JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2019; 3:63-68
https://doi.org/10.30621/jbachs.2019.580

Original investigation

Serum Levels of Trace Elements, Vitamin D and Oxidant Status in Children with Asthma

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Received: 08.01.2019; **Accepted:** 05.03.2019; **Available Online Date:** 28.05.2019

Cite this article as: Akyüz Özkan E, Göçmen AY, Küçükbağrıaçık Y, Akyüz M. Serum Levels of Trace Elements, Vitamin D and Oxidant Status in Children with Asthma. J Basic Clin Health Sci 2019; 3:63-68. https://doi.org/10.30621/jbachs.2019.580

Abstract

Purpose: The aim of this study was to detect serum levels of trace elements, vitamin D and oxidant status in asthmatic children and to understand whether these elements reduce the number of attacks or use them as supplementation.

Methods: Fifty children with asthma and 50 healthy children were included in the study. We studied serum levels of vitamin D, selenium (Se), manganese (Mn), chromium (Cr), chlorine (Cl), iron (Fe), calcium (Ca), zinc (Zn), sodium (Na), magnesium (Mg), potassium (K), copper (Cu), total antioxidant capacity (TAS), total oxidative status (TOS) and oxidative stress index (OSI) in both group.

Results: In asthmatic group, the serum level of Cr, Se, Zn and TAS levels were lower (p=0.040, p=0.000, p=0.000, p=0.000 respectively) and serum Cu, TOS and OSI levels were higher than controls (p=0.000, p=0.000, p=0.000 respectively). Serum TAS level showed negative correlation with TOS, OSI, Fe, Cu, Mn, Se, Zn and positive correlation with Se and Vitamin D.

Conclusion: The supplementation of Se, Zn and Cr may provide better control of attacks and can be use treatment of asthma in children and modulating plasma antioxidants may have a beneficial effect on asthma progression.

Keywords: Childhood asthma, trace elements, vitamin D, oxidative status

INTRODUCTION

Childhood asthma is a chronic respiratory disorder characterized by increased airway responsiveness, changeable airflow obstruction and chronic inflammation which take places mast cells, eosinophils and T lymphocytes (1). The prevalence of asthma has been increasing in recent years. This increase can be explained by the environmental factors. These factors are toxicants, infections, tobacco smoke and allergens. The toxicant sources are inhale airborne metallic particulates and take metals with food and in drinking water, contaminated dust and soil, painted surfaces (2). Also the studies showed that air pollution increases the risk of asthma and wheezing in recent years (3).

It has been shown that there is an association between decreased antioxidants intake and increased asthma prevalence (4). Trace elements and Vitamin D can affect the host immune system by antioxidant and anti-inflammatory features. The dietary changes between regions may reduce trace elements and vitamin D uptake and that may result in decreased intake of antioxidant micronutrient, therewithal increase oxidant damage and deterioration of the immune system (5).

Trace elements are found in the structure of the antioxidant enzymes. These enzymes play role as a part of the immune system and can alter the viral genome by regulating the host immune system. Poor antioxidants diets may leave the person vulnerable to reactive oxygen species. Major trace elements and vitamin D have immunomodulator effects and thus have an important effect on the course of respiratory tract infections (6).

In this study, we aimed to reveal the levels of serum vitamin D, Selenium (Se), manganese (Mn), potassium (K), sodium (Na), chromium (Cr), chlorine (Cl), iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), copper (Cu), total antioxidant capacity (TAS), total oxidative status (TOS), Oxidative Stress Index (OSI) and vitamin D in asthmatic children and healthy group. Also to assess the possible interrelation between plasma concentrations of oxidative system, trace elements and vitamin D. The second goal was to show whether these associations would be used in the follow-up and treatment of asthma.

It is important to identify additional risk factors for asthma because of the developing more effective treatment strategies can be effective in asthma control.

MATERIALS AND METHODS

In current study, asthmatic group consisted of fifty patients admitted to the Pediatric Department, between October 2015 and October 2016. The study was approved by the local ethics committee of Bozok University (Date: 06/08/2015, No: 604–500). The control group included fifty children who applied to the pediatric polyclinic. They had no lower respiratory-tract disease story, chronic disease or recurrent wheezing attacks.

The patients included in the study who had received asthma diagnosis in the past. Children who suffer from gastro-oesophageal reflux, malnutrition, cystic fibrosis, pneumonia or any other chronic illness were excluded. The presences of diseases or complaints out of asthma, during acute asthma attack, history of febril episode or acute illness in the preceding 7 days, mild asthma and individuals who take supplementation of trace elements or vitamin D were also excluded.

Venous blood samples were collected after 12-h fasting between 8:00 and 10:00 a. m. Serum samples were separated after centrifugation for 10 minutes and kept in −80°C until the day of analysis.

Serum trace elements concentrations were measured by an atomic absorption flame emission spectrophotometer (Agilent Technologies 240Z AA, GTA-120 Graphite Tube Atomizer).

25 (OH) Vit D was analyzed using an enzyme linked immunosorbent assay (ELISA) kit (Immunodiagnostic System Ltd., Boldon Business Park, Boldon, Tyne and Wear, UK) based on the manufacturer's instructions and values expressed in ng/ml.

Serum TOS and TAS were determined with kits (Rel Assay Diagnostics kit; Mega Tip, Gaziantep, Turkey) developed by Erel and Oxidative Stress Index (OSI) values were calculated.

Measurements of TOS and TAS were performed on microplate reader Multiscan GO (Thermo Scientific, USA).

The serum TAS value measured by the generation of 2.2′-azino-di-(3-ethylbenzthiazoline sulphonate) (ATBS) radical cation with the commercial kit TAS in accordance with the manufacturer's manual.

TOS was measured as described by manufacturer's protocol. In current method, the iron ion-o-dianisidine complex is oxidized to the ferric ion with the oxidants present in the sample. Ferric ion forms a colored complex with xyleneol orange in the acidic environment. The measurable spectrophotometrically color intensity is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results expressed as μmol H2O2 equivalents/L serum.

The TOS: TAS ratio was identified as the OSI, and was calculated as: OSI (arbitrary units)=[(TOS, μmol H2O2/L)/(TAS, mmol Trolox equiv. /L)].

Statistical Analysis

The mean values obtained in the both groups were compared by the unpaired Student's *t*-test and chi-square test. All statistical analyses were done with the program Statistical Package for the Social Sciences (SPSS) for Windows (version 18.0). Bivariate associations of the variables were assessed using Pearson's correlation coefficients. Multivariate linear regression analysis was performed to correlated parameters with TAS and *P* value <0.05 was accepted as a sign of statistical significance.

RESULTS

The study was conducted with 100 children. There were 50 children in the asthmatic group (mean age 7.86±3.43; 24 boys and 26 girls) and 50 children in healthy group (mean age: 8.08±4.21; 22 boys and 28 girls). There was no difference between the groups in terms of age and gender. The clinical characteristics of asthmatic and healthy children were showed in Table 1.

Table 1. Comparison (mean ± SD) of clinical characteristics of asthmatic and healthy children

Variable	Asthmatic children	Healthy controls	p value
Age (years)	7.86±3.43	8.08±4.21	0.220[a]
Body height (cm)	122.8±9.2	123.5±10.4	0.110[a]
Body weight (kg)	20.6±8.5	21.6±9.5	0.280[a]
Males/Females	24/26	22/28	0.230[b]

[a] student's t test.
[b] Chi-square test.

Table 2. Comparison (mean ± SD) of serum level of vitamin D, trace elements, TAS (Total antioxidant status), TOS (Total oxidant status), OSI (Oxidative Stress Index) in children with asthma and healthy controls

Variable	Asthmatic children	Healthy controls	p value
Ca (mg/mL)	9.30±0.96	9.44±1.09	0.480
Cl (mmol/L)	103.80±3.20	102.40±4.73	0.082
Fe (μg/dL)	82.74±24.41	87.40±21.30	0.306
Na (mmol/L)	143.45±6.12	142.61±6.16	0.112
K (mmol/L)	3.92±0.31	3.90±0.50	0.791
Mg (mg/dL)	1.97±0.27	2.06±0.34	0.156
Cr (μg/dL)	0.84±0.35	1.00±0.40	0.040
Cu (μg/dL)	68.51±10.86	59.26±7.78	0.000
Mn (μg/L)	1.61±0.31	1.64±0.30	0.686
Se (μg/L)	121.50±8.89	141.40±15.36	0.000
Zn (μg/dL)	88.28±10.22	123.08±35.91	0.000
Vit D (ng/mL)	26.59±13.22	30.99±14.28	0.110
TAS	2.17±0.47	2.62±0.45	0.000
TOS	1.44±0.27	1.06±0.16	0.000
OSI	0.70±0.21	0.41±0.09	0.000

Selenium (Se), manganese (Mn), chromium (Cr), iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl) and copper (Cu), TAS (Total antioxidant status), TOS (Total oxidant status), OSI (Oxidative Stress Index)

Table 3. Pearson's correlations between TAS (Total antioxidant status), TOS (Total oxidant status), OSI (Oxidative Stress Index), serum trace elements and vitamin D in asthmatic children

	TAS	TOS	OSI	Ca	Cl	Fe	K	Mg	Na	Cr	Cu	Mn	Se	Zn
TOS	-0.021													
OSI	-0.784**	0.608**												
Ca	0.026	0.010	-0.042											
Cl	-0.099	0.073	0.103	0.152										
Fe	-0.441**	-0.063	0.335*	0.186	0.018									
K	-0.105	-0.092	0.008	-0.001	0.185	0.203								
Mg	-0.566**	0.256	0.562**	0.287*	0.117	0.000	-0.001							
Na	-0.111	0.058	0.162	-0.046	0.151	-0.093	0.020	0.011						
Cr	-0.737**	0.141	0.657**	0.309*	0.075	0.666**	0.148	0.738**	-0.031					
Cu	-0.822**	0.017	0.676**	0.183	0.076	0.825**	0.144	0.395**	0.031	0.856**				
Mn	-0.827**	0.163	0.754**	-0.179	0.006	0.492**	0.116	0.691**	0.036	0.865**	0.777**			
Se	0.638**	-0.040	-0.537**	0.670**	0.015	0.005	0.023	-0.180	-0.145	-0.167	-0.373**	-0.584**		
Zn	0.174	0.178	-0.071	0.540**	0.053	-0.038	0.042	0.570**	-0.140	0.364**	-0.118	0.077	0.643**	
Vit D	0.909**	-0.065	-0.720**	-0.195	-0.140	-0.463**	-0.131	-0.704**	-0.012	-0.842**	-0.811**	-0.813**	0.434**	-0.092

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Selenium (Se), manganese (Mn), chromium (Cr), iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl) and copper (Cu), Total antioxidant status (TAS), Total oxidant status (TOS), Oxidative Stress Index (OSI).

Table 4. Multiple linear regression analysis of total antioxidant status (TAS) as dependent variable in asthmatic children

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	7.945	3.003		2.646	0.011
OSI	-0.113	0.150	-0.052	-0.754	0.455
Fe	0.012	0.006	0.632	2.084	0.043*
Mg	0.317	0.485	0.188	0.655	0.516
Cr	1.452	0.835	1.093	1.738	0.089
Cu	-0.052	0.015	-1.199	-3.430	0.001*
Mn	-1.506	0.599	-1.018	-2.516	0.016*
Se	-0.026	0.015	-0.491	-1.774	0.083
Vit D	0.022	0.004	0.629	5.974	0.000*

Dependent Variable: total antioxidant status **(**TAS) *Correlation is significant at the 0.05 level (2-tailed). Selenium (Se), manganese (Mn), chromium (Cr), iron (Fe), magnesium (Mg), copper (Cu), Oxidative Stress Index (OSI)

In asthmatic group, the serum level of Cr, Se and Zn were lower than healthy group and these parameters were found to be statistically significant (p=0.040, p=0.000, p=0.000, respectively). The serum level of Cr was 0.84±0.35, Se was 121.50±8.89, Zn was 88.28±10.22 in asthmatic group, while the serum level of Cr was 1.00±0.40, Se was 141.40±15.36 and Zn was 123.08±35.91 in control group. Serum Cu level was higher in asthmatic group than controls (68.51±10.86, 59.26±7.78, p=0.000).

In asthmatic group serum TAS level was lower (2.17±0.47, 2.62±0.45), serum TOS (1.44±0.27, 1.06±0.16) and OSI (0.70±0.21, 0.41±0.09) was higher and statically significant (p=0.000, p=0.000, p=0.000, respectively) (Table 2).

In asthmatic group, 25 (OH) Vit D level was lower than healthy group but did not show any statistically significant difference among two groups (p=0.110).

Serum TAS level showed negative correlation with TOS, OSI, Fe, Cu, Mn, Se, Zn and positive correlation with Se and Vitamin D (Table 3).

Multivariate regression analyse was performed to correlated parameters with TAS. TAS was chosen as dependent variable TOS, OSI, Fe, Cu, Mn, Se, Zn and Vitamin D were independent variables in the regression analysis. Fe, Cu, Mn and Vitamin D were found as independent factors on TAS (Table 4).

DISCUSSION

It has been shown that the prevalence of asthma has increased in recent years. This increase can be explained by some theories: western life-style, changing environmental factors and eating habits (7). It has also found that there is a positive relationship between increased incidence of asthma and decreased intake of antioxidants (4).

In current study, we aimed to investigate the association between vitamin D, trace elements and TAS, TOS, OSI in asthmatic children. We found a significant difference between the asthmatic group and controls in terms of serum level of Se, Cu, Cr, Zn, TAS, TOS and

OSI. The level of serum Se, Cr, Zn and TAS were lower, whereas serum Cu, TOS and OSI were higher in asthmatic group.

It is suggested that decreasing in antioxidant intake in the diet makes pulmonary airways vulnerable to reactive oxygen, so increasing incidence of asthma in recent years can be explained with this theory (5).

There was a direct correlation between TAS and Se, vitamin D level and an inverse relationship with OSI, Mg, Cr, Fe, Cu and Mn. When regression analysis is performed vitamin D, Fe, Cu, Mn were independent predictors of TAS.

An oxidant-antioxidant imbalance in asthma and resulting inflammation can manifest in different shapes. It may be caused by increase or decrease of oxidative stress, depending on whether the changes are caused by a defense response (increase) or neutralization with oxidants (reduction) (6). There may be no change if the reserves are sufficient. In our study, we found low level of TAS and high level of TOS in asthmatic children. An absent of compensatory TAS elevation, instead of increasing oxidative stress, may be one of the possible determinants of the clinic in asthma.

There are some studies demonstrated alterations in the antioxidant activity and formation of systemic oxidative stress in asthma (7). An oxidant/antioxidant imbalance caused by altered plasma antioxidants may contribute to oxidative stress and lung inflammation.

Zn have important roles in many bodily processes; energy production, building of DNA and RNA, regulation of the immune system and cell metabolism. There are different findings about the relationship between Zn and asthma. Researches generally found low plasma Zn concentrations in asthmatics similar to our trial and thus they suggested that children with asthma are at risk of zinc deficiency (5, 8). However, Malo et al (9) demonstrated that Zn can lead to occupational asthma.

Zn and Se have antioxidant properties, also these elements deficiency changes the Th1 immune response to a Th2 type response, thus suggesting that this feature plays a role in the asthma pathophysiology (10). Zn and Se deficiency inhibit Th1 cells and stimulate Th2 type immune response and normal Th1: Th2 ratio becomes unbalanced. Also Se and Zn strengthen Th1 immunity response against viral infections (11). Asthma and Zn deficiency are both associated with an upregulation of the production and release of various pro-inflammatory cytokines and skewing toward the pro-inflammatory Th2 response, asthmatics with Zn deficiency are likely to have increased inflammation (10). There is an imbalance between Th1 and Th2 functions in Zn deficiency and results in a decrease in the production of IFN-gamma, TNF-alpha and IL-2 (12). Due to these effects of Zn on the immune system, individuals with Zn deficiency are more susceptible to respiratory tract infections. We showed in line with the literature that, asthmatic children had low serum Zn levels and Zn deficiency may play a role in the asthma pathogenesis and respiratory viral infections. So supplementation of Zn and Se may lead to better control of asthma during childhood.

Several studies reported lower blood levels of Se in asthmatic patients in agreement with our study (13). Se supplementation was observed to improve clinical symptoms in asthma patients (14). In another study it is found that serum Se levels were lower in wheezy children and there was a negative correlation between the number of wheezy attacks and serum Se levels (13).

We found increased Cu concentrations in childhood asthma. Cu may effect antioxidant status by its presence in sodium dismutase and thus lung function in theory (15). Some studies demonstrated that higher levels of Cu concentration in children with bronchial asthma and recurrent wheezing similar to our study (8). However, in a study conducted to Nigerian children showed lower plasma Cu levels in asthmatic patients (16).

Lower Zn levels and higher Cu levels in asthmatic children are reported mostly in the literature (5, 10). Uysalol et al (17) reported that serum Cu, Cu/Zn ratio was significantly higher and vitamin D and Zn levels were significantly lower in patients with recurrent wheezing. El-Kholy et al (8) assessed serum Zn and Cu levels in atopic dermatitis patients, bronchial asthma and healthy controls and found that Zn levels were lower and Cu levels were higher than control group. They suggested that Zn deficiency worsened allergic diseases and Zn administration improved symptoms. It is shown in several trials that, Zn level was significantly lower in children with recurrent wheezing compared with healthy children and this lower Zn level could be a risk factor for wheezing in infants (12).

Fe is an essential element and has several crucial functions in the human body. Deficiencies and excesses of this mineral are both recognized as important health problems. Although Fe is an essential nutritional element for all life-forms, Fe overload may cause various diseases (17). It has been suggested that high body stores of Fe is associated with increased risks of asthma (18). We could not find any difference in Fe between the two groups in this study.

Mn is a trace element that acts as a cofactor in most enzymes and has antioxidant activity. Southar et al (5) reported that bronchial reactivity is inversely related to the intake of Mn in the diet. They have also been found that low intake of Mn and C vitamin was associated with five-fold increase in bronchial reactivity. Koçyiğit et al (19) found lower levels of serum Mn and higher Fe levels in asthmatic patients than controls. We found lower serum level of Mn in asthmatic group but it was not statically significant.

Vitamin D affects the immune system through its anti-inflammatory and antioxidant properties. It may affect the course of respiratory tract infections since it has immune modulator effect and thus the incidence of wheezing can increase and led to asthma attacks (20). There was no difference between healthy children and recurrent wheezy children in some studies in terms of serum vitamin D (21). In another study it is found

that the serum level of Vit D was significantly lower in patients with asthma (22). Although in current study Vitamin D level was lower in asthmatic group and it was not statically significant, there was a significant positive correlation between the TAS and vitamin D levels in asthmatic children. There are some studies suggested that 25 (OH) D3 have a positive impact on chronic asthma and have a protective effect from recurrent respiratory infections in asthmatic children, supporting our results (23). It is suggested that 25 (OH) D3 plays a role in antioxidant production and thus may be an effect on fighting oxidative stress, produced as a result of asthmatic inflammation (24). Factors that increase antioxidant production capacity can remove the harmful effects of inflammation in asthma (25). Some studies found that low Vitamin D levels were found to be related with the exacerbation of asthma in adolescents (25).

In vitro and in vivo exposure to Cr can lead to lung tissue inflammation and release of inflammatory cytokines. It has been also shown that, Cr inhalation forms increase the severity of allergic asthma in rats (26). In a study conducted Huang et al (27) suggested that there was a positive correlation between prevalence of adult asthma and urinary Cr, Cu and Se and negative correlation was found with Mn, Fe, Zn levels. Previous epidemiological studies have reported a relationship between asthma risk and Cr and cadmium exposure (28). In current study serum level of Cr was lower in asthmatic patients than controls.

In conclusion, our study showed that children with asthma had low serum levels of total antioxidant capacity, selenium, zinc, chromium and high level of copper, total oxidative status and oxidative stress index. We suggested that supplementation of selenium, zinc and chromium may provide better control of attacks and can be use in the treatment; and modulating plasma antioxidants may also have a beneficial effect on progression of asthma.

Informed Consent: Written informed consent was obtained from patient who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - EAÖ, AYG; Design - EAÖ; Supervision - EAÖ; Materials - AYG, YK; Data Collection and/or Processing - YK, MA; Analysis and/or Interpretation - EAÖ; Literature Search - EAÖ, MA; Writing Manuscript - EAÖ, YK; Critical Review - EAÖ, AYG, YK, MA

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was supported by Bozok University Scientific Research Projects Coordination Unit (Project Number: 6602b-tf/16-44).

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