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Original Article

Evaluation of Some Trace Elements in Sera of Asthma Patients: a Case Control Study

Falah S. Al-Fartusie[1], Manal J. Abood[1,2], Hassanain K. Al-Bairmani[1], Ahmed S. Mohammed[3]

[1] *Department of Chemistry, College of Science, Mustansiriyah University, Baghdad, Iraq*

[2] *Al-Salam Specialized Rehabilitation Center, Baghdad Health Department, Ministry of Health, Baghdad, Iraq*

[3] *Consultation Center for Allergy and Asthma in Rusafa, Baghdad, Iraq*

Corresponding author: Falah S. Al-Fartusie, Department of Chemistry, College of Science, Mustansiriyah University, Palestine Street, Baghdad, Iraq; E-mail: sci.falah.al_fartusie@uomustansiriyah.edu.iq, chemfalah@yahoo.com; Tel.: (+964) 7901661275

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Abstract

Introduction: Asthma is a chronic inflammatory disease of the airways characterized by recurrent respiratory symptoms of dyspnea, wheezing, chest tightness, and cough.

Aim: This study aims to investigate the concentrations of Cu, Zn, Mg, Mn, Fe, Cr, Ni, and Al in the serum of asthmatic patients.

Materials and methods: An atomic absorption technique was used to determine the levels of trace elements. The study included sixty asthmatic patients and ninety healthy individuals as control group their ages ranging from 20 to 45 years.

Results: We found a significant increase of the levels of copper, iron, and aluminum by 20%, 54%, and 47% ($p<0.01$), respectively, in the asthmatic patients as compared with the controls. On the other hand, in comparison with the controls, the levels of zinc, magnesium, manganese, and nickel were found to be significantly decreased ($p<0.01$) by 24%, 16%, 53%, and 81%, respectively, in the asthmatic patient group. Moreover, chromium level showed non-significant differences ($p>0.05$) between patients and control group.

Conclusions: The increased level of Cu and Fe may reflect their potential role in the pathogenesis of asthma. Furthermore, the serum iron levels tend to be increased as a result of the inflammatory process that occurs in asthma. Low levels of Zn, Mg, and Mn may contribute to allergic diseases due to their role in the synthesis of certain antioxidants or to their effect on the immune system.

Keywords

asthma, Cu, Fe, Ni, trace elements

INTRODUCTION

Asthma is a chronic disease that affects human airways. This disease can be characterized by frequent respiratory symptoms such as dyspnea, wheezing, chest tightness, and coughing. Asthma has been found to be associated with a variable limitation of airflow.[1,2] Although the initial symptoms of asthma sometimes begin later in life, it usually begins in childhood.[3] Asthma is a disease that is caused by a combination of genetic and environmental factors. Environmental factors mean any nongenetic cause, not just pollution. Common environmental factors that contribute to asthma include exposure to air pollution and allergens, and other potential factors may include medications such as aspirin and beta blockers.[4]

Asthma is one of the most common chronic diseases that cause morbidity and, in some cases, death. In fact, asthma is now causing a significant burden on health services and economies around the world.[5] Although it is known that developed countries have the highest prevalence of asthma, the rate is also increasing in other countries which may be due to the adoption of western lifestyles.[6] The World Health Organization (WHO) has estimated that 15 million of disability-adjusted life years (DALYs) are lost each year due to asthma accounting for about 1% of the global disease burden.[7]

Trace elements and minerals are chemical elements present in living tissues in small amounts. Some trace elements are nutrient minerals essential for the proper growth and physiology of the human body.[8] It has been revealed that trace elements have several important roles in human body, some of which are essential for enzyme reactions as they attract and facilitate the conversion of substrate molecules into specific end products. Some mineral ions, such as Fe and Cu, participate in oxidation-reduction reactions to generate energy at metabolism.[8] Some of them have structural roles and are responsible for stabilizing important biological molecules. Trace elements mainly act as catalysts in enzyme systems. Moreover, these elements have a fundamental role in various physiological processes, and are important for the proper functioning of the immune system.[9,10] Deficiency of trace elements and infectious diseases are frequently concurrently perceived and result in complex interactions. Some elements make up an important part of the structure of antioxidant enzymes, such as superoxide dismutase (SOD). Cu/Zn superoxide dismutase system contains Cu and Zn in its structure. Manganese-SOD contains Mn, and iron-SOD contains Fe in their structures. These minerals are essential to SOD activity in reducing the harmful effects of ROS. The reduction in these trace elements results in lowering the effects of antioxidant systems and thus to hyperactivity and inflammation in the respiratory tract.[11-13] It has been found that some trace elements such as Cu, Zn, and Mn, may play important roles in the genesis of asthma because they participate in oxidative stress reactions as cofactors of antioxidant enzymes.[14] Excessive production of ROS has been shown to lead to oxidative stress and play a major role in initiating and amplifying asthma. The overproduction of ROS leads to a change in key enzymatic antioxidants such as superoxide dismutase, and glutathione peroxides resulting in an oxidant-antioxidant imbalance in asthma.[15] It has been found that increased oxidative stress in asthma can lead to increased superoxide dismutase inhibition, which may amplify inflammation and block progressive airflow.[16]

Asthma prevalence is found negatively related to manganese and zinc levels.[17] It has been reported that the presence of copper, zinc, and manganese in medicinal plants plays a vital role in managing and controlling asthma.[18] In fact, zinc supplements can be used as a treatment for asthma because they may provide better control of asthma attacks, plus, adjusting the antioxidants in the plasma may have a beneficial effect on the development of asthma.[19]

Recently, atomic absorption spectrometry has become one of the most common techniques for biochemical analysis of trace elements. It is used mainly to measure the concentrations of trace elements in the samples of cancer patients[20], toxoplasmosis patients[21], schizophrenia patients[22] and major depressive disorder patients.[23] Moreover, some studies examined the relationship between trace elements levels and the most common trace element-associated diseases like anemia[24] and hypothyroidism.[25] Despite considerable efforts that have been made by a lot of researchers, there are still delays in the diagnosis and treatment of asthma.

AIM

Therefore, this research has been focused on studying whether monitoring of concentrations of trace elements is helpful in predicting the probability exposure to asthma. In this study, a quantitative analysis of the elements (Cu, Zn, Mg, Mn, Fe, Cr, Ni, and Al) was performed in the sera of patients with asthma and define the relationship with their levels measured in the serum of healthy volunteers, thus to investigate their potential clinical importance as biomarkers of the disease.

MATERIALS AND METHODS

Subjects and specimen collection

The study included 60 asthmatic patients aged 20 to 45 years (43 females and 17 males). Patients were registered at Consultation Center for Allergy and Asthma in Rusafa, Baghdad from October 2019 to January 2020. In addition, the study included 90 healthy individuals as controls their ages ranging from 20 to 45 years (60 females and 30 males).

The blood samples were taken from all study subjects (patients and control) using plastic disposable syringes. Blood samples were placed in a gel tube and left for 30 minutes at room temperature. After coagulation, the sera were separated by centrifuging at 2000 g for 10 minutes. The sera were stored frozen at about −20°C until analysis.

Methods

An atomic absorption spectroscopy technique was used to determine the levels of trace elements. It is a sensitive, accurate technology that can be used to detect only one element at a time. Elemental determination is fast and accurate once the sample is in the proper shape. It is now clear that in addition to offering good specificity and sensitivity, AAS instruments are readily available and easily usable. In this study, measurements of Cu, Zn, Mg, and Fe were conducted using Flame Atomic Absorption Spectrophotometer (FAAS), Model AA646 (Shimadzu Corporation, Kyoto, Japan). The measurements of Cr, Ni, Mn, and Al were performed using Flameless Atomic Absorption Spectrophotometer (GFAAS), Model 210VGP (Buck Scientific, USA). The settings of AAS were performed and single element hollow cathode lamps were used as line radiation sources which were operated at currents or energies recommended by the manufacturer. The optimum working conditions regarding the wavelength and slit width of the instrument for each element are listed in **Table 1**. Serum samples were diluted with 10-fold deionized distilled water for Cu, Zn, and Fe measurements. The samples were diluted 50-fold by lanthanum chloride heptahydrate for Mg measurement and the serum samples of Ni, Mn, Cr, and Al were measured directly by injection into the graphite tube of the GFAAS. The certified reference material, NIST SRM 909 trace elements in serum (National Institute of Standards and Technology (NIST), USA), was used to confirm the accuracy of the measurements.

Table 1. Optimum working conditions of FAAS for the elements (Cu, Zn, Mg and Fe) and GFAAS for the elements (Cr, Ni, Mn, and Al)

Element	Flame Mode	Wave length (nm)	Slit width (nm)	Detection limits (μg/ dl)
Cu	Flame	324.7	0.7	0.003
Zn	Flame	213.9	0.7	0.007
Mg	Flame	285.2	0.7	0.004
Mn	Flameless	279.5	0.2	0.002
Fe	Flame	248.3	1.0	0.003
Cr	Flameless	357.9	0.7	0.001
Ni	Flameless	232	0.2	0.001
Al	Flameless	309	0.7	0.001

Statistical analysis

SPSS program, version 22.0, was used to analyze all data collected. The mean values, standard deviation (SD) and range were calculated for each element included in the study. Statistical analysis was performed using Independent-Samples Student *t*-test for assessment of mean differences between two groups (patients and control groups). In addition, the correlations between all study trace elements were determined using a Pearson correlation analysis. Statistical analysis was considered significant at $p < 0.05$ and highly significant at $p < 0.01$ with 95% confidence interval.

RESULTS

The mean values, standard deviation (SD), range, and probability (p) values of the elements for the asthmatic patients were calculated using SPSS program. The findings of this analysis were then compared with those of the control group and the collective results are presented in **Table 2**. From these results, a 20%, 54%, and 47% significant increase can be observed in copper levels (patients: 143.21±9.85 μg/dl, controls: 119.84±4.97 μg/dl), iron levels (patients: 156.32±11.95 μg/dl, controls: 101.76±13.37 μg/dl), and aluminum levels (patients: 0.166±0.002 ng/mL, controls: 0.113±0.005 ng/mL), respectively, of asthmatic patient group compared to the control group.

The results showed that although the estimated chromium levels of the asthmatic patient group (0.12±0.05 μg/dl) were 8% lower than those of the control group (0.13±0.07 μg/dl), these differences were not statistically significant. Moreover, the results confirmed that the concentrations of zinc (patients: 77.45±5.15 μg/dl, controls: 101.39±6.67 μg/dl), magnesium (patients: 1.12±0.13 mg/dl, controls: 1.33±0.29 mg/dl), manganese (patients: 0.09±0.05 μg/dl, controls: 0.19±0.07 μg/dl), and nickel (patients: 0.026±0.01 μg/L, controls: 0.14±0.03 μg/L) were found significantly decreased by 24%, 16%, 53% and 81%, respectively, in the asthmatic patient group compared to the control group.

In addition, the relationship between all parameters included in the present work was studied using Pearson's correlation analysis. The collective results are presented in **Table 3**, where the values are above the diagonal for controls and below diagonal for patients. Pearson's correlation analysis revealed a significant positive correlation between zinc and nickel levels in the control group. In contrast, the correlation analysis revealed the presence of a significant positive correlation between the levels of Zn and Mg in patients with asthma. The analysis also revealed a significant negative correlation between the levels of Mn and Ni, and between the levels of Ni and Al in patients with asthma. In contrast, no significant correlation was observed between all the rests of parameters.

DISCUSSION

Asthma is a chronic inflammatory disease that causes bronchial hyper-responsiveness, and since trace elements play major roles in inflammation, they may have a direct and/or indirect effect on the pathogenesis of asthma.[30] Asthma has been found to be closely related to high levels of free radicals, especially reactive oxygen species such as superoxide and hydrogen peroxide. Recent clinical studies indicate an important link between sporadic excessive oxidative processes and various inflammatory diseases, especially asthma.[31] The pathophysiology of bronchial asthma and lung inflammation can result from a lack of activation of antioxidants. SOD activity (an enzyme that diffuses widely in aerobic cells) is significantly lower in asthmatics than in healthy people, and accordingly it was suggested that Cu/Zn SOD and Mn SOD activity is associated with asthma.[32] Recently, after establishing the role of ROS in causing asthma, most studies have focused on antioxidant defense systems and trace elements. It has been revealed that disturbance in the levels of trace elements has an important role in the pathogenesis of asthma.[33]

The current work clearly highlighted the high levels of Cu in asthma patients. It has been reported previously that Cu concentration in the serum of asthma patients was

Table 2. Serum levels of elements (Cu, Zn, Mg, Mn, Fe, Cr, Ni, and Al) in patients with asthma and control group

Element	Sample	Mean	SD	Range	*p* value	Reference values*
Cu, μg/dl	Control	119.84	4.97	99-145	0.002	60-180
	Asthma	143.21	9.85	130-173		
Zn, μg/dl	Control	101.39	6.67	77-122	0.001	58-122
	Asthma	77.45	5.15	50-98		
Mg, mg/dl	Control	1.33	0.29	1.12- 1.63	0.009	1.2-2.2
	Asthma	1.12	0.13	0.85-1.52		
Mn, μg/dl	Control	0.19	0.07	0.13- 0.23	0.004	0.01-0.15
	Asthma	0.09	0.05	0.05- 0.18		
Fe, μg/dl	Control	101.76	13.37	76 - 142	0.000	82-209
	Asthma	156.32	11.95	111 - 193		
Cr, μg/dl	Control	0.13	0.07	0.1- 0.21	0.32	0.04-0.48
	Asthma	0.12	0.05	0.05- 0.21		
Ni, μg/L	Control	0.14	0.08	0.09-0.19	0.000	0.13-2.8
	Asthma	0.026	0.009	0.011-0.040		
Al, ng/mL	Control	0.113	0.005	0.91-0.126	0.006	0.1-6
	Asthma	0.166	0.002	0.98-0.215		

* The ranges involved data collected from references [26-29]

Table 3. Correlations between all variables studied in the control group above the diagonal and the patient group below the diagonal (R value)

Parameter	Cu	Zn	Mg	Mn	Fe	Cr	Ni	Al
Cu		−0.125	−0.098	−0.175	−0.124	−0.152	−0.078	−0.156
Zn	−0.206		0.113	0.059	−0.014	−0.118	0.314*	−0.021
Mg	−0.110	0.599**		0.079	−0.148	0.041	−0.018	−0.103
Mn	0.200	0.050	0.035		0.038	−0.028	−0.017	0.247
Fe	0.030	−0.166	−0.085	0.032		0.129	0.052	0.063
Cr	0.035	0.005	0.000	−0.018	0.195		−0.112	0.122
Ni	−0.065	0.088	0.062	−0.288*	0.175	0.105		0.030
Al	−0.083	0.023	−0.138	−0.226	0.128	0.208	0.263*	

**: correlation is significant at the 0.01 level (2-tailed).

higher than that in the controls[11,34,35] and these findings are consistent with the findings of the present work. Copper has an essential role in the activities of many physiological enzymes including cytochrome *c* peroxidase and superoxide dismutase, which are important in the antioxidant defense systems.[36] In fact, although Cu is an essential element for the human body, high Cu concentrations can lead to cell damage via increasing the formation of toxic free radicals.[37] Copper is a redox-active transition metal that can participate in single electron reactions and catalyze formation of free radicals, including the undesirable hydroxyl radicals.[38] So, any increase in its levels will lead to increase formation of toxic free radicals, which in turn lead to increase the risk of asthma.

The current results of low Zn levels in asthma patients are consistent with the results of previous studies[11,39-41] which confirmed a lower zinc level in asthma patients compared to controls. Zn is an essential nutrient that plays a special role in the conductive airways and partly contributes to the structure and function of many biological enzymes. Zinc is known to exhibit strong antioxidant activity in the lungs and many parts of the body, and it is often used to evaluate inflammatory diseases.[42,43] It has been found that zinc deficiency can affect the regulation of T-cell lymphocytes leading to increased inflammation and eosinophilia, and thus may play a role in the development of allergies.[44,45] Since zinc levels were significantly lower in patients than in the control group, we concluded that zinc deficiency may reduce antioxidant function and tend to play a role in the onset of asthma. Previously, an association has been reported between an increased risk of developing asthma and decreased intake of dietary zinc.[35]

Cu-Zn SOD is an antioxidant enzyme that contains Cu and Zn as primary components. The current study showed that the copper level was higher while zinc levels were lower in asthma patients than those in the healthy control group. It has been suggested that there are two major changes in the lungs associated with a decrease of the level of zinc: cell membrane damage and changes in Cu-Zn SOD structure.[46] Moreover, a previous study indicated that elevated serum copper level associated with low zinc level could lead to decreased Cu-Zn SOD activity.[35] Therefore, it is important to note that this work gives further evidence and strongly supports the fact that there are significant correlations between copper and zinc status and pathogenesis of asthma.

Previous studies suggested that perturbation in the copper/zinc ratio (Cu/Zn ratio) can be used as a useful indicator for patient evaluation rather than using copper or zinc alone.[23,47] In this study, it was found that the Cu/Zn ratio increased significantly (p<0.01) in the serum of asthma patients (1.85) compared to the control group (1.18). Indeed, there is a strong correlation between oxidative stress and the Cu/Zn ratio.[48] Therefore, it can be suggested that a deficiency in the Cu/Zn ratio here may be of great value in diagnosing and predicting asthma, and a good indicator for evaluating patients' antioxidant defense system.

The current findings for Mg accompany the results of previous studies which reported that the serum levels of Mg were significantly lower in asthmatic patients.[40-42] Magnesium deficiency has been shown to play an important role in the pathogenesis of asthma[49], as Mg is known to act as an anti-inflammatory in addition to its role of inhibiting the effect of calcium on smooth muscle contractions.[50,51] A previous study indicated that serum Mg could have an effect on lung function in chronic obstructive pulmonary disease patients.[52] In fact, magnesium intake (daily recommended intake) has protective effects against loss of lung function. It has been found that individuals with asthma-chronic obstructive pulmonary disease overlap (ACO) have a significantly lower level of Mg than do healthy people. Also, it has been found that individuals with severe ACO have significantly lower Mg levels than those with mild to moderate ACO.[53] Medically, magnesium sulfate is used as a bronchodilator mainly to treat people suffering from severe asthma attacks. It relaxes the bronchial muscles and can relieve asthma symptoms leading to improved lung function.[54,55]

Manganese is an essential element in the human body and has the ability to act as a cofactor with several cellular enzymes, such as those that have antioxidant activity. It is an essential component of the antioxidant manganese superoxide dismutase (Mn-SOD).[56] A previous study indicated a lower level of manganese in childhood asthma patients compared to the control group[32], which is consistent with the results of the current study. A decrease in the level of manganese will lead to a decrease in the level of Mn-SOD which can increase the oxidative stress in the body and thus, the development of allergic diseases. From the results of the present work, it appears likely that manganese is independently linked to the risk of developing asthma. The explanation is that asthma may be related to an overall less healthy diet and/or a less healthy lifestyle.

The elevated serum iron levels of asthma patients recorded in this study were also reported by Ismaheal et al.,[39] which is explained by the fact that the mediators, including nitrous oxide released during chronic inflammation, showed an increase in heme oxygenase expression in asthmatic patients. The reaction of heme oxygenase, with its products bilirubin, carbon monoxide and free iron products, is interpreted as an antioxidant defense mechanism due to the release of bilirubin. However, free iron, which is a catalyst during the production of reactive oxygen species, is another product of this reaction and thus may have some inflammatory effects. In line with a recent study conducted by Ali et al.,[56] the current study confirmed findings about the critical role of iron in the pathogenesis and severity of asthma. The study concluded that in asthma patients, altered levels of iron in the airways are associated with lung functions and that a high accumulation of iron in the lungs may lead to asthma.[56]

Although the current study showed a slight decrease in the Cr level of asthmatic patients compared to the control group, these results give the impression that there is no relationship between chromium level and asthma disorder. In fact, no direct causal relationship has been reported between Cr inhalation and the development of allergic asthma, even for those working in the chromium industries.[57]

Nickel is known to be a carcinogen, as exposure to nickel can have a variety of negative effects on human health such as modifications to DNA, lipid peroxidation enhancement, and reactive oxygen production.[58] In fact, according to the available information, little is known about the relationship between nickel and asthma. Although nickel allergy was demonstrated and clearly identified in allergic diseases[59], it is surprising that the results here, in this work, showed a low level of nickel among the group of asthma patients. Therefore, a comprehensive study is needed to address the relationship between nickel levels in blood and asthma.

Aluminum usually enters the human body through food. It is distributed to different tissues in different proportions. Approximately half of the amount of Al is concentrated in the bones and a quarter in the lungs, and reaches the brain through the cerebrospinal fluid.[60] Although the function of Al in the human body is still not well understood, it has been found that asthma is associated with inhalation of aluminum sulfate, aluminum fluoride, potassium aluminum tetrafluoride and exposure to a complex environment in potrooms during the production of aluminum electrolytic in aluminum industries.[61] For the current work, it is important to point out that high levels of aluminum may be associated with an increased risk of developing asthma.

CONCLUSIONS

The present work indicated that the elements Cu, Fe and Al are significantly elevated in serum samples of asthmatic patients in comparison with controls. The increased level of these elements may reflect their potential role in the pathogenesis of asthma. Serum iron levels tend to be increased because of the inflammatory process that occurs in asthma. The mean values of Zn, Mg, and Mn were lower in the serum samples of asthmatic patients compared with those of healthy control subjects. The reduction in these elements may contribute in the manifestation of the allergic diseases due to their roles in the synthesis of some antioxidants or due to their impact on the immune system. Therefore, it is suggested that a diet rich in magnesium and zinc may be helpful in preventing or treating asthma. Although the elements involved in the present work may have a vital role in the complex disorders that lead to asthma, the exact mechanism responsible for changes in the levels of these parameters in asthma is unclear and requires additional evaluation. Therefore, further studies are warranted to address this issue.

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Оценка некоторых микроэлементов в сыворотках больных астмой: исследование случай-контроль

Фалах С. Ал-Фартуси[1], Манал Дж. Абоод[1,2], Хассанаин К. Ал-Баирмани[1], Ахмед С. Моахмед[3]

[1] Кафедра химии, Колледж наук, Университет Мустансирия, Багдад, Ирак

[2] Специализированный реанимационный центр Ал-Салам, Министерство здравоохранения, Багдад, Ирак

[3] Консультационнный центр аллергии и астмы в Русафе, Багдад, Ирак

Адрес для корреспонденции: Фалах С. Ал-Фартуси, Кафедра химии, Колледж наук, Университет Мустансирия, ул. „Палестина“, Багдад, Ирак; E-mail: sci.falah.al_fartusie@uomustansiriyah.edu.iq, chemfalah@yahoo.com; Тел.: (+964) 7901661275

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Резюме

Введение: Астма – хроническое воспалительное заболевание дыхательных путей, характеризующееся повторяющимися респираторными приступами одышки, хрипов, стеснения в груди и кашля.

Цель: Это исследование направлено на изучение концентраций Cu, Zn, Mg, Mn, Fe, Cr, Ni и Al в сыворотке крови пациентов с астмой.

Материалы и методы: Для определения содержания микроэлементов использовался метод атомной абсорбции. В исследование были включены шестьдесят пациентов с астмой и девяносто здоровых людей в качестве контрольной группы в возрасте от 20 до 45 лет.

Результаты: Мы обнаружили значительную разницу в уровнях меди, железа и алюминия с 20%, 54% и 47% (p<0.01), соответственно, у пациентов с астмой по сравнению со здоровыми людьми из контрольной группы. С другой стороны, по сравнению с контролем, уровни цинка, магния, марганца и никеля были значительно снижены на 24%, 16%, 53% и 81% соответственно в группе пациентов с астмой (p<0.01). Кроме того, не было обнаружено значительных различий между пациентами и контрольной группой по уровням хрома (p>0.05).

Заключение: Повышенные уровни Cu и Fe могут выявить их потенциальную роль в патогенезе астмы. Кроме того, уровень железа в сыворотке повышен в результате воспалительного процесса, вызванного астмой. Низкие уровни Zn, Mg и Mn могут способствовать развитию аллергических заболеваний из-за их роли в синтезе определённых антиоксидантов или их влияния на иммунную систему.

Ключевые слова

астма, Cu, Fe, Ni, микроэлементы