Serum Trace Element Levels in Bronchial Asthma

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Abstract

Background: Free radicals have been proposed to be responsible from the pathogenesis of many diseases because of their harmful effects on the cells and tissues. There are some defence mechanisms in the organism to avoid the harmful effects of free radicals. The enzymes responsible from antioxidant defense have trace elements like selenium, zinc and copper within their structure. Decreased levels of these elements lead to a reduction in antioxidant activity. This study was conducted to determine serum levels of some trace elements (Se, Zn, Cu) in asthmatic patients during acute attacks, after inhaled corticosteroid therapy and during asymptomatic periods in order to evaluate the relation of these elements to different clinical phases of asthma.

Medhods: The study and control cases included 40 asthmatic children being followed up in Department of Pediatric Allergy Dokuz Eylül Universty Medical Faculty, and 19 children presenting with enuresis nocturna and having no personal or family history of allergy. Patients presenting with acute asthmatic attacks (study group 1, n=22) were evaluated before the treatment (pretreatment) and after three months of Fluticasone Propionate (400 mg daily) therapy (posttreatment).The other study group included asthmatic patients who were asymptomatic (study group 2, n=18). Control group was the children presenting with enuresis with (group 3, n=19). Serum selenium, zinc and copper levels were measured in all cases. In addition, pulmonary function tests were performed and FVC and FEV1 were determined in all of the children.

Results: Serum selenium and zinc levels during acute attacks and after inhaled corticosteroid therapy were significantly different in group1, being lower during acute attacks ($p<0.05$). However, pre and posttreatment serum copper levels did not differ ($p>0.05$). FVC and FEV1 were also statistically different in the pre and posttreatment periods ($p<0.05$). Although serum selenium and zinc levels were significantly lower when group 1 (pretreatment) was compared with groups 2 and 3 ($p<0.05$), serum copper levels did not differ between the groups ($p>0.05$). FVC and FEV1 were also significantly different between the three groups ($p<0.05$).

Conclusion: These results show that, there is an oxidant stress in bronchial asthma, and antioxidant capacity decreases in parallel to a decrease in serum selenium and zinc levels. This leads to a further increase in oxidant stress, and as a result, inflammation and hyperreactivity in the airways becomes more evident.

We speculate that, dietary deficiencies in selenium and zinc should be avoided with proper supplementations in the management of airway inflammation due to free oxygen radicals in asthmatic patients to increase the effect of antioxidant defense system.

Turkish Respiratory Journal, 2001;2 (3):10-15

Key words: *asthma, free oxygen radicals, antioxidant defense mechanism, trace elements*
Abbreviation: *Cu: Copper, Zn: Zinc, Se: Selenium, FEV1: Forced expiratory volume in 1 sec, FVC: Forced vital capacity, GSH-Px: Glutathione peroxidase, SOD: Superoxide dismutase*

Introduction

Bronchial asthma is a chronic inflammatory disease of the respiratory tract. Different genetic and environmental factors are responsible in the pathogenesis of asthma (1). In the recent studies, free oxygen radicals were accused for the pathogenesis of bronchial asthma (2).

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There are some defense mechanisms to escape from the effects of oxidant radicals. These defense systems prevent the production of free radicals, decrease their activities or destroy them. The most important antioxidant endogen systems are mitochondrial cytochrome oxidase, superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) systems. Also albumin, seruloplasmin, ferritin and hemoglobin which are found in the extracellular space have antioxidant properties (3) Glutathione peroxidase has selenium and Cu/Zn superoxide dismutase system has copper and zinc in their structure which diminish the harmful effects of free oxygen radicals. Decreasing of these trace elements causes the effects of antioxidant systems to be lower and this leads to hyperactivity and inflammation in the respiratory tract (4,5).

In this study, we aimed to define the relation between bronchial asthma and serum levels trace elements (selenium, zinc and copper)

Materials and Methods

The study group consisted of 40 asthmatic children who were followed by Dokuz Eylül University Faculty of Medicine Pediatric Allergy Department and 19 control group of children who attend the pediatric outpatient clinic by the complaint of enuresis nocturna.

The diagnosis of bronchial asthma depends on the history, family history, physical examination and laboratory results. Pulmonary function tests were performed to all children who were in the study. Epidermal skin tests were performed to all bronchial asthma patients to detect the atopy with the allergens which are met frequently in their daily lives. PA chest x-ray, Water's x-ray, complete blood count and eosinophil count were performed on all patients.

Cromalin or nedocromil sodium for prophylaxis were given to all patients who have the diagnosis of bronchial asthma.

None of the patients in the study used systemic or inhaled steroids and acetyl salicylic acid for the last 2 weeks or other nonsteroidal antiinflammatory drugs. Bronchial asthmatic patients were allowed to take beta-2 agonist drugs when needed. Blood samples were taken at least 12 hours after the last dose of beta-2 agonists. Serum trace element levels were measured in 3 different groups in the study.

First group: These patients have presented with acute asthma attacks (n=22). Degree of respiratory tract obstruction was measured by pulmonary function tests in acute asthmatic patients and 5 cc of blood was taken to a special acidified tube to measure the serum trace element levels. After the treatment of acute asthma attack was performed appropriately, the patients were given 400 mg inhaled form of fluticasone propionate daily (200 mg am, 200 mg pm). This treatment modality was continued for 3 months with the same dosage. The patients routinely were controlled with physical examination, pulmonary function tests. Inhaled corticosteroid treatment was stopped after 3 months and venous blood samples were taken to estimate the serum trace element levels. Thus trace element levels have been determined before and after the steroid treatment.

Second group: Eighteen patients who were followed by allergy department with the diagnosis of asthma. Venous blood samples were taken monthly for the measurement of trace elements.

Third group: The patients have admitted to pediatric outpatient clinic for enuresis nocturna and none of the 19 children had allergic family history.

Serum was determined from the 3 groups of peripheric venous blood samples and samples were preserved at –20°C.

Serum Zn and Cu levels were measured by Flame atomic absorbtion spectroscopy. Serum Se level was measured by atomic absorbtion (6,7).

Statistical analysis: All results were given as the mean ± standard deviation value and data analysis were performed by SPSS 6.0 statistical programme.

Triple results were compared with Kruskal Wallis Variant Analysis and $p<0.05$ value was accepted as statistically significant. Mann Whitney U Test was performed for the estimation of the group that creates the statistically significant difference in Kruskal Wallis Test and p results were divided in 3 groups and $p<0.016$ was accepted as statistically significant. Wilcoxon test was performed to the group before and after the treatment and $p<0.05$ value was accepted as statistically significant. Differences between the genders were analysed by chi-square test and $p<0.05$ value was accepted as statistically significant.

Results

We have evaluated the serum trace element levels of asthmatic patients during the acute attack after the treatment of inhaled steroids, during the stable period of asthma and compared the results with the healthy children; respectively each group consisted of 22 patients in the first, 18 in the second and 19 in the third one, with the sum of 59 children. The mean ages of children 8.93± 2.08 (6-14)years, 8.53±1.79 (6-12) years and 9.24±2.18 (6-14) years in the first, second and third group, respectively. There was no significant difference regarding the mean ages of the groups ($p>0.05$). Mean weights of the patients were 29.23±8.11(7-49) kg, 28.08±8.07 (16-45) kg and 30.76±9.65 (19-51) kg in the first, second and third group, respectively and there was no significant difference between the groups ($p>0.05$). Mean heights of the patients 131.36±10.59 (111-161) cm, 128±13.42 (105-152) cm, 144.82±13.31(117-165) cm in the first, second and third group, respectively. There was no statistically significant difference between the groups ($p>0.05$).

All groups were examined in view of the differences in gender. Of the 22 children in the first group 13 were male and 9 were female, of the 18 children in the second group 10 were male and 8 were female, of the 19 children in the third group 10 were male and 9 were female. There was no statistically significant difference between the groups ($p>0.05$).

Selenium, zinc and copper serum levels were examined in all groups. There was statistically significant difference between serum selenium levels examined during the asthma attacks and the treatment of 400 mg inhaled form of fluticasone propionate for 3 months when compared with Wilcoxon Test ($p=0.006$) (Table 1).

Table 1. Comparison of pre and post-treatment trace element levels in the first group.

	Pre Treatment	Post Treatment	p
Selenium (mg/L)	50.38 ± 15.18	55.99 ± 23.42	**p:0.006**
Zinc (mg/dl)	89.26 ± 20.29	124.05 ± 40.35	**p:0.001**
Copper (mg/dl)	104.5 ± 23.49	99.91 ± 32.42	p:0.087
FEV_1 (%)	62.68 ±12.26	93.77 ± 8.02	**p:0.0001**
FVC (%)	58.95 ± 12.03	90.73 ± 6.38	**p:0.0001**

There was statistically significant difference between the variables of FEV_1 and FVC in pulmonary functional tests performed during the attacks of asthma and after the treatment of inhaled steroids ($p=0.0001$). These parameters were significantly decreased during the attacks (Table 1).

Serum selenium, zinc and copper levels which are taken before the treatment of asthma during the stable period of asthma and in healthy children were compared to each other and statistically significant difference was detected between serum selenium and zinc levels ($p<0.05$), (Table 1) Between the first (before treatment) and the third groups this difference is more prominent in plasma selenium levels ($p=0.0001$). When the plasma zinc level were compared in two group the difference between first and third groups were significant ($p=0.0058$). But there was no statistically significant difference between serum copper levels ($p>0.05$). There was also a statistically significant difference between the variables of FEV_1 and FVC in these 3 groups ($p<0.05$) (Table 2).

Table 2. Comparison of the levels of trace elements between the first (pre-treatment), the second and the third groups.

(Pre Treatment)	1. Group	2. Group	3. Group	p
Selenium (mg/L)	50.38 ± 15.18	70.09 ± 24.11	77.03 ± 14.52	**p:0.0004**
Zinc (mg/dl)	89.26 ± 20.29	101.56 ± 30.05	121.21 ± 36.85	**p:0.020**
Copper (mg/dl)	104.5 ± 23.49	107.50 ± 22.39	109.26 ± 20.25	p:0.079
FEV_1 (%)	62.68 ±12.26	100.89 ± 9.65	92.95 ± 9.82	**p:0.0001**
FVC (%)	58.95 ± 12.03	93.17 ±13.45	91.11 ± 8.34	**p:0.0001**

Serum selenium, zinc and copper levels were compared between the 3 groups. Only the serum levels of selenium between the groups was statistically significant ($p=0.019$), (Table 3). This difference was originated from the serum selenium levels of the first and third groups ($p=0.004$). Serum selenium levels in the first group after therapy were measured significantly lower than the healthy children (Table 3).

Discussion

The free radicals were considered as responsible for hundreds of diseases because of their disturbing effects on the cells and tissues.

Table 3. Comparison of the levels of trace elements between the first (post-treatment), the second and the third groups.

(Pre Treatment)	1. Group	2. Group	3. Group	p
Selenium (mg/L)	55.99 ± 23.42	70.09 ± 24.11	77.03 ± 14.52	**p:0.019**
Zinc (mg/dl)	124.05 ± 40.35	101.56 ± 30.05	121.21 ± 36.85	p:0.169
Copper (mg/dl)	99.91 ± 32.42	107.50 ± 22.39	109.26 ± 20.25	p:0.088
FEV_1 (%)	93.77 ± 8.02	100.89 ± 9.65	92.95 ± 9.82	p:0.061
FVC (%)	90.73 ± 6.38	93.17 ±13.45	91.11 ± 8.34	p:0.807

There are defense mechanisms for protection from the injury of these free radicals. These mechanisms were taken apart in the pathogenesis of many respiratory tract diseases.

Eosinophils also have a stronger effect on the synthesis of the free oxygen radicals than neutrophils (8). In bronchial asthma enhanced release of free oxygen radicals from eosinophils shown in studies underscores the potential role of eosinophils in tissue destruction. Recently after understanding the role of free oxygen radicals in the pathogenesis of asthma, most studies pay attention to antioxidant defense systems and selenium which is a serum trace element. The enzyme glutathione peroxidase is one of the antioxidant defenses which protect the lungs from the potentially damaging effects of free oxygen radicals. Glutathione peroxidase catalyses the reduction of hydrogen peroxide and lipid hydroperoxides by glutathione. This cellular enzyme is a tetrameric protein with 4 identical subunits, each containing 1 atom of selenium in the form of selenocysteine at the active site. Selenium is an integral component of all these enzymes, thus, reduced intake of this trace element may potentially lead to reduced activity of glutathione peroxidase and decreased antioxidant defense mechanisms (9). Therefore, serum selenium level is very important for the activity of the glutathione peroxidase in the pulmonary antioxidant systems (10).

In order to investigate further link between low selenium status and asthma, a study was undertaken in New Zeland. This study showed that asthmatic patients in New Zeland had lower whole blood selenium concentrations than the control subjects had and was in contrast to the previous British study, which showed that this result was associated with a reduced whole blood glutathione peroxidase activity. A six-fold increased risk of asthma was observed among subjects with the lowest range of whole blood glutathione peroxidase activity (11). The studies show that the reduced selenium status with associated lowered glutathione peroxidase activity may contribute to the pathogenesis of asthma, an inflammatory disorder which is characterized by the influx and activation of a number of inflammatory cells within the airways. Following either immunologic or non-immunologic stimulation, polymorphonuclear leukocytes, macrophages, monocytes, eosinophils and mast cells release oxygen radicals. Oxygen radicals may also react with a component of plasma to form a product which is chemotactic for neutrophils and increase capillary wall permeability.

In our study serum selenium levels in patients with acute asthma attacks (all atopic asthma) were significantly lower than the other groups.

In recent years the beneficial effect of steroids in inducing a recovery of antioxidant capacity has been suggested in two different studies. Rahman et al. have shown the evidence from *in vitro* studies, also suggested that among the inflammatory actions of steroids is a reduction in the generation of reactive oxygen species by neutrophils and stimulation of the synthesis glutathione in liver (12). In another study Raeve et al. have shown that corticosteroids decrease oxidant levels by decreasing the number of oxidant-generating cells present in the asthmatic airway mucosa and reducing macrophage oxidant production (5). In the same study, it is evidenced that inhaled corticosteroid treatment lowers asthmatic airway oxidant levels.

In our study after the appropriate asthma attack treatment, fluticasone propionate was started with the dosage of 400 mg/day. After the 3 months of treatment, serum selenium levels were established significantly higher than the pretreatment levels. These results showed similarity with Rahman's and Raeve's studies.

Intracellular antioxidants are crucial for protection of cells against oxidant induced injury. Superoxide dismutase enzyme is one of these systems. This enzyme is widely spread in aerobic cells and protect cells with the dismutation of the superoxide radicals. The enzyme superoxide dismutase mutates superoxide radicals to hydrogen peroxide and molecular oxygen 10,000 times more rapidly than

spontaneous dismutation in physiological pH. Copper and zinc containing superoxide dismutase (Cu-Zn SOD), manganese containing SOD and ferrum containing SOD are the 3 types of this enzyme.

Two main changes might be considered in the lungs with decreased serum zinc level (13,14).

(1) Cell membrane damage. (Shortage of Zn lowers the stability and integrity of the cell membrane, leading to release of enzymes from lysosome and histamine from mastocytes. The ability of the membrane to resist free radicals is impared).

(2) Cu-Zn SOD structure changes. (Lower Zn damages the structure and effects the activity of Cu-Zn SOD, concequently reduces the ability of Cu-Zn SOD to scavenge free radicals.)

(3) When the contents of Cu and Zn decrease Cu-Zn SOD activity also diminishes and free radicals cause increased lung injury.

Serum Zn and Cu levels and Cu/Zn ratio of 22 intrinsic asthma patients were compared to that of 33 healthy control subjects by 5 of the intrinsic asthma patients were aspirin intolerant. The zinc content of serum was found to be significantly lower in asthmatic patients than in control individuals (15).

Raeve et al. investigated bronchial epithelial cell response to the antioxidant agents in chronic airway inflammation in asthma. Cu-Zn SOD, Mn SOD, catalase and glutathione peroxidase were major intracellular antioxidants detected in the bronchial epithelial cells of the asthmatic patients and healthy controls. Catalase and glutathione peroxidase levels were similar in the bronchial epithelium of the asthmatics and control group, but superoxide dismutase activity was significantly lower in the asthmatic patients who are not treated by inhaled corticosteroid when compared to control group and the patients treated with inhaled corticosteroid. Investigators demonstrated that the chronic airway inflammation in asthma is associated with bronchial epithelial superoxide dismutase deficiency and decreased inflammation with corticosteroids is associated with normal SOD activity (5).

In our study, zinc levels during the asthma attacks were detected significantly lower than the other groups. Zinc level was found normal after the teatment with corticosteroid, during stable asthma period and in the healthy control group. Our results resemble the other studies. We found the serum copper level to be in the normal levels during acute asthma attacks, after the inhaled steroid treatment and during stable asthma. There was no significant difference between asthmatic patients and healthy control group.

In the treatment studies related with diet, it was aimed to increase the consumption of antioxidant agents like vitamin E, A, C, beta carotene, selenium, zinc and magnesium rich foods for increasing intracellular antioxidation system activity.

As a result, in our study serum selenium, zinc and magnesium were found in low levels during acute asthma attack and increased after inhaled steroid treatment that increases the antiinflammatory and antioxidant effects. These results show that oxidant stress is present and increases in the bronchial asthma because of the low levels of serum selenium, zinc and magnesium. Bronchial airway inflammation and hyperreactivity increase with the oxidant stress. Selenium and zinc-rich diet which are thought to increase the antioxidant system activity can be useful in the treatment of the airway inflammation.

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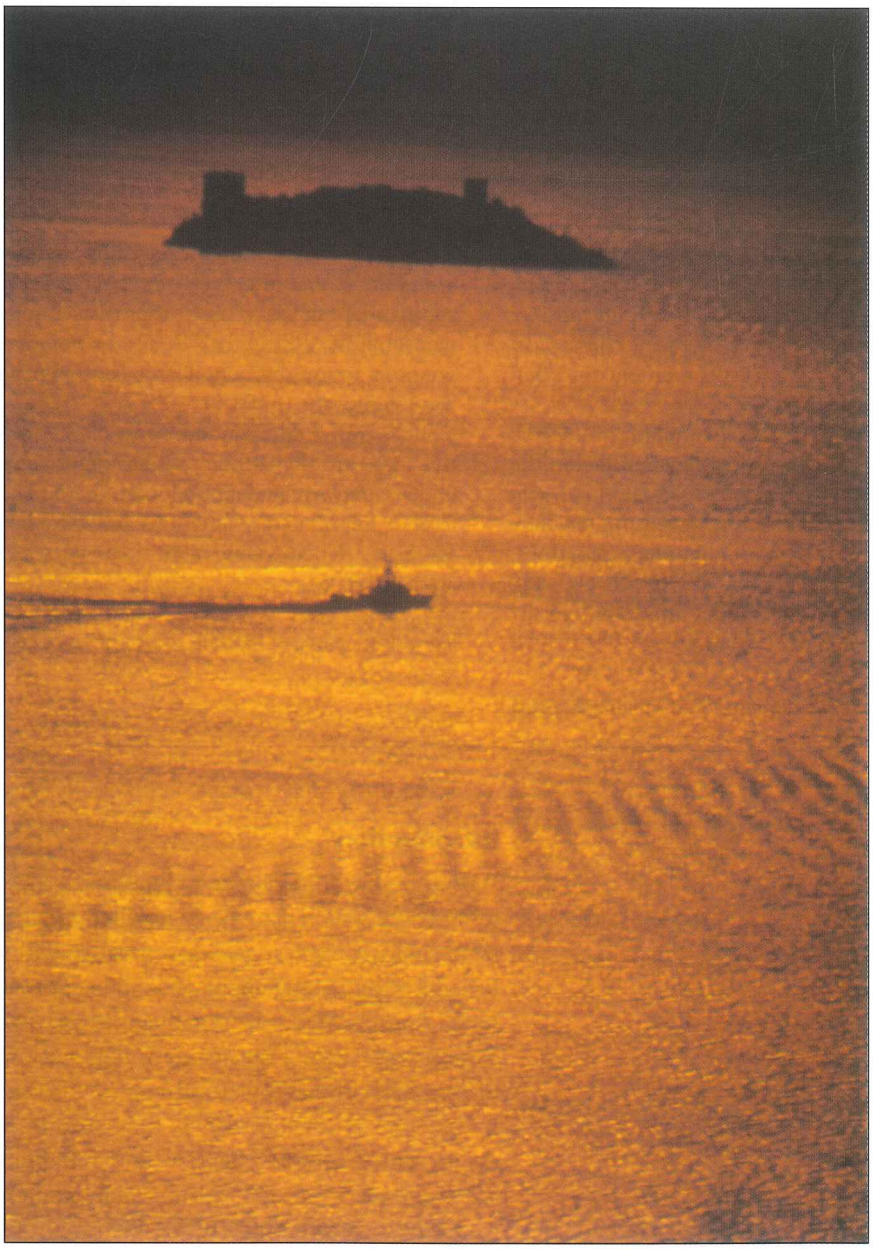

A view from Büyükada; Photography by Kamil Levent Arslan, MD