Comparison of Plasma MDA, Vitamin C and Serum Trace Elements Status between Bronchial Asthma Patients and Healthy Subjects

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(ABSTRACT)

plasma vitamin C, malondialdehyde (MDA), serum copper and zinc were investigated in (40) patients with bronchial asthma (BA) (Age range:20 to 50 years) and in (50) healthy subjects. This study confirms the fact that in (BA) disease, the status of plasma oxidants and antioxidants shifts from normal. The status of oxidants in plasma as represented by (MDA) levels increased significantly in the conditions of (BA) ($p<0.001$). The vitamin antioxidant (vitamin C) showed decreased levels than in controls.

On the other hand, we aimed to define the relation between (BA) and serum levels of two trace elements (Zn & Cu). Zinc levels were significantly decreased in comparison to the control values($p<0.01$), but serum copper levels were found to be increased in patients with (BA) compared to the control group ($p<0.01$). The changes in trace element status may be the effect of chronic disease state.

Introduction

Asthma is a chronic relapsing inflammatory disorder of the airways and a major health problem worldwide (Seaton et al,2000).Different genetic and environmental factors are involved in the pathogenesis of asthma (Fraenkel& Holgate, 1996).The prevalence and morbidity rate of asthma have increased in the past decade, despite improved knowledge about its pathophysiology and treatment. As inflammatory cells generate and release reactive oxygen species, asthmatic airways are liable to oxidative stress (Shanmugasundaram et al,2001) .Moreover, inflammatory cells from asthmatic patients generate more reactive oxygen species than those from controls. The extent of oxidative stress will depend, in part, on the antioxidant defences available within the respiratory tract lining fluid (Kelly et al,1999).

The potential of oxidants to damage pulmonary tissue is dependent on the local antioxidant defense mechanisms. Antioxidants are physiologic substances that are derived from both endogenous and exogenous sources and that act against oxidant stress. Antioxidants may delay or prevent direct oxidation of oxidizable substrates or scavenge oxidant free radicals and thus neutralize the physiologic oxidant burden created by both exogenous and endogenous free radicals (Buhi et al, 1996).

The dominant antioxidant in the epithelial lining fluid of the lung is glutathione (Cross et al, 1994) . (Poly) aromatic substances; which are obtained mainly from the diet, include vitamins and their precursors such as vitamin A(beta carotene), vitamin E (‹-tocopherol), and vitamin C(ascorbic acid).It is believed that in order to maintain normal lung function, there must be a balance between toxic oxidants and protective antioxidants(Halliwell et al, 1992).

It has been suggested that antioxidants might have an etiologic role in asthma and, if so, this could lead to the development of new therapeutic strategies. The data are strongest for vitamin C, which is one of the key antioxidant vitamins(Kaur et al. , 2001).It is abundant in the extracelluar fluid lining the lung, and adequate vitamin C intake has been associated with the protective effects of airways responsiveness and lung function (Shanmugasundaram et al,2001; Omenaas et al,2003) .Vitamin C reduces the number and severity of attacks in patients with asthma and reduces the severity of the bronchial responses to exercise (Cohen et al, 1997).

Further research suggests that oxidant stress may contribute to the pathogenesis of many different lung disorders, including asthma, chronic obstructive pulmonary disease(COPD), cystic fibrosis CF), pulmonary fibrosis, and pneumoconiosis, lung cancer, and the acute respiratory distress syndrome (ARDS) (Halliwell et al, 1992).
There have been numerous reviews focusing the importance of vitamin C for respiratory disease such as asthma, other studies are important in the role of zinc and copper in bronchial asthma, it is clear that profound variations in copper and zinc status occur during the course of acute and chronic inflammation ,and both zinc and copper are required for numerous biochemical functions and for optimal activity of the immune system. Zinc plays an important role in DNA and protein synthesis and is intimately involved with copper as cofactors in several important enzyme systems (Beisel,1982; Fraker et al,1986). Zinc and copper are involved in cell and tissue growth. Changes in patterns of dietary consumption, associated

with development of a more affluent lifestyle, may have contributed to the rise in asthma over the past few decades (Burney,1987 ; Seaton et al,1994).

The aim of the present study was, to study the plasma oxidant- antioxidant and serum trace elements status in patients with bronchial asthma and values were compared with normal healthy controls.

Material And Method

The study group consisted of 40 patients with bronchial asthma who were followed at AL-diwaniya hospital (Age range:20 to 50 years). The diagnosis of asthma was established from a documented clinical history of recurrent cough and/or wheezing and previous demonstration of improvement of symptoms in response to asthma medication. Subjects were selected after each patient had been assessed clinically Pulmonary function tests were performed in asthmatic patients and in control subjects . The control group included 50 (Age range:22 to 55 years) healthy ,non-smokers without any history of lung disease and had normal pulmonary function tests. Fasting venous blood samples were collected for the study of asthmatic patients and taken in EDTA vial. Samples were used for the estimations of plasma vitamin C ,serum MDA,serum copper and serum zinc.

Vitamin C was measured at 254 nm by using high performance liquid chroma-tographic technique (Rose and Nahrwold,1981).

The zinc and copper concentrations in serum were measured by means of an Atomic Absorption Spectrometer. The zinc and copper concentrations in the samples diluted 1:5 with ultra deionised water and values were expressed in µg/dl. Each measurement was performed twice and avera- ges were taken (Mahalingam et al,1997).

Serum MDA was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of Fong et al.,1973 The thiobarbituric acid reaction is used to measure serum MDA. In this test, the chromogen is formed by the reaction of one molecule of MDA with two molecules of TBA. The method involves heating the sample (serum) with trichloroacetic acid and thiobarbituric acid under acidic conditions, and reading the absorbance of the MDA-TBA adduct at 532 nm.

Statistical analysis was carried out using unpaired‘t’ test. P value less than 0.05 ($P<0.05$) was considered as significant.

Results And Discussion

Oxidants-antioxidants balance is essential for the normal lung function. Both, an increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant balance in favor of oxidants, leading to lung injury.A number of diseases involving the lung, such as COPD,emphysema, bronchiectasis and bronchial asthma have been associated with a disturbance of these balances (Buhi et al, 1996).

In this study table(1) shows significantly lower plasma vitamin antioxidant (vitamin C) levels in patients group as compared to control subjects. The results of this study confirm previous observations that there is low plasma antioxidants (vitamin C) in BA patients (Kelly et al, 1999).

Vitamin C is a water soluble free radical scavenger.Vitamin C can directly scavenge $O_{2.}^-$ and •OH with the formation of semidehydroascorbate-free radical that is subsequently reduced by GSH to generate dehydroascorbate and GS and it can also neutralize several oxidants present in the blood of smokers (Heffner & Repine, 1989). In the present study, it was found that concentration of vitamin C significantly decrease (0.84 ± 0.165 mg/dl) in patients with bronchial asthma as compared to control group, our results are in agreement with the data of Zhou et al , 1997 who reported decreased plasma vitamin C in asthmatic smokers , also this study was consistent with the findings of other studies (Hu et al, 1998). Vitamin C, as an important antioxidant , has been hypothesized to have a role in preventing the increased production of oxidants in asthma and the resulting oxidative stress in the lungs (Forastiere et al, 2000) . Other studies showed that lower intake of vitamin C was associated with the presence of respiratory symptoms and pulmonary diseases such as asthma (Huang and Pan,2001).Trenga et al,2001 in a double-blind crossover study reported that vitamin C plus vitamin E can decrease the ozone - induced bronchial hyper-responsiveness in adults with asthma .

On the other hand serum copper levels were higher (141 ± 19.8 µg/dl) than normal subjects(table 1). In another study, serum copper has been found to be elevated while serum zinc levels were found to be low (Vural et al,2000). It is debatable whether an increase in the copper level has an impact upon SOD absorption; however, it is clear that a decrease in this element impairs the enzyme activity. Such a condition may cause oxidative stress or may further increase the existing stress (Ermis et al,2004).

Our study (table 1) was showed relatively low serum levels of zinc (69.6 ± 7.3 µg/dl) in subject with asthma. Chronic inflammation causes a characteristic decline in serum zinc levels in experimental studies (Milanion et al,1993). The first consistent study investigating the Zn status of bronchial asthmatics was that of Goldey *et al.* in 1984 who reported a reduction in the Zn content of hair in asthmatics and Kadrabova *et al.* 1996 reported similar results, but extended the observations to show a significant drop in serum Zn levels. It was proposed that adequate dietary intake and Zn supplementation may decrease the severity of

asthmatic attacks by correcting this underlying hypozincaemia (el-Kholy et al,1990).

It was proposed that several intrinsic factors may contribute to a low Zn status in asthmatics. First, like other inflammatory diseases, a redistribution in plasma Zn to the liver can occur during excessive stress. This has been attributed to the release of leucocyte endogenous mediator from activated phagocytes, which then stimulates movement of Zn from plasma to hepatocytes in allergic reactions (el-Kholy et al, 1990). Second, the immune system is extremely dependent on the availability of Zn for maintaining its homeostasis. Inflammatory diseases can cause an increase in the demand for Zn as: (i) Zn is essential for producing the thymic hormone thymulin necessary for regulating T-cell development and activation; and (ii) Zn is crucial for the activation of natural killer cells, phagocytic cells and for granulocytes, such as mast cells and eosinophils. As a result, greater demand for Zn by the immune system could be a contributing factor to the Zn deficiency noted in inflammatory diseases. Zinc deficiency itself is detrimental for inflammation as it results in dramatic increases in the number, size and activation state of mast cells. This further exacerbates damage via increasing chemotaxis of eosinophils and neutrophils, which creates a continuous cycle of oxidative damage, all of which have been implicated in promoting the pathogenesis of allergic diseases such as asthma. Third, although reactive oxygen species are formed as a normal component of cellular respiration, in asthma there is a reported imbalance between the flux of oxidants generated and the presence and/or activation of cellular anti-oxidant defence mechanisms. This especially relates to Cu-Zn SOD, which is normally required to detoxify superoxide anions(Wellinghausen&Rink,1998). At least three studies have demonstrated a significant decrease in Cu-Zn SOD activity in erythrocytes(Tekin et al,2000) and respiratory epithelial cells.(De Raeve et al,1997 ; Smith et al,1997). One possible explanation for the decrease in activity of Cu-Zn SOD may be that in order for the body to compensate for increased oxidative stress, it must upregulate its anti-oxidant production, hence increasing its need for biochemically active Zn. Therefore, if a hypozincaemia exists and tissue Zn becomes limiting during inflammation, the activity of Cu-Zn SOD may be compromised. Finally, because the respiratory epithelial cells are rich in Zn their loss, through shedding into the airways during asthmatic episodes, will further deplete Zn reserves.

MDA is a representative of thiobarbituric acid reacting substances (TBARS). The amount of MDA is a measure of lipid peroxidation and its measure provides an estimate of free radical activity (Bonnefont et al,1989). In the present study MDA was found to be having statistically significantly higher values (0.61 ± 0.177 µmol/l) in BA patient group as compared to control group(table 1) . Similarly, Nadeem et al,2003 reported increased lipid peroxidation products in patients with bronchial asthma . Thus our study confirmed, the presence of an oxidant-antioxidant imbalance in patients with bronchial asthma, supporting the concept of systemic oxidative stress in this condition.

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Table1. Levels of copper, zinc, vitamin C, vitamin E and (MDA) in bronchial asthema (BA) patients and control.

	Cases	Control	p value
Serum copper µg/dl	141 ± 19.8	129 ± 22.6	p<0.01
Serum zinc µg/dl	69.6 ± 7.3	77.3 ± 8.2	p<0.01
Plasma vitamin C mg/ dl	0.84 ± 0.165	1.01 ± 0.133	p<0.01
Serum (MDA) µ mol/l	0.61 ± 0.177	0.433 ±0.122	p<0.001

Values expressed as mean ± SD; NS= Not significant

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مقارنة بين المصابين بالربو المزمن والأصحاء من حيث مستويات المالون داي الديهايد وفيتامين C في البلازما والعناصر الضئيلة في المصل

زينب نجم عبدا لله

جامعة القادسية/ كلية العلوم/ قسم الكيمياء

(الخلاصة)

في هذه الدراسة أجريت مقارنة بين وضع المالون ثنائي الديهايد (MDA) ، فيتامينC ، ،النحاس والزنك لدى (٤٠) من البالغين المصابين بالربو المزمن المسيطر عليه (BA) تتراوح أعمارهم (٢٠- ٥٠ سنة)، ووضعه لدى ٥٠ من الأصحاء الذين تم انتقاؤهم عشوائيا.

في هذه الدراسة تم تأكيد تغير مستوى المؤكسدات ومضادات الأكسدة عن الطبيعي في بلازما مرضى (BA)، حيث وجد أن هناك زيادة معنوية في مستوى الأكسدة (مستوى MDA) في حالة مرضى (BA) ($p<0.001$)، بينما اظهر مستوى (فيتامينC) في المرضى نقصانا عن مستوى الأصحاء.
من جهة أخرى تم توضيح العلاقة بين مرضى (BA) ومستويات المصل من كلا العناصر الضئيلة(Zn,Cu) حيث أظهرت الدراسة نقصانا معنويا في مستوى الزنك مقارنة بالأصحاء ($p<0.01$) بينما وجد زيادة في مستويات النحاس في مرضى (BA) مقارنة مع الأصحاء ($p<0.01$).حيث وجد أن التغير في مستوى العناصر الضئيلة محتمل أن يكون نتيجة لتأثير الحالة المرضية المزمنة عليه.

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