SERUM LEAD, COPPER AND ZINC IN CHILDREN WITH BRONCHIAL ASTHMA

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ABSTRACT

Background: Bronchial asthma is chronic inflammatory of the lung airways resulting in episodic airflow obstruction. Lead exposure results in alteration of immune system function resulting in asthma. Zinc, copper are component of antioxidant system and alteration of their level affect increase risk of asthma.

Objective:. Determine the serum level of lead, zinc, copper in children with bronchial asthma and study their relation to development of asthma

Patients and methods: The patient group included 38 full-term newborn infants diagnosed as having bronchial asthma who were selected from pediatric department Benha Teaching Hospital. The control group included 17 healthy child age and sex matched C-reactive protein (CRP) were measured by the ELISA technique (highly sensitive CRP). ESR was performed by the Wintrobe method, Serum lead was determined by atomic absorption method. Serum copper and zinc were determined by calorimetric method.

Results: Serum copper and zinc were elevated in asthmatic children compared to controls with no relation to clinical manifestations or laboratory parameters .Serum lead showed no difference among patients and controls however it increased in severe cases compared moderate and mild cases and correlated positively with duration of admission.

Conclusion:. : We determined serum copper, zinc and lead in children with bronchial asthma. We found elevated serum copper and zinc in asthmatic children compared to controls with no relation to clinical manifestations or laboratory parameters .Serum lead showed no difference among patients and controls, however it increased in severe cases compared moderate and mild cases and correlated positively with duration of admission. These results indicate importance of environmental exposure as a risk factor of asthma in children

Keywords: Bronchial asthma ,Children, Copper Zinc ,Lead

INTRODUCTION & OBJECTIVE

Asthma is a chronic inflammatory condition of the lung airways resulting in episodic airflow obstruction. Different etiological factors including genetic , environmental are involved in development of asthma (1)Epidemiological study of asthma revealed that asthma still common among lower socioeconomic classes including African Americans and Hispanics two to four times relative to Caucasians. Among possible environmental factors of asthma lead and cupper exposure. (2)

The epidemiology of pediatric asthma and that of lead poisoning are similar. Both are prevalent among minority of children, and some elements in the physical environment increase risk of disease (3).Low socioeconomic stander and residing in an urban setting are associated with increased risk for both conditions (4).

Published an analyses suggest that lead exposure may result in alterations to immune system components known to be associated with asthma (5).Lead has been associated with the increased production of total immunoglobulin E (IgE),which is also observed in atopic and non-atopic individuals with asthma (6) In experimental animals lead exposure resulted in asthma like disease (7) .

Children are at risk of chronic lead exposure because of their proximity of ground and soil and putting objects in mouth .Other possible sources, dust, and contaminated diet, breast milk of mother, contaminated drinking water, improper manufactured Chocolate, and candy (8)

There is increasing evidence that reactive oxygen species can be of particular importance in the pathophysiology of several lung diseases. Reactive oxygen species can even induce an autonomic imbalance between muscarinic receptor-mediated contraction and the beta-adrenergic-mediated relaxation of the pulmonary smooth muscles (9). Increased tissue

vulnerability to oxidant stress is likely to increase the risk of development of asthma (10).

Copper , zinc are important component of anti-oxidant system , super oxide dismutase (11) and Changes in their level decrease the efficiency of this antioxidant systems and this leads to hyper-reactivity and inflammation in the respiratory tract(12)

Copper was supposed to have anti-oxidant(13) and anti-inflammatory(14) function related and protects against chronic obstructive airway disease in animal models (15). While some reports described its higher level may be associated with development of asthma and infant wheezes possible due to environmental exposure (16&17&18), other reported low level (19)

Zinc is an essential trace element acquired by dietary means. It plays a central role in modulating the immune system and is essential for cellular function in the immune response as well as acting as an antioxidant [20]. While some reports described zinc deficiency in children with asthma (18) , others described high (19) and normal level (21)

For the possible role of these elements in pathogenesis in bronchial asthma in children, which may be reflected on management, we determined serum lead, copper, and zinc in children with bronchial asthma.

SUBJECTS&METHODS

Patients group: Thirty eight children diagnosed as bronchial asthma with age ranged from 24 - 101 months was[i] chosen from pediatric department in *Banha Teaching Hospital*. Detailed history, physical examination was done for all cases .Complete *nutritional history* was done at infant ,toddler ,preschool ,school ages. *Socio-demographic data* was obtained including: localities, housing, parent occupation, educational level, smoking. These patients were compared with 17 healthy controls.

Diagnosis of asthma based on history of recurrent acute attack of cough ,dyspnea and wheezes in child more than 2 years in response to provocation which is associated with impaired lung function " in 6ys or older " and improved with bronchodilator β agonist in addition to family history and eosinophilia. Respiratory function (FEV) was assessed in children older than 6 years in absence of other causes of wheezing (16)

Exclusion criteria cases with possible causes of wheezes other than asthma as congenital lung lesion ,reflex disease, cardiac , chest diseases other than asthma ,.Also cases with severe other systemic illness, protein energy malnutrition were excluded .

Severity of acute attack of asthma was classified as:1-*mild* : presented with mild distress only with excretion, end expiratory wheeze ,pulse 100/minute , O_2 saturation >95%. .2-*moderate*: presented with distress on rest with difficult feeding, loud whole expiratory wheezes pulse rate 100-120/minute , O_2 sat 90-95 3-*severe*: distress on rest stop feeding ,loud wheezes expiratory ,inspiratory , pulse > 120 /minute ,O_2 saturation < 90% (1)

The control group: were selected from 1ry health care center coming for routine care as immunization, complains not related to infection, infestation, allergy or chest diseases. Those with history suggest respiratory illness in last one month or any history suggest asthma or recurrent cough were excluded**.** In addition, those with family history suggest asthma or atopic diseases were excluded.

Laboratory and radiological investigations:

Laboratory investigations including CBC with peripheral smear was done, CRP was determined by ELIZA "high sensitive CRP". ESR done by Win Trobe method.

Blood sampling: Three mls of blood were taken at 24 hours of age from both cases and controls and left for 20 minutes at 37 ^{o}C. Serum was separated after centrifugation and kept at -20 ^{o}C until analysis.

Radiological investigations : chest x ray was done routinely for all cases included in the study. C. T. scan was done for cases suspected to have another cause of wheezing other than asthma .

Determination of serum lead: Serum lead was determined **by atomic** absorption spectroscopy**.**

*Principle of test***:** atomic **a**bsorption depends on that elements in ground "atomic" state absorb light at specific wavelengths. Metal ions in a solution are converted to atomic state by means of a flame. Light of the appropriate wavelength is supplied and the amount of light absorbed can be measured against a standard curve. The technique of flame atomic absorption spectroscopy (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases

Steps of measurement of lead ::a-open power and gas pressure regulator of the device b-set item to surrounding lamb, adjust wave length of element c-introduce sample for measurement d-the computer assigns element concentration in the sample .

Determination of serum Copper: Serum copper was measured by calorimetric method by kit provided Centronic GmbH (Germany).

*Principle of test***:** Copper forms with-(3,2Dibromo-2-pyridyiazo) Methyl-N-sulfa-propyl-aniline a chelate complex. The increase of absorbance of the complex can be measured and is proportional to the total copper in the sample.

Determination of serum zinc: was measured by a colorimetric method by a kit from Centronic GmbH (Germany).

Principle of test:

Zinc forms a red chelate complex with 2- (5-Brom -2- pyridyazo) -5-(-V-Propyl-N—Sulfo-prpylamino)-pbeal The increase of absorbance of the complex can be measured and is proportional to the total zinc in the sample**.**

Legal, ethical aspect: Written consents have been taken from the parents of all the children included in this study. The study also was approved from the Research Ethics Committee in General Organization for Teaching Hospital and Institutes in Cairo

Statistical Analysis: Data were analyzed using *SPSS 20 computer program (IBM, Endicott, Broome County, New York, United States)*. Data were expressed as mean ± SD for parametric data .When data are non-parametric data expressed as median ,range . Tests of significance "Chi-square and T tests" and correlation "Pearson's" study were done for parametric data where appropriate $P < 0.05$ was statistically significant. Non-parametric tests "Man-Whitney for significance, Spearman's correlation " were used when data are non-parametric

RESULTS

The study included 55 children 38 cases diagnosed as bronchial asthma, 17 healthy children taken as controls. No significant statistical differences was found between cases and controls regarding age ,sex and weight (P was 0.801, 0.949 and 0.195 respectively) **."Table 1"**

***Clinical characteristics of patients group*: "Table 2"**

Ten (26.32 %) cases had naso-pharyngitis with low-grade fever, 6 cases had naso-pharyngitis without fever. (15.79 %) None of cases or controls showed evident clinical manifestation of anemia.. Nine (23.68 %) asthmatic cases presented in mild attack, 19 (50 %) cases presented in moderate attack and 10 (26.32 %) cases presented in severe attack.

Laboratory parameters of the patient and controls: "Table 3"

Asthmatic children showed lower Hb ,Hct , RBC, (P was 0.001 ,0.008 ,0.004, respectively)

Asthmatic children showed higher ESR compared to controls (P was 0.000) .No significant differences between cases and controls regarding WBCs and platelets (P was 0.069, 0.829 respectively).

Also Asthmatic children showed higher level of serum Zinc and copper compared to controls.(P was 0.00&0.00respectively). While no difference in serum lead was found, (P was 0.457). Serum lead it was detectable in 26 of 38 cases (68.4 %) compared to 11 of 17 controls (64.7%) " P was 0.786 ".

***Clinical data relation to Zinc, Copper and Lead: "*Table 4"**

Asthmatic cases who had **fever** did not show significant differences with those without

fever regarding serum Zinc, Copper and Lead (P was 0.232, 0.882 and 0.105 respectively). Cases with naso-pharyngitis showed no significant difference with those without naso pharyngitis regarding these parameters (P was 0 .0.79, 0.782 and 0.311, respectively.)

Mild, Moderate and severe cases of asthma showed no differences regarding serum zinc, copper using one-way ANOVA (P was 0.153, 0.491 respectively).

Serum lead was significantly different among mild, moderate and severe cases of asthma using non-parametric Kruskal-Wallis test (P was 0.002). Subgroup analysis using Mann-Whitney test revealed no significant difference between mild and moderate cases (P 0.3318) while severe cases showed higher lead values compared to mild and moderate cases using the same test (P 0.0019 was 0.0061 respectively) "data not shown"

***Zinc, Copper and Lead correlation with Age ,Weight , Duration of Admission and Hematological Parameters* ."Table 5"**

Serum zinc, copper, and lead had no significant correlation with age, weight, RBCs, Hb and WBCs

Serum zinc showed significant positive correlation with platelets (P was 0.015) while copper and lead had no significant correlation.

Serum lead has significant positive correlation with duration of admission (P was 0.002), while zinc and copper has no significant correlation.

Table (1): Clinical Parameters in Patients, Controls

		Cases n=38	Controls n=17	P
Age (months)	Ẍ ±SD	39.68±17.55	38.35± 19.16	0.801 NS
	Range	24-101	24-99	
Sex	Females	16- 42.1%	7- 41.2%	0.949 NS
	Males	22- 57.9%	10- 58.8%	
Weight (kg)	Ẍ ±SD	14.64±3.48	16± 3.71	0.195 NS
	Range	10-27	12-29	

Table (2): Clinical data of the patients group

C inical data		Number	Frequencies
Naso pharyngitis	+ve with Fever	10	26.31 %
	+ve no fever	6	15.79 %
	No Nasopharyngitis	22	57.9
Fever	+ve	12	31.5%
	-ve	26	68.5%
Severity of attack	Mild	9	23.68%
	moderate	19	50 %
	Severe	10	26.32%

Table (3): Cases versus controls regarding the laboratory data

Laboratory data		Cases= 38	Controls = 17	**P**
Hb (gm/dl)	Ẍ ±SD	10.72 ±1.49	12.16±1.11	0.001
	Range	8-14	10-14	
Hct (%)	Ẍ ±SD	33.329±6.02	37.76±3.58	0.008
	Range	22-47	33-49	
RBCs ($\times 10^6$)	Ẍ ±SD	3.9±0.86	4.43±0.41	0.004
	Range	2.7-6.22	3.8-5.1	
WBCs ($\times 10^3$)	Ẍ ±SD	9.01±2.95	11.92±16.27	0.069
	Range	4-15	5-11	
Platelets ($\times 10^3$)	Ẍ ±SD	361.26±139.36	352.94±111.9	0.829
	Range	110-625	150-560	
CRP(mg/dl)		00-00	00-00	
ESR	Ẍ ±SD	20.26±6.77	9.71±2.37	0.00
	Range	10-40	5-15	
Serum Zinc	Ẍ ±SD	249.63±93.97	148.47±60.924	0.00
	Range	115-556	104-170	
Serum Copper	Ẍ ±SD	183.18±44.228	126.06±15.986	0.00
	Range	90-277	108-170	
Serum Lead "Man whitny U test" Non-parametric	Median	1.215	1.1	0.457
	Range	00-8.21	00-3.1	
	Mean rank	29.05	25.65	

Table (4) Clinical data relation to with Zinc, copper and lead

		Zinc	Copper	Lead
Fever	+ve 12	222.5±69.95	181.58±34.27	Median1.095(0-4)
	-ve 26	262.15±101.976	183.92±48.75	Median1.56(0-9)
	P	0.232 NS	0.882	0.105 NS
Acute naspharyngitis	+ve 16	254.5±112.46	185.56±44.118	Median 0.95(0-4)
	-ve 22	248.09±80.75	181.54±45.264	1.005(0-9)
	P	0.79 NS	0.782 NS	0.3117 NS
Asthma severity	Mild	210.56±49.518	188.44±37.74	Median 0(0-2.38)
	Moderate	245.16±87.504	174.74±43.292	Median 0.73(0-8.21)
	Severe	293.3±122.96	183.18±44.228	Median 3.88(0-9)
	F"ANOVA"	1.979	0.727	Kruskal-Wallis test
	P	0.153	0.491	0.002

Table (5) Zinc, Copper, and Lead correlation with Age, Weight, Duration of Admission, and Hematological Parameters

		Age	Weight	Duration of admission	**RBCs**	Hb (gm/dl)	WBCs	Platelet
Zinc "Persons"	R	0.044	0.061	0.048	0.004	0.037	0.042	0.392*
	P	0.792	0.715	0.776	0.98	0.827	0.802	0.015
Copper "Persons"	R	0.027	0.066	-0.136	-0.134	-0.066	-0.271	0.100
	P	0.872	0.694	0.334	0.424	0.694	0.10	0.552
Lead "Spearman,s"	R	0.222	0.084	0.485**	-0.194	-0.213	0.037	0.262
	P	0.181	0.617	0.002 HS	0.244	0.198	0.827	0.112

DISCUSSION

In this study, we investigated 38 children diagnosed as asthma for serum lead, copper and zinc compared to 17 age, sex and weight matched control. Zinc and copper are considered trace elements and are important component of antioxidant enzymes (22), they are also heavy metals and are possible source of environmental pollutants and toxicity (23).

Asthmatic children showed lower Hb , Htc ,RBCs and ESR compared to controls .No difference was observed regarding WBCs and platelets between both groups . Our previous reports of revealed similar results in asthmatic children. (24, 25) Association of anemia with asthmatic children can be explained on basis that asthma is associated with acute or chronic inflammation which causes anemia (26) , also anemia may be a possible risk factor of asthma in many reports (25,27,28). ESR is expected to be elevated in asthma because of associated acute and chronic inflammatory reaction (1)

Asthmatic children showed higher serum copper compared to controls .Also it showed no differences or correlations in relation to clinical or laboratory parameters among asthmatic children .While some studies in agreement with our results (18, 29 ,30), other studies showed low level of copper in asthmatics (19 ,31) .However these last two studies done in adult asthmatics . Copper contaminant now is public health problem. Children are more risky than adults for copper exposure .Beside common environmental exposure risks of children as small body size, hand mouth behavior, drinking excess water (32)., infants and children up to 10 years of age have increased susceptibility to copper toxicity because of a normally high concentration of copper in the liver during early life and the lack of a fully developed homeostatic mechanism of copper contaminant in children under 10 years of age. (33). Water and food are the main sources for copper exposure beside air and soil (34).

May studies proposed that reactive O_2 species (ROS), oxidative stresses play important role in pathogenesis of asthma and allergic diseases (35, 36, and 37). Copper oxide nanoparticles in ambient air were found to cause increased oxidative stresses in human respiratory epithelial cells (HRECs) compared to iron and silicon (38). Copper ion in ambient air induced increased ROS and pro inflammatory cytokine release (IL-6 and IL-8) in HRECs (39)

Asthmatic children showed higher serum zinc in relation to controls . Among asthmatic children serum zinc did not show any relation to clinical manifestations however serum zinc showed positive correlation with platelets with no relation to other laboratory parameters. No available explanation for positive correlation of zinc with platelets. Further studies is required to explain this correlation.

Elevated serum zinc in asthmatic children is unexpected. However, some reports support this finding. Hussein et al 2008**(19)** found elevated serum zinc in adult asthmatics. They attributed their finding to low serum copper . Another study in elderly asthmatics has the

same finding (40).One Japanese study found that asthmatic adult female has higher zinc level similar controls (41). No available study has similar finding in children. , however some reports support our finding. Ambient metals exposure , fine particle "PM 2.5" was associated with increased risk of development of asthma in children (42). Increased Ambient air zinc concentration was associated with increased asthma morbidity including emergency department "ED" visits ,/hospital admissions (42). Intra-tracheal exposure to metals particulate matter filter extracts "including zinc" was associated with acute pulmonary toxicity including asthma like pathology (44). Zinc oxide nanoparticles induced pathological changes occurs in asthma as pulmonary eosinophilia, release of IL-13, IL1 β and goblet cell hyperplasia, increased serum IgE in rat model (45).

Increased zinc level in children is not surprising. Zinc is not only trace element but also considered as heavy metal and is a source of environmental pollutant as mentioned before (23)**.** Certain populations receive greater-than-average exposures to zinc from environmental sources. For example, higher levels of zinc have been reported in soil and water near waste sites, metal smelters, and areas exposed to untreated waste water, those who have galvanized plumbing in their residences are at risk of zinc contamination Children are at more prone to risk of exposure and contamination than adult. Children drink more water breath more air (46) Child behavior as playing in ground ,soil putting hand in mouth , more proximal to ground.(32). Our previous study in wheezy infant revealed no difference in zinc level between wheezy infant and healthy controls . The difference in result from current study is expected .Wheezy infants have lower age range (0-24 mo.) and possible different etiology, pathogenesis and outcome from asthmatic children (47). Older children in current study with age range 24-101 mo) are at more risk of environmental contaminants. Young infant are easily controlled in feeding and in keeping away from soil, dust exposure.

Contrarily to our results low level of zinc were found in asthmatic children (48, 49) and adults (22). Also zinc supplementation reduced airway inflammations and hyper-responsiveness to common allergen in mouse (50) and reduced asthma severity in asthmatic children (51). Normal level of zinc was found in asthmatic children by **Ghaffari** et al (52).

The explanation of contradiction in the literature about zinc level in asthmatic children has different possibilities including methods of determination, different socioeconomic classes, and environmental conditions, and effect of treatment (19). Low serum zinc levels was found in corticosteroid-treated asthmatic patients (children and adults)compared to non-treated asthmatics(53).Another possibility that zinc deficiency as well zinc excess may be a risk factor of asthma in children . As mentioned before, ROS, oxidative stresses play important role in pathogenesis of asthma and allergic diseases (35, 36, and 37). Superoxide dismutase is antioxidant enzyme contain zinc and copper in their structure. It possible that any disturbance in zinc and copper level will affect activity and function of the enzyme (18) Increased zinc level or its derivative is hazardous and toxic to lung (54, 55, and 56) and generates oxidative stresses and pulmonary eosinophilia (44).

Asthmatic children showed no differences regarding serum lead with controls. Severe , moderate and mild asthmatics showed differences in their serum level . In agreement with these finding Mohammed et al 2015 found no difference between asthmatic patients and healthy controls in serum lead while serum lead > 10 mg/dl associated with increased severity of asthma and development of eosinophilia and elevated IgE (57) , however they found higher mean values (13.3 , 11.4 µgm/dl) of lead among patients and controls compared with our study . Previous studies showed that higher blood lead has no relation with development of asthma (57, 58) .while other studies (29, 51) showed that higher lead were found in asthmatic patients compared to controls. These last 2 studies despite apparent

similar finding demonstrated variation in serum lead in both patients and controls (8.2±3.1, 5.7±2.3 µg/dl." Egyptian study " vs 5± 2.46, 3.68±1.83 µg/d "Iranian study") but still has higher values of than our study. In **Argentina** serum blood level of lead in children was found in range 1.1-3.6 µg/dL with median value 2.2 µg/dL (60) These data showed the distribution and median value near our value. It is expected to find different values of serum lead among different studies in different populations with different risk factors of exposures and local governmental precautions against exposure.

In our study no correlation between serum lead and hematological or other laboratory parameters as shown in other studies (57, 29). This can be explained because of lower values of lead in our patients compared with previous studies. Our values still considered lowest values among Egyptian studies. No apparent explanation for this difference apart from this study is not epidemiological study with small number of patients with no available study in same localities for comparison. Joseph et al 2005 (61) found that the threshold of blood lead as a risk of asthma development varies among African Americans and Caucasians . They suggested different factors including asthma phenotype, race, and genetic susceptibility interact with lead exposure for development of asthma. This can explain contradiction in previous studies in different localities and different population in this issue.

Despite our lower values of serum lead, severe asthmatic cases showed higher serum lead compared to mild and moderate cases, also serum lead correlated with admission days. Lead is not natural element in human body. So it is supposed that no threshold below which no adverse effect are observed (62, 63). It is possible that lead at certain level is not a risk factor for asthma but can increase it severity. This require further investigation with larger population study. Admission days is expected to correlate with asthma severity for longer time of management, this can explain its correlation with serum lead in asthmatic children

Some limitation in our study that we studied 3 elements in one study. At the study plan, we did not expect environmental contaminant as determinant of our results. Environmental determinants needs larger epidemiological studies including bigger sample size for each element alone with comprehensive investigation of environmental exposure. Also we included younger pediatric age randomly which is more risky for environmental exposure than older age. Larger epidemiological studies including all pediatric age with detailed social, nutritional history about environmental exposure and possible contamination is required .

Conclusion: We determined serum copper, zinc and lead in children with bronchial asthma. We found elevated serum copper and zinc in asthmatic children compared to controls with no relation to clinical manifestations or laboratory parameters .Serum lead showed no difference among patients and controls, however it increased in severe cases compared moderate and mild cases and correlated with duration of admission. These results indicate importance of environmental contaminants in development of asthma in children.

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CONFLICTS OF INTEREST

None of the authors has any conflicts of interest to declare

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