Original article

Serum levels of lead and copper in a group of Egyptian children with bronchial asthma

Background: *Copper and lead are trace elements required for the activity of antioxidant enzymes and changes in their levels may lead to reduction in antioxidant activities in asthma.*
Objective: *Our study aims to investigate the serum levels of copper and lead in asthmatic children in correlation to disease severity to anticipate their role as oxidant defenders in this disease.*
Methods: *We enrolled 45 children who were divided into two groups: group 1 included 30 asthmatic children during disease quiescence and group 2 included 15 clinically healthy children matched for age and sex as a control group. Patients were subjected to: history taking, clinical examination, spirometry before and after bronchodilator therapy, complete blood counting, and measurement of serum levels of total IgE, copper and lead (in patients and controls).*
Results: *Patients' group had significantly higher serum levels of lead (mean 8.2±3.1 µg/dl) and copper (122±31.5 µg/dl) in comparison to controls (mean 5.7±2.3 µg/dl and103.3±21.1 µg/dl respectively). Serum lead and copper levels were higher among patients with moderate persistent asthma than those with mild asthma. Serum total IgE levels correlated positively with serum lead levels among the asthmatic children. However, serum lead and copper levels did not correlate with any of the measured pulmonary function parameters tested.*
Conclusion: *Increased serum level of lead and copper were high in a group of children with bronchial asthma in children and this was more evident in moderate than mild cases.*

Keywords: children; trace elements; lead; copper; spirometry; bronchial asthma; antioxidants; severity.

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INTRODUCTION

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation.[1] Trace elements are essential micronutrients that exist in very low concentrations in the body, forming less than 0.01% of the total body weight. They play an important role in various physiological processes, and are crucial for proper functioning of the immune system.

Deficiency of trace elements and infectious diseases are often concomitantly observed and result in complex interactions.[2] This is mainly due to the fact that these elements are part of the structure of antioxidant enzymes. These enzymes act as antioxidant defense and are able to regulate the host immune system, and alter viral genome. There is increasing evidence that reactive oxygen species can be of particular importance in the pathophysiology of several lung diseases.[3] The antioxidant mechanisms that protect the lung against these oxidants include: superoxide dismutases (SODs) and Glutathione peroxidase (GSH-Px). GSH-Px has selenium component and the super oxide dismutases have copper and zinc components in their structure. Changes in the level of these trace elements decrease the efficiency of antioxidant systems and this leads to hyper-reactivity and inflammation in the respiratory tract.[4] The epidemiology of pediatric asthma and that of lead poisoning are strikingly similar. Published analyses suggest that lead exposure may result in alterations to immune system components known to be associated with asthma. Blood lead levels ≥0.48 pmol/L (10 microg/ dL) has been associated with the increased production of total immunoglobulin E (IgE), which is also observed in atopic and nonatopic individuals with asthma.[5]

All the mechanisms mentioned above suggest that copper and zinc are important elements in oxidant/antioxidant path ways and therefore have a critical role in patients with BA. A number of studies reported have shown the association and or correlation between serum trace elements and asthma disease.[6] With this as a background, we aimed to investigate the serum levels of copper and lead in asthmatic children in correlation to disease severity to anticipate their role as oxidant defenders in this disease.

METHODS

This is a cross sectional controlled study that was conducted at the specialized Pediatric Chest Clinics of Benha and Zagazig University Hospitals during the period from March 2014 to June 2014. Two groups of subjects were enrolled; group 1 included 30 children above 5 years of age having physician diagnosed bronchial asthma during disease quiescence.

The diagnosis was verified according to the Global Initiative for Asthma (GINA) Guidelines for Asthma Severity and Control[7] after consideration of the exclusion criteria which include receiving systemic corticosteroid treatment during the last month; chronic illness involving liver, kidney disease or thyroid dysfunction; and uncontrolled asthma. Group 2 included 15 clinically healthy children without personal or family history of asthma or other allergic conditions. The study gained approval of local ethical committee of the Pediatric Department, Benha University. Informed written consent was obtained from the parents or care givers of enrolled children after explanation of the study.

Methods:

Enrolled patients were subjected to full history taking with special attention to intermittent attacks of cough, expectoration, wheezy dyspnea and chest tightness. Detailed physical examination was performed including tachypnea, signs of hyperinflation, prolonged expiratory phase and expiratory rhonchi. Evaluation of lung functions by Spirometry (performed by Erich jaejre 95 GmbH 1992-1997 for measurement of pulmonary function) were performed before and after bronchodilator (administration of four separate puffs at 30-second intervals of the short acting B2-agonist salbutamol (total of 400 mcg) using a spacer device) [8] with measuring the following indices: Forced vital capacity (FVC), forced expiratory volume in 1st second (FEV1) and FEV1/FVC and post bronchodilator change in FEV1 which are displayed automatically by the apparatus.

Laboratory investigations were done for all enrolled subjects including complete blood count analyzed by sysmex Kx-21N with microscopic manual differential count, total serum IgE level measurement by ELISA (DiaMed Eurogen, Turnhout, Belgium) and determination of serum levels of copper and lead using Perkin-Elmer 2380, USA atomic absorption spectrophotometer.

Statistical analysis:

Data were tabulated, coded then analyzed using the computer program SPSS (statistical package for social science) version 16. Quantitative data were presented as mean ± SD. Student t-test was used to compare between two groups. Pearson's correlation coefficient was used to test correlation between variables and chi-square test was used to compare frequency of qualitative variables among the different groups. For all analyses, the level of significance was set at $p < 0.05$.

RESULTS

The current study included 30 asthmatic children and 15 matched healthy children as a control group. Their clinical and demographic data are shown in table (1). Patients pulmonary function parameters are depicted in table (2). Concerning grades of asthma severity; there were 12 cases (40%) with mild persistent asthma, 15 cases (50%) with moderate persistent asthma and 3 cases (10%) with intermittent asthma. In comparison to controls, patients had significantly higher serum total IgE and hemoglobin levels and higher eosinophils count (table 3). Patients' group showed significantly higher serum levels of lead and copper in comparison to controls (table 4). Among enrolled patients, serum lead and copper levels were higher among those with moderate persistent asthma in comparison to patients with mild asthma (table 6). However, serum lead and copper levels did not correlate with any of the measured pulmonary function parameters tested (table 7).

Serum total IgE levels correlated positively with serum lead levels among enrolled patients (table 5).

Table 1. Epidemiological and clinical data among patients and controls.

Variable		Patients (No.=30)		Controls (No.=15)	
Age (years)	mean± SD; (range)	7.8±1.3; (5.5-11)		7.7±1.1; (6-10)	
Sex	Female	13	43.3%	7	46.7%
	Male	17	56.7%	8	53.3%
Consanguinity	No	23	76.7%	10	66.7%
	Yes	7	23.3%	5	33.3%
History of allergy	No history of allergy	15	50.0%	15	100.0%
	Allergic conjunctivitis	4	13.3%	0	0.0%
	Allergic rhinitis	8	26.7%	0	0.0%
	Atopic dermatitis (eczema)	3	10.0%	0	0.0%

Table 2. Pulmonary function test results among enrolled patients.

Variable (No.=30)	Mean± SD	Range
FEV1%	76.9±8.1	60-90
FVC%	81.4±9.9	60-98
FEV1/FVC	82.8±4.8	76-90
Percentage of change in FEV1 post bronchodilator	15± 2.3	13-20

FEV1%= forced expiratory volume in the first second percent predicted, FVC%= forced vital capacity percent predicted

Table 3. Serum total IgE, HB, and eosinophil counts in the studied sample.

Variable	Patients (No.=30)		Controls (No.=15)		Test	p -value
	mean± SD	Range	mean± SD	Range		
IgE (IU/ml)	471.2±484.8	32-1920	86.5±44.4	44-176	z= 3.49	<0.001 (HS)
Eosinophils count (cells per microliter)	320±170	100-570	112±25	65-126	z= 4.79	<0.001 (HS)
HB (g/dl)	11.7±1.0	9.6-13.7	10.8±1.0	9.2-12.5	t=2.80	0.008 (S)

IgE = immunoglobulin E, HB=hemoglobin

Table 4. Serum lead and copper levels in the studied sample.

Variable	Patients (No.=30)		Controls (No.=15)		t-test	p-value
	Mean ± SD	Range	Mean ± SD	Range		
Serum lead (µg/dl)	8.2±3.1	2.3-12	5.7±2.3	2.1-9.6	2.75	0.01 (S)
Serum copper (µg/dl)	122±31.5	73-165	103.3±21.1	78-138	2.16	0.04 (S)

Table 5. Correlation between serum lead and copper levels and other laboratory parameters in the patients' group.

Variable (No.=30)	Lead		Copper	
	r	p	r	p
IgE (Iu/ml)	0.40	0.03	(-) 0.01	0.94
Eosinophils (cells/mcL).	0.45	0.03	0.09	0.58
HB (g/dl)	0.06	0.73	(-) 0.23	0.21

IgE: immunoglobulin E, HB: hemoglobin

Table 6. Variation of serum lead and copper levels according to asthma severity grades.

Variable (No.=30)		Serum lead (µg/dl)			Serum copper (µg/dl)		
		mean± SD	F	P	mean± SD	F	P
Asthma severity	Mild persistent (n=12)	7.99±3.36	3.73	0.04	116.2±30.5	3.45	0.046
	Moderate persistent (n=15)	9.34± 2.42			128.1±31.8		
	Intermittent(n=3)	5.27±2.89			123.3±41.9		

Table 7. Correlation between serum lead and copper levels and pulmonary function test parameters among the asthmatic children.

Variable (No.=30)	Lead		Copper	
	r	p	r	p
FEV1% (pre-bronchodilator)	0.08	0.67	0.05	0.78
FVC %(pre-bronchodilator)	(-) 0.02	0.93	0.05	0.79
FEV1/FVC (pre-bronchodilator)	0.10	0.61	(-) 0.09	0.62
FEV1% (post-bronchodilator) *	(-) 0.07	0.74	(-) 0.11	0.63

FEV1%: forced expiratory volume in the first second percent predicted, FVC%: forced vital capacity percent predicted

DISCUSSION

Different studies have suggested that trace elements such as lead, zinc and copper might be involved in acute and chronic inflammatory diseases such as bronchial asthma. Furthermore, zinc and copper are required for optimal activity of the immune system.[9] We sought to investigate serum copper and lead levels among children with bronchial asthma in comparison to matched healthy controls to investigate if these elements are related to asthma in the pediatric age group. In our study, eosinophils count was significantly higher among patients in comparison to control group. This result is supported by study of Fahy, who found that increased numbers of eosinophils in peripheral blood and in airway secretion are a characteristic feature of asthma. Moreover, patients with more severe asthma differ from patients with mild asthma in having more eosinophils in their peripheral blood and in their airways.[10] In contrast, Wenzel et al, have pointed out that not all patients with severe asthma have airway eosinophilia.[11]

The current study revealed significant increase in the total serum IgE levels in asthmatic patients compared to control group. In a study done by Kovac et al, they reported that asthmatic children with higher asthma severity have higher serum concentration of both total IgE (>288.0kIU/L) and specific IgE to Dermatophagoides pteronyssinus (>44.1 kIUA/L).[12]

In the present study, hemoglobin level was significantly lower among studied asthmatic children when compared to control group. This was in agreement with Ramakrishnan and Borade, who found that anemic children are 5.75 times more susceptible to develop childhood asthma compared to the non-anemic children.[13] This may be explained by that in anemic persons tidal volume is decreased while reserve volumes increase or remain unchanged, however total lung capacity markedly decreases in these subjects suffering from anemia. Weakness of accessory muscles of respiration adds to a decrease in Peak expiratory flow rate, forced expiratory volume in one second (FEV1). Maximum voluntary ventilation is markedly reduced due to the decreased depth of respiration as well. Anemia leads to decreased pulmonary functions which further hampers the oxygenation of the tissue and may worsen the physical and mental capabilities.[14] Serum lead and copper levels were significantly higher among enrolled patients in comparison to controls; however the mean value of lead level (8.2±3.1µg/dl) was below the toxic level (toxic levels are those above 10 µg/dl).[15] These results probably reflect that high lead level might be a result rather than an etiopathogenic factor in asthma. It may also represent one of potential risk factors for persistent bronchial asthma. Increased blood lead level can damage the structure of bronchial mucosa, which causes chronic inflammation and bronchial hyper responsiveness; at the same time, a wide range of toxic effects of lead can affect the function of the body defense system, in particular, inhibiting the T lymphocyte functions, which lead to imbalance between Th1 cell and Th2 cell. Increased blood Lead can also increase the level of serum IL-4 levels and serum IgE levels, which lead to the increased incidence of bronchial asthma.[6] Joseph et al, reported that among African Americans, blood lead level (BLL) ≥ 5 and BLL ≥ 10 µg/dL were not associated with asthma. The association of BLL ≥ 5 µg/dL with asthma among Caucasians was slightly elevated, but not significant [adjusted hazard ratio = 1.4; 95% confidence interval (CI), 0.7–2.9; p =0.40] and they

stated that the racial differences observed illustrate the need for further exploration of the role of race in the interrelationships between genetic susceptibility, socio environmental exposures, and risk of asthma. Lead exposure may be associated with excessive production of immunoglobulin E, possibly increasing asthma risk and contributing to racial disparities.[16] On the other hand, a previous study reported that there was no significant association between lead poisoning even in blood lead level 25 μg/dl and asthma diagnosis or asthma symptoms.[17] El-Kholy et al, studied Zinc and copper status in children with bronchial asthma and atopic dermatitis and found a significant increase in serum and hairs copper in both allergic groups compared to the controls. Mean serum and hair copper values were 79.5 ± 8.06 μg/100 ml and 18.7 ± 1.9 μg/gm respectively in asthmatic cases, and 81.4 ± 8.4 μg/100 ml and 17.8 ± 2.08 μg/gm in cases with atopic dermatitis. The control mean serum and hair concentrations were 67.95 ± 6.37 μg/100 ml and 14.5 ±2.53 μg/gm respectively.[18]

The current study showed that among studied patients, total serum IgE levels and eosinophils percentage correlated significantly with serum lead levels but not serum copper levels. It has been proposed that lead acts to increase production of IgE through direct or indirect stimulation of B-cells or through the binding and subsequent alteration of allergens that stimulate the allergenic immune response.[19] In previous studies, lead has shown a positive correlation with IgE levels by acting upon IL-4 synthesis (cytokine controlling immuno-globulin class switch to IgE production) and leading to an up- regulation of IgE in B cells.[5] Mohammed et al, found that elevated blood lead levels among patients with asthma was associated with higher frequency of eosinophilia and increased total IgE.[20]

In the current study there was no statistically significant correlation between hemoglobin and serum lead levels. The Environmental Protection Agency has suggested a threshold lead level of 20–40 μg/dl for a decrease in hemoglobin in children.[21] Our study does not contradict their report as all of our studied children had serum lead level not exceeding 20 μg/dl.

In the present study there was significant difference between different asthma grades regarding serum lead and copper levels, however there was no significant correlation between serum lead and copper levels and pulmonary function test parameters. Mohammed et al, found that elevated blood lead levels among patients with asthma was associated with increase in the severity of asthma compared to the patients with asthma with blood lead level <10μg/dL and several authors concluded that although elevated blood lead level had no significant correlation with asthma diagnosis, it was significantly associated with increased asthma severity.[20]

Our results did not show clear relation between serum lead levels and pulmonary function test parameters, mostly due to the fact that most of our enrolled patients' blood lead levels were below10 μg/dl so the effect was not clear. Larger study samples and inclusion of patients with severe persistent or uncontrolled asthma might show more prominent effect of lead and copper levels on asthma disease severity and control and their impact on the required inhaled steroid dose.

CONCLUSION

Increased serum levels of lead and copper may be associated with bronchial asthma in children and might correlate with disease severity. The conclusions are limited by the sample size especially of the control group. The lack of local reference levels is another limiting factor. Further wider scale studies are needed for better assessment of these trace elements' status in childhood asthma and for understanding their possible role in asthma pathogenesis.

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