Determination of Magnesium, Zinc, and Reduced Glutathione Levels in Serum of Asthmatic Patients

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Abstract

Asthma is a disease characterized by hyper reactivity of the trachea and bronchi to reversible either spontaneously or as a result of treatment .

In this study serum magnesium, Zinc, and reduced glutathione levels were measured in order to determine their levels in the asthmatic patients and comparing these levels with healthy controls.

Fifty- four asthmatic patients were enrolled in the study and had their pulmonary function test, and the levels of Mg,Zn, and GSH.

Serum magnesium and serum zinc were determined by using Atomic Absorption Spectrophotometer . While serum glutathione was determined by spectrophotometric analysis.

The asthmatic patients were divided into four groups according to the interpretation of the pulmonary function test : severe expiratory flow limitation, moderate expiratory flow limitation , slight expiratory flow limitation and normal lung values .

In this study, it was found that serum magnesium levels in severe cases of asthmatic patients were significantly lower than the healthy control population [0.92 ± 0.17mmol / L *vs.* 1.01 ± 0.19 mmol /L; ($p< 0.05$)] while the other cases did not show any significant differences compared with the healthy control .

As for serum zinc levels, it was found that the slight cases of asthmatic patients were significantly higher than the healthy control [19.48 ± 1.93 μmol/L *vs.* 17.01 ± 2.94 μ mol/L; ($P<0.05$)]. However, the other cases did not show any significant differences compared with healthy control.

While serum glutathione levels in severe and slight cases of asthmatic patients were lower than the healthy control . [2.08 ± 0.51

mmol/L *vs.* 2.42 ± 0.21 mmol/L ; (P< 0.001), and 1.95 ±0.36 mmol/ L *vs.* 2.42± 0.21 mmol/L ; (P< 0.01)],respectively.

It was found that there is a correlation between the severity of asthma as measured by pulmonary function test and levels of magnesium and glutathione levels, the more severe one showed low levels of magnesium and glutathione .

Introduction

Magnesium (Mg) is the second most abundant intracellular cation (1) only a small proportion of the body's content being in the extracellular (2) .

Magnesium has a wide range of biological actions that are of potential relevance to the airways (3). Intravenous magnesium has also shown a brochodilator effect in patients with severe chronic obstructive pulmonary disease (4). Its low cost, widespread availability, and low incidence of side effects make magnesium an attractive addition to bronchodilator therapy (5). Magnesium has been shown to have a relaxant effect on smooth muscle *in vitro*. In the last decade, the potential role of intravenous magnesium in acute asthma has gained a renewed interest (6,7).

A relevant epidemiological study has shown that a low dietary intake of magnesium in the general population is associated with impaired of FEV1 and a higher risk of wheezing and of airway hyper reactivity (8).

Zinc (Zn) is an essential trace mineral (9). Zinc is an intracellular divalent cation that is found in almost every cell. It stimulates the activity of approximately 100 enzymes whose reaction and biological function are depended on its chemical characters (10,11). Zinc is an essential element in our diet. Too little zinc can cause health problems, but too much zinc is also harmful (9). A report showed zinc level to be lower in patients with asthma. Another showed a positive correlation between low dietary zinc intakes and bronchial hyper reactivity. However, caution is needed. Occupational exposure to zinc has been reported to cause asthma (12).

Glutathione (GSH) is a tripeptide compound of three amino acids: cysteine, γ - glutamyl, and glycine (13). Glutathione is found in blood and almost every cell of the body (14) with the highest levels found in the liver, the lenses of the eyes, pancreas, spleen, and kidneys (13). Glutathione is a powerful antioxidant. It exists in the reduced form

(GSH), and is in equilibrium with its oxidized form (GSSG) a disulfide. The principle of action as an antioxidant is based on the fact that it reduces other toxic substances before they can damage other molecules or important parts of the cell (14). Glutathione is a naturally occurring tripeptide that protects every cell, tissue, and organs from toxic free radicals and disease. Without glutathione the cells would have little resistance to bacteria, viruses, and cancer, and the liver would shrivel up from the eventual accumulation of toxins (15).

Glutathione in asthma may be beneficial. Asthmatic information results in increased oxygen free radical generation, a glutathione is a powerful antioxidant itself, as well as assisting other important antioxidants in their efficiency (16).

Materials and Methods

A- Grouping

Group one contains fifty- four patients from both sexes and with different types of asthma. These types are severe expiratory flow limitation (n= 29), moderate expiratory flow limitation (n= 10), slight expiratory flow limitation (n= 13), and normal lung function values (n=2). The age range was (18-75) years. The patients were attended the Educational Hospital in Al-Kadhimia Iraqi College of Medicine. The serum samples were used for the determination of magnesium, zinc, and reduced glutathione.

The second group contains thirty non- asthmatics healthy controls from both sexes and different ages, and their serum samples were used in the same tests.

B- Sample collection

5 ml of blood was drawn from the vein of each patient and healthy control without torniqua, and immediately transferred to a plain tubes and were allowed to stand at room temperature for 10 minutes to permit clot information. After centrifugation for 15 minutes at 3000 (r.p.m) revolution per minute, the serum was transferred to a second tube a micro-pipette and stored at (-20°C) until the day of analysis.

Methods

Serum magnesium and serum zinc were measured using Atomic Absorption Spectrophotometer by acetylene air flame and hollow cathode lamp of each metal.

Serum glutathione was measured by Calorimetric method using DTNB [5,5- dithio bis (2-nitrobenzoic acid)] as a disulfide chromogen.

Result

Fifty- four patients were enrolled during six- month study period. All patients were diagnosed as asthmatics and receiving their medication [ventoline (tab or nubulizer), butadiene, aminophylline, and prednisolone]. The asthmatic patients are divided into four groups according to the interpretation of the pulmonary function test done at the university hospital at Iraqi College of Medicine (Pulmonary Function Lab.). The subgroups were severe expiratory flow limitation (n= 29), moderate expiratory flow limitation (n= 10), slight expiratory flow limitation (n= 13). And normal lung function values (n= 2).

Magnesium levels in the serum of asthmatic patients compared with healthy controls

In this study, the results indicated that the serum magnesium levels in severe cases of asthmatic patients were significantly lower than the healthy control population [0.92 ± 0.17 mmol/L *vs.* 1.01 ± 0.19 mmol/L; ($P< 0.05$)]. While slight, moderate, and normal PFT showed no significant differences compared with the healthy control population as shown in the table (1).

As for the subgroups of the asthmatic patients, the seum magnesium levels in severe asthmatic patients are significantly lower than the magnesium level in moderate asthmatic patients [0.92 ± 0.17 mmol/L *vs.* 1.05 ± 0.19 mmol/L; ($P< 0.05$)], and are also lower with the serum magnesium level in slight asthmatic patients [0.92 ± 0.17 mmol/L *vs.* 1.08 ± 0.14 mmol/L; ($P< 0.05$)]. Comparison of serum magnesium levels in the other subgroups of the asthmatic patients showed no significant differences with each other, as shown in table (2).

Zinc levels in the serum of asthmatic patients compared with healthy controls

The results revealed that the serum zinc levels in patients with slight asthmatic attack of asthmatic patients were significantly higher than the healthy control population [19.48 ± 1.93 μmol/L *vs.* 17.01 ± 2.94

μmol/L; (P< 0.05)]. While the severe, moderate, and normal (PFT) cases of asthmatic patients showed no significant changes compared with healthy control as shown in table (3).

As for the subgroups cases of asthmatic patients, the serum zinc levels in severe cases of asthmatic patients are slightly significantly lower as compared with the slight asthmatic patients [17.94 ± 3.03 μmol/L *vs.* 19.48 ± 1.93 μmol/L]. Serum zinc levels with the other subgroups of the asthmatic patients showed no significant differences with each other, as shown in table (4).

Glutathione levels in the serum of asthmatic patients compared with healthy controls

The results revealed that the serum glutathione in severe cases of asthmatic patients were significantly lower than the healthy control [2.08 ± 0.51 mmol/L *vs.* 2.42 ± 0.21 mmol/L; (P< 0.001)], and in the slight cases of asthmatic patients, the serum glutathione were lower than the healthy control [1.95 ± 0.36 mmol/L *vs.* 2.42 ± 0.21 mmol/L; (P< 0.01)].

While the other cases of severity showed no significant changes compared with the healthy control as shown in table (5).

Serum glutathione levels showed no significant differences between the subgroups of asthmatic patients as compared with each other, as shown in table (6).

Discussion

In this study, serum magnesium levels were found to be lower than in the healthy control population. Haury and Coworkers (1940) found that the serum magnesium levels in asthmatic patients were lower than normal, but the study did not describe the patients demographic data or a confounding factor such as alcohol abuse or renal disease (17). Falkner and Coworkers (1992) studied 23 asthmatic exacerbation cases and found that serum magnesium in an asthmatic population did not differ from a nonasthmatic control group, but they did not describe the severity of asthma in their patients (18). Chaiwat and Coworkers (2001) found that serum magnesium levels in asthmatic patients were significantly lower than in normal population (19). Nural and coworkers (2000) studied various trace elements in serum of patients with bronchial asthma and no changes were found in serum

magnesium levels in patients with bronchial asthma as compared with controls (20). The mechanism of magnesium action in asthma is currently unknown. It has been postulated that the magnesium relaxes bronchial smooth muscles and produces dilation of the airways (21).

Also, it was found in this study that the slight cases of asthmatic patients were significantly higher than the healthy control. These results do not agree with the results of Kardrabove and coworkers (1996) who studied plasma zinc levels in 22 intrinsic asthmatic patients and compared it with plasma zinc in 33 healthy control subjects. The zinc content of plasma was found to be significanty lower in patients than in control individuals (22). Also, Aural and coworkers studied serum zinc and other various trace elements in 40 patients with bronchial asthma, and in 43 healthy subjects, and found that the serum zinc level was significantly lower in healthy subjects ($P< 0.01$) (20).

While the serum of glutathione was lower in both severe and slight cases of asthmatic patients as compared with the healthy control population. These results agree with results of Eric Braverman who found that low glutathione has been associated with asthma (23). Pennings and coworkers (1999) who stated that the antioxidant defense is decreased in asthmatic patients (24).

References

1. Teinhart, R. (1988) Arch. Intren. Med., 148: 2415-2420.
2. Whitby, L. G.; Smith, A. F. and Beckett (1988) Lecture notes on clinical chemistry. Forth Edition, chapter 19, p. 270.
3. Britton, J. ; Pavord, I. ;Richards, K. and Yasue, H. (1994) Lancet. 344: 357-362.
4. Skorodin, M. ; Tenholder, M. and Yetter, B. (1995) Arch. Intern. Med., 155: 496- 500.
5. Tifany, B. ; Berk, W. and Todd, I. (1993) White S. Chest., 104: 831-834.
6. Bloch, H.; Silverman, R.; Mancherje, N. ; Grant, S.; Jagminas, L. and Scharf, S. (1995) Chest. Ann. Emery. Med., 107: 1576-1581.
7. Mathewson, H. (1992) Respir. Care., 37: 361-362.
8. Spivey, W.; Skobeloff, E. and Levin, R. (1990) Ann. Emerg. Med., 19: 1107-1112.
9. Judith, Wheian, Health writer. (2000) Dators change advice as paracetamol is linked to asthma_ smh_ com_ au_ htm. Mar.22.

10. National Institutes of Health Clinical Center: Facts about Dietary Supplements (Zinc) (Internet).
11. Lao, T.; Chin, R.; Swaminathan, R. and Mak, Y. (1989) Eur. J. Obstrt. Gynaecol. Reprod. Biol., 30: 117-122.
12. Complementary and Alternative Medicine: Does it have a role treating asthma? Dietary Supplements, heroal therapies. ADAM PERLMAN, MD < MPH AND JEFFREYS. SERBIN, MD. Womenheal in primar caree. Vol. 4, April (2001).
13. Untitled Document. Html. Glutathione: What is it ? (Internet).
14. Glutathione- Acetycylsteine. The new old supper drug!! Txt. http://www.acetylcysteine.org/glutathione.html.
15. Gabriele, Wills B.Sc., E. Ed. Immunotec Research authorized distributor of Immunocal. (Glutathione and Immunocal). http://www.raiseglutathione.com.
16. Immunocal and Immunity: Conditions Associated with Low Glutathione Levels. Green Door Wellness Center. http://www.greendoorwellness.com.
17. Haury, V. (1940) J. Lab. Clin. Med., 26: 340-344.
18. Falkner, D.; Glauser, J. and Allen, M. (1992) Am. J. Emerg. Med., 10: 1-3.
19. Bumroongkit, C. and Charoenpan, P. (2001) Chiang. Mai. Med. Bull., 40: 1-5.
20. Aural, H.; Uzun, K.; Kocyigit, A.; Cigli, A. and Akyol,O. (2000) J. Trace Elem. Med. Biol., 14: 88-91.
21. Rolla, G.; Bucca, C.; Bugiani, M. and Arossa, W. (1987) Spinaci S. Allergy,. 42: 186-188.
22. Kadrabova, j.; Madaric, A. ; Podivinsky, F. ; Kovacikova, Z. ; Ginter, E. and Gazdik, F. (1996) J. Trace. Med. Biol., 52: 241-248.
23. Eric, Braverman: Asthma. The Wellness Manual. Chapter 1, p. 3-4 (2001).
24. Penning, H. ; Borm, P. and Evelo, C. (1999) Eur. Respir. J., 13: 1260-1266.
25. Ellman, G. (1958) Arch. Biochem. Biophys., 443: 70-77.

Table (1) Comparison between serum Magneesium Levels in healthy controls and asthmatic patients at different severity of the disease

Individual	Mean ± SD mmol/L	Significance
Healthy	1.01 ± 0.19	
Severe	0.92 ± 0.17	Healthy *vs.* severe P< 0.05
Moderate	1.05 ± 0.19	Healthy *vs.* moderate N.S
Slight	1.08 ± 0.14	Healthy *vs.* slight N.S
Normal (PFT)	1.11 ± 0.16	Healthy *vs.* normal N.S

Table (2) Comparison between levels of serum Magnesium of asthmatic patients group depending on their severity of the disease

Comparison between groups	Significance
Severe *vs.* moderate	P< 0.05
Severe *vs.* slight	P< 0.05
Severe *vs.* normal (PFT)	N.S
Moderate *vs.* slight	N.S
Moderate *vs.* normal (PFT)	N.S
Slight vs. normal (PFT)	N.S

Table (3) Comparison between serum zinc levels in healthy controls and asthmatic patients at different severity of the disease

Individual	Mean ± SD µmol/L	Significance
Healthy	17.01 ± 2.94	
Severe	17.94 ± 3.03	Healthy *vs.* severe P< 0.05
Moderate	18.63 ± 2.48	Healthy *vs.* moderate N.S
Slight	19.48 ± 1.93	Healthy *vs.* slight N.S
Normal (PFT)	17.41 ± 4.64	Healthy *vs.* normal N.S

Table (4) Comparison between levels of serum zinc of asthmatic patients group depending on their severity of the disease

Comparison between groups	Significance
Severe *vs.* moderate	N.S
Severe *vs.* slight	0.07449
Severe *vs.* normal (PFT)	N.S
Moderate *vs.* slight	N.S
Moderate *vs.* normal (PFT)	N.S
Slight vs. normal (PFT)	N.S

Table (5) Comparison between serum glutathione levels in healthy controls and asthmatic patients at different severity of the disease

Individual	Mean ± SD mmol/L	Significance
Healthy	2.42 ± 0.21	
Severe	2.08 ± 0.51	Healthy *vs.* severe P< 0.001
Moderate	1.91 ± 0.3	Healthy *vs.* moderate N.S
Slight	1.95 ± 0.36	Healthy *vs.* slight N.S
Normal (PFT)	1.82 ± 0.59	Healthy *vs.* normal N.S

Table (6) Comparison between Levels of serum glutathione of asthmatic patients group depending on their severity of the disease

Comparison between groups	Significance
Severe *vs.* moderate	N.S
Severe *vs.* slight	N.S
Severe *vs.* normal (PFT)	N.S
Moderate *vs.* slight	N.S
Moderate *vs.* normal (PFT)	N.S
Slight vs. normal (PFT)	N.S

قياس مستويات المغنيسيوم، الزنك، والكلوتاثايون المختزل في امصال مرضى الربو

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الخلاصة

ان مرضى الربو يشخص بوساطة زيادة نشاط الشعيبات الهوائية تلقائيا او كنتيجة للعلاج، وفي هذه الدراسة تم قياس مستويات المنغيسيوم الزنك، والكلوتاثايون المختزل في امصال مرضى الربو نسبة الى مستوياتها في الاشخاص الاصحاء.

حيث تم اخذ اربع وخمسون مصابا بعد اجراء اختبار الفحص الرئوي الوظيفي وقياس مستويات المغنيسيوم والزنك بأستخدام جهاز مطياف الامتصاص الذري، ومستوى الكلوتاثايون المختزل بأستخدام تحليل الطيف.

تم تقسيم المرضى على اربع مجاميع اعتمادا على اختبار الفحص الرئوي الوظيفي وهي مجموعة الجريان التنفسي الشديدة والمتوسطة والخفيفة والطبيعية على التوالي. وقد وجد في هذه الدراسة ان هناك انخفاضا في مستوى المغنيسيوم في مصل مرضى الربو للمجموعة الاولى مقارنة مع الاشخاص الاصحاء.

$(0.92 \pm 0.17$ mmol/L vs 1.01 ± 0.19 mmol/ L)

وان قيمة $P<0.05$ بينما لم توجد اية فروقات معنوية في المجاميع الثلاثة الاخرى مقارنة مع الاصحاء.

اما بالنسبة لمستوى الزنك فقد وجد ان هناك ارتفاع معنويا في مستواه في مصل دم مرضى الربو في المجموعة الثالثة مقارنة مع الاشخاص الاصحاء.

$(19.48 \pm 1.93$ µmol/L vs 17.01 ± 2.94 µmol/L)

وان قيمة $P< 0.05$ في حين لم تواجد اية فروقات معنوية في المجاميع الثلاثة الاخرى مقارنة مع الاصحاء .

بينما لوحظ ان هناك انخفاضاً معنويا في مستويات الكلوتاثايون ي حالات المجاميع الاولى والثالثة مقارنة مع الاشخاص الاصحاء.

and 1.95 ± 0.36 mmol/L vs 2.42 ± 0.21 mmol/L; ($P < 0.01$)

نستنتج من هذه الدراسة بأن هناك علاقة بين شدة مرض الربو المقاس بالاختبار الرئـــوي الوظيفي مع مستويات المغنيسيوم والكلوتاثايون فكلما ازدادت حدة المرض قلت مستويات المغنيسيوم والكلوتاثايون.