genders, diagnosed by the specialized medical staff in the Consultant Center for Allergy and Asthma, in addition to 28 apparently healthy individuals as a control group.

A sample of ten milliliters of venous blood was drawn aseptically into plastic disposable syringes for each individual in the study and control groups, and transferred to acid – washed centrifuge tubes provided with plastic cups which were immersed in HNO3 5% V/V for 24 hours, then allowed to stand 2 hours at room temperature and centrifuge at 3000 rpm for 30 minutes to separate the serum. The samples were preserved at -20° C till the time of analysis.

Apparatus

- A Shimadzu model AA-670 Flame Atomic Absorption Spectrophotometer (FAAS) was used for the determination of Cu, Zn, Mg levels
- A Shimadzu model AA-670 Flameless Atomic Absorption Spectrophotometer (GFAAS) was used for the determination of Se level.

Chemicals

All chemical substances used were of the highest purity (analytical-reagent grade), obtained from Fluka and BDH companies. The standard stock solutions are listed in Table (1).

Table (1)
Concentrations of stock solutions and their commercial sources.

Standard stock solution	Manufacturer
1000µg.ml^{-1} Copper in 1% HNO3	Riedel-de Haen
1000µg.ml^{-1} Magnesium in 1% HNO3	BDH
1000µg.ml^{-1} Selenium in 1% HNO3	BDH
1000µg.ml^{-1} Zinc in 1% HCl	Riedel-de Haen

Analytical method for FAAS

Serum samples were diluted 5-fold with deionized water and introduced into nebulizer-burner system by the injection method. In view of viscosity of diluted serum, 3% (v/v) Glycerin was added to the standard solutions for matching the surface tension between samples and calibrators for Zn and Cu, to determine their levels at wave length 213.9, 324.8 nm respectively.

1% (w/v) Lanthanum chloride was added to the sample (which was diluted 50-fold with deionized water) and standard solution to overcome or minimize the effect of phosphate and other species on absorbance signals (usually depression) for Mg determination at wave length 285.2nm (2).

Analytical methods of GFAAS

A nitrate mixture of [0.1% Ni^{+2}, 0.1 % Mg^{+2} and 0.1 Cu^{+2}] was added as a modifier to both standard solution and serum samples (which was diluted 2-fold with deionized water) for determination of Se at wave length 196.0 nm (8).

A blank was used for setting of zero absorbance of spectrophotometer. Calibration curves have been prepared for each element, separately. Finally, serum content of elements being determined was estimated.

Statistical analysis

The upper and lower limits were recorded for each element in this study, and all results were given as the mean ± standard deviation (SD) value and data analysis were performed by SPSS 11.0 statistical program. If P value was less than 0.05, it was considered statistically significant. Other statistical calculations, such as correlation coefficient (r) was also performed.

Results and Discussion

The high attainable sensitivity in the determination of Mg by FAAS has resulted in its widespread application, it is precise and accurate, provided proper care is taken to maintain burner and nebulizer performance. Fig. (1) represent calibration graph for determination of Mg. Standard solutions were prepared in range of (0.2 to 0.8 µg/ml), and correlation coefficient (r) was 0.9954.

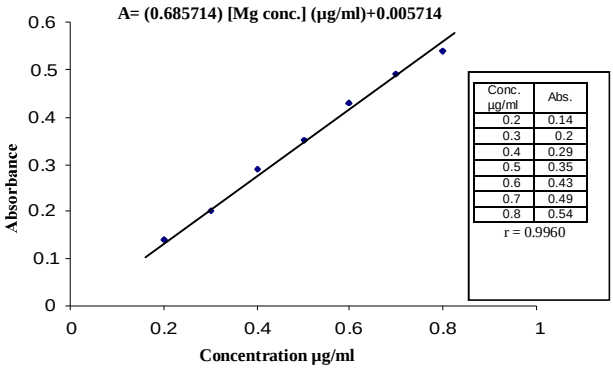

Fig.(1): Calibration curve of Mg in aqueous solution.

The difference in mean serum Mg concentration between control and patient groups is highly significant ($p<0.001$) as shown in the Table (2). These results are in agreement with the data of other studies (9-13) confirming the beneficial effect of this element on lung function.

Table (2)
Serum traces elements concentration in asthmatic patients and control subjects.

Element	Group	Upper limit	Lower limit	Mean±SD	P
Mg	Patients Control	11.00 25.58	0.75 17.05	5.995±0.350 21.412±0.459	p<0.001
Zn	Patients Control	0.20 0.35	0.03 0.02	0.115± 0.007 0.155±0.013	p<0.05
Cu	Patients Control	1.10 2.19	0.10 0.08	0.332±0.027 0.802±0.068	p<0.001
Se	Patients Control	0.09 0.12	0.01 0.01	0.041±0.003 0.060±0.005	p<0.001

Cu deficiency is a significant risk factor for asthma (14, 15), as it has been linked to inability to produce the important anti-oxidant enzyme, superoxide dismutase (SOD) which contain Cu and Zn. This enzyme is extremely important in defense, so decreasing these trace elements causes the effects of anti-oxidant system to be lower and this leads to hyperactivity and inflammation in the respiratory tract (16). Fig.(2) shows the linear relationship between absorbance and Cu concentration over a range of (0.1 to 0.8 µg/ml) and (r) was 0.9967. The results of our study clearly show the difference in serum Cu concentration of controls and patients. This significantly decreased level (p<0.001) of this element was also found by other studies (12, 17, and 18).

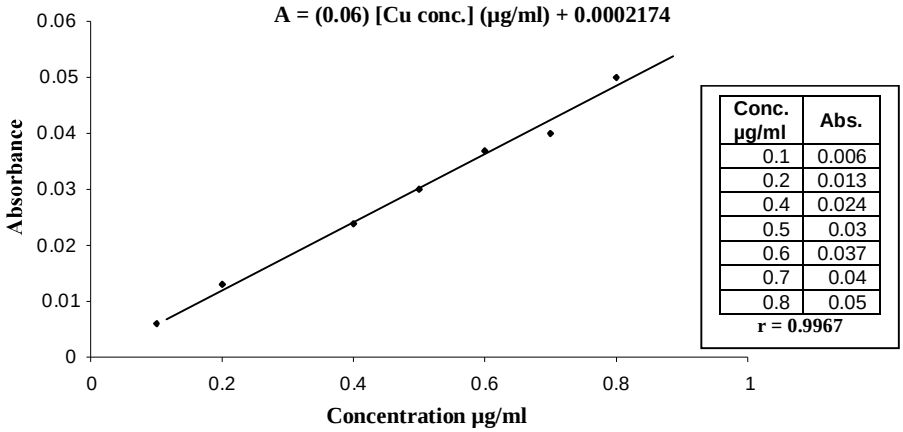

Fig.(2): Calibration curve of Cu in aqueous solution.

Se is an essential element of one of the main anti-oxidant enzymes in the human body, glutathione peroxidase (GSH-Px) which prevents the production of free radicals, decreases their activity or destroys them. (14, 15), and the most suitable technique for quantitating microgram amounts of Se in biological fluids is ETAAS (2). Fig.(3) shows calibration curve for standard solution for Se, ranging from 20 to 150 ng/ml, and (r) was 0.9991.

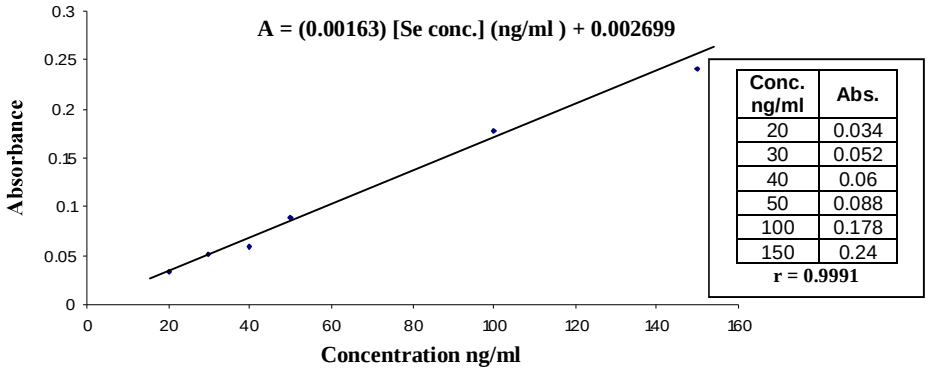

Fig.(3) : Calibration curve of Se in aqueous solution.

In recent years quite enough evidence has accumulated that links Se deficiency with increased risk of asthma. The obtained results here are in agreement with those of others (19-21) who also observed declines in serum Se values.

FAAS is the best method for the determination of Zn in human blood serum (22); it is characterized by many of advantages such as, simple, rapid, accurate, precise and sensitive. To determine Zn level, calibration curve was obtained from standard solution ranging from 0.1 to 0.9 µg/ml, and (r) was 0.9985 Fig.(4). The results obtained for Zn concentration in control and patient groups which are shown in the Table (2), show relatively significant difference ($p<0.05$). The results were so similar to those published in other literatures (12, 17, and 18).

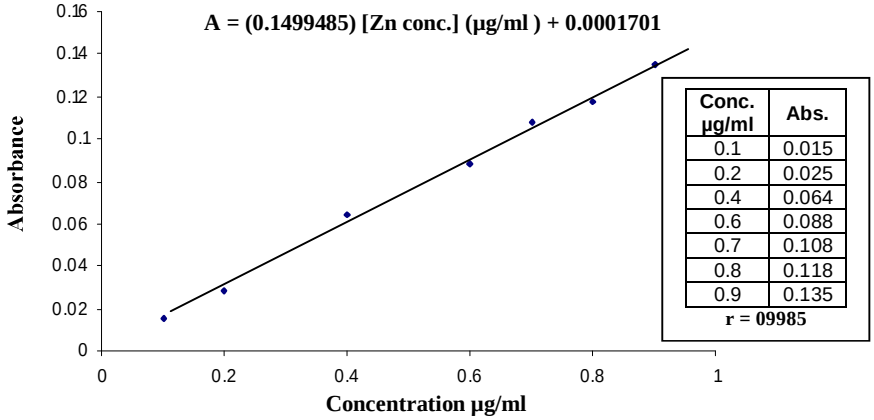

Fig.(4): Calibration curve of Zn in aqueous solution.

The results of this study confirm the previous observations, that there is low blood serum concentration of Zn, Cu, Se, and Mg in asthmatic patients. We conclude that quantitative determination of these elements in the sera of asthmatic patients assisting in the early detection of the disease and evaluation of therapeutic agents, adding to that our results underscore the efficacy and applicability of AAS methods in determination of their levels.

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الخلاصة

ان هدف الدراسة هو تقدير مستويات العناصر النزرة، المغنيسيوم، الزنك، النحاس، والسيلينيوم في مصل دم المرضى المصابين بالربو. تم استعمال مطيافية الأمتصاص الذري اللهبي (FAAS) في تقدير عناصر المغنيسيوم، الزنك، والنحاس وتقدير مستوى السيلينيوم بواسطة مطيافية الأمتصاص الذري غير اللهبي (GFAAS). شملت الدراسة (55) مريضاً مصابا بالربو، و(28) شخصاً سليما ظاهريا ومن كلا الجنسين.

أظهرت النتائج زيادة في مستويات المغنيسيوم، النحاس، والسيلينيوم في مجموعة الأصحاء بالمقارنة مع مجموعة المرضى وكانت ذات دلالة احصائية عالية، وبالنسبة لعنصر الزنك فقد اظهرت النتائج دلالة احصائية نسبية للزيادة في مستواه.

ان نتائج هذه الدراسة تؤكد على أن طريقة الأمتصاص الذري هي طريقة فعالة وملائمة جدا في تقدير مستويات العناصر النزرة في امصال دم مرضى الربو، وتؤكد على وجود علاقة بين مستويات هذه العناصر وشدة المرض وعلاجه.