# SERUM COPPER, MAGNESIUM AND ZINC LEVEL IN WHEEZY INFANTS

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## ABSTRACT

Trace elements are micronutrient present in small amount in the body they have antioxidant and immune-regulatory function and possibly have role in pathogenesis in infant wheezes. We estimated serum level of copper .zinc magnesium in 30 wheezy infants versus 10 control Both groups were age, sex and weight matched. Serum copper, magnesium was significantly higher in patient versus control (48.6±36.8 &2.57± 0.69 respectively in cases vs. 21.7±5.7 &2.18± 0.11 respectively in control ) p was 0.00 &0.006 respectively. Serum zinc was not significantly different between patients and control (83.7±12 in cases vs 85.3±36.2 in control ) ,p was 0.832 .Serum copper was significantly lower in wheezy cases with bronchopneumonia than wheezy cases without (29.5±19.5 vs 55.5±39.5) p was 0.028 ,while zinc was higher in these cases than those cases without (108.2±34.5 vs 67.9±33,8) p was 0.034 .There was no significant difference between wheezy bronchopneumonia and non-infection cases regarding magnesium (2.97±1.2 vs 2.4±0.3) p was 0.232 .Their results prove that possible environmental zinc, copper accumulation in children may play role pathogenesis in infant wheezes ,also proves ant infection role of copper

## INTRODUCTION

Infant wheezes has different phenotypes Three of these phenotypes are transient early wheezing, non-atopic wheezing, and IgE mediated wheezing.[1] Transient wheezing in infants is not associated with a family history of asthma or allergic sensitization.[2]The primary risk factor for this phenotype seems to be reduced pulmonary function.[1,2] Other risk factors for transient wheezing include: prematurity and exposure to siblings and other children at day-care centers.[3,4] Maternal smoking during pregnancy and postnatal exposure to tobacco smoke increases the risk for transient wheezing in children younger than 3 years of age[5,6] Other risk factors for transient infantile wheezes are unknown

Epidemiological pattern of bronchial asthma recently have been changed, owing to environmental, dietary factors, and feeding practice in early life[7]The relation between trace elements level in prenatal, natal, postnatal, infancy period and development of asthma ,atopy, and infant wheezes was discussed in some literatures with non conclusive results[8;9;10]

The pattern of diet in some countries has been changed in favor of development of asthma and atopy[11] A proposed mechanism related changes in dietary antioxidant intake to reduced lung antioxidant defenses, with increased airway susceptibility to oxidant damage resulting in airway inflammation and asthma.[12] Some investigators attributed increase in incidence in atopy and wheezes to decrease antioxidants in diet[13]

Trace elements are essential micronutrients that exist in very low concentrations in the body, forming 0.01% of the total body weight [14]. 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 [15]

The major trace elements such as selenium, zinc, copper, and magnesium have Immune-modulatory effects and thus influence susceptibility and the course of a variety of infections. 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[15].There is increasing evidence that reactive oxygen species can be of particular importance in the pathophysiology of several lung diseases[16].

Zinc (Zn) is one of the dietary antioxidants[17] Zn is essential for many components of the immune system, including phagocytic function, cellular immunity ,humeral immunity, and natural killer cell function.[18]

Magnesium has a role in several enzymatic reactions, helping maintain cellular homeostasis.[19,20] .It has relaxant effect on smooth muscle in bronchial wall. Its role in asthma has not been clearly defined, but there have been studies to explain its mechanisms of action. In 1912, **Trendelenburg(21)** observed magnesium's bronchodilator effects in cows; in 1936 **Rosello and Pla** demonstrated the same on human patients. In vitro studies demonstrated the role of magnesium in the relaxation of bronchial cells. In smooth muscle, magnesium decreases intracellular calcium by blocking its entry and its release from the endoplasmic reticulum and by activating sodium-calcium pumps. Furthermore, inhibition of calcium's interaction with myosin results in muscle cell relaxation. Magnesium also stabilizes T cells and inhibits mast cell degranulation, leading to a reduction in inflammatory mediators (22)

No available reports about level in magnesium in wheezy infants or asthmatic however some reports described role of IV magnesium in refractory wheezes and asthma.

Copper is also involved in antioxidant defenses both extracellular, when bound as ceruloplasmin (23), and intracellular as a coenzyme for superoxide dismutase (24). Copper is also necessary in the diet to prevent inflammation (25) and emphysema in animal models (26), as well as reducing the adverse effects of maternal nicotine exposure on lung development in rats (27), and so may also have a protective effect against chronic obstructive pulmonary diseases (COPD).

Trace elements are not commonly monitored in health and disease Also, Some commercial formula not adequately fortified for them [28] To study their role in infant health and diseases and development of asthma, atopy, we will measure serum, zinc, selenium, copper and magnesium in different types of infant wheezes

**Patients & methods:**

About 30 infants (1mo-2ys) with different types of wheezes was taken from Banha Teaching Hospital. Detailed history including family history of asthma, antenatal maternal smoking, pregnancy complications Natal history was also discussed,. Questionnaire about frequency of wheezes, severity ,relation to cold ,smokes, irritants, presence atopy i.e. allergic rhinitis ,conjunctivitis, atopic dermatitis ,family history of asthma ,atopy Those with preterm labor , LBW, admission in NICU ,were excluded from study .Also Those with clinical suspicion of malnutrition were also excluded. Blood film for eosinophil was done to identify those with atopic wheezes Chest radiography including plain x-ray , C.T. scan was done when required to identify those with non-atopic wheezes

5 ml of blood were collected from each patient and control subject, allowed to clot at room temperature. Then serum was separated and stored at -20$^{o}$C until analysis was performed

Copper was measured by a colorimetric method by a kit from Centronic GmbH: 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.

Zinc was measured by a colorimetric method by a kit from QUIM1CA CLINICA APLICADA, S.A :In an alkaline solution, the zinc ions of the sample will produce a red color with 2-(5- Brom -2- pyridyazo) -5-[-N-Propyi-N-(3-Sulfo the zinc ions concentration present in the sample.

Magnesium was measured by a colorimetric end point method by a kit from Centronic-GmbH:In alkaline solution magnesium forms a colored complex with xylidyl blue, which is determined photo metrically . The color is proportional to the magnesium concentration.[29]

Written consent have been taken from parents of all children in this study , The study also was approved from research ethics committee in general organization for teaching hospital and institutes in Cairo

**Statistical Analysis**

Data were entered and analyzed by using spss 20.Data were expressed as mean ±SD for categorized variables .Chi-square, student-T test and correlation when appropriate P< 0.05 was statistical significant

## RESULTS

**1-Trace elements in wheezy infants**

**Table(1) demographic data**

| | | Control n=10 | Cases n=30 | p |
|---|---|---|---|---|
| Age (months) | Ẍ ±SD | 10.5±6.3 | 10.3±6.2 | 0.94 |
| | range | 3-24 | 1.5-24 | |
| sex | ♀ | 8 80% | 27 90% | 0.58 |
| | ♂ | 2 20% | 3 10% | |
| Wt.(kg) | Ẍ ±SD | 8.7±2.2 | 8.5±2.1 | 0.82 |
| | range | 5-12 | 3.7±12 | |

**2-Frequency of symptoms**

| Symptoms | Number | percentage |
|---|---|---|
| Recurrent wheezes | 14 | 46.6% |
| Bronchopneumonia | 8 | 26.7% |
| Eczema | 1 | 3.3% |
| Respiratory distress | 7 | 23.3% |

N.B. Bronchopneumonia case identified by clinical, radiological and laboratory finding. Distressed infants identified by increased respiratory rate for age and increase respiratory efforts (30)

**3--Trace éléments patients versus control**

| | | Control n=10 | Cases n=30 | p |
|---|---|---|---|---|
| Copper(ug/dL) | Ẍ ±SD | 21.7±5.7 | 48.6±36.8 | 0.000 |
| | range | 11.7-29.6 | 11.45-132 | |
| | median | 22 | 31.1 | |
| Magnesium(mg/dl) | Ẍ ±SD | 2.18± 0.11 | 2.57± 0.69 | 0. 006 |
| | range | 2 .03-2.32 | 1.39-4.44 | |
| | median | 2.15 | 2.43 | |
| zinc(ug /dL) | Ẍ ±SD | 83.7±12 | 85.3±36.2 | 0.832 |
| | ra | 63.42-97.95 | 36.5-158.24 | |
| | median | 84 | 74 | |

**4-Trace elements according to bronchopneumonia**

| | | -ve n=22 | +ve n=8 | P |
|---|---|---|---|---|
| cupper(ug/dL) | Ẍ±SD | 55.5±39.5 | 29.5±19.5 | 0.028 |
| | range | 11.45-132 | 14.41-75.5 | |
| | median | 46.65 | 22.67 | |
| magnesium(mg/dl) | Ẍ±SD | 2.4±0.3 | 2.97±1.2 | 0.232 |
| | range | 1.67-2.83 | 1.39-4.44 | |
| | median | 2.435 | 2.735 | |
| zinc(ug/dL) | Ẍ±SD | 67.9±33,8 | 108.2±34.5 | 0.034 |
| | Range | 36.5-156.51 | 70.1-158.24 | |
| | Median | 66.49 | 91.53 | |

**5-Trace elements regarding recurrent wheezes**

| | Nonrecurring(16) | Recurring(14) | |
|---|---|---|---|
| Cupper(ug/dL) | 47.9±35.7 | 49.5±39.4 | 0.9 |
| | 16.76-131.75 | 11.45-132 | |
| Magnesium(mg/dl) | 2.6±0.9 | 2.48±0.31 | 0.52 |
| | 1.39-4.44 | 1.67-2.83 | |
| zinc(ug/dL) | 94.2±36.2 | 75.1±34.7 | 0.15 |
| | 36.85-158.24 | 36.5-150.51 | |

**6-Tracec Elements In Distressed versus Non -distressed Infants**

| | Distressed (7) | Non-distressed (23) | p |
|---|---|---|---|
| Cupper(ug/dL) | 30.32±16.7 | 54.23±39.7 | 0.03 |
| Magnesium(mg/dl) | 2.4±0.33 | 2.6±0.76 | 0.46 |
| zinc(ug/dL) | 73.1±21.46 | 89±39.28 | 0.32 |

**7-Zinc versus cupper in wheezy infants**

| | zinc | r | p |
|---|---|---|---|
| cupper | | 0.338 | 0.068 |

We selected 30 patients from Banha Teaching Hospital .The mean age of patient was 10.3±6.2 months ,27 female and 3 were male ,their mean wt were 8.7±2.2kg .No significant statistical difference between patients ,control regarding age ,weight, sex(table 1)

14 of our patients had recurrent wheezes(46.6) ,8 had chest infection in the form of pneumonia(26.7%) ,one case had eczema (3.3%) table (2)

We found no significant statistical difference between between patients and control subjects regarding zinc (P was 0.832) ,However cupper and magnesium were higher in patients compared to control( P was 0.000&0.006 respectively) table (3)

Among patients group we found no significant difference between in magnesium between patients with bronchopneumonia and those without ( p 0.232). However cupper was significantly higher in those without. Zinc was higher in cases with bronchopneumonia than cases without (p was 0.028&0.034 respectively

No significant difference regarding cupper, magnesium and zinc between those with recurrent wheezes and those with non-recurrent attacks of wheezes (p was 0.9&0.52&0.15 respectively)

## Discussion

In this study we investigated cupper ,magnesium ,and zinc in wheezy infants (aged from 1-24 months).Our patients were age ,sex and weight matched with control .14 of our patients had recurrent wheezes.8 had clinical radiological sign bronchopneumonia ,one had atopic dermatitis and 7 had clinical criteria of respiratory distress We used term wheezy infants in this age rather bronchial asthma because because different risk factors contributing to wheezes in this age including atopy and considerable overlap between asthma and other types of wheezes in this age (31) .

We found significant increase in serum cupper in wheezy infants compared to control p was 0.000. Few reports described serum copper in wheezy infants and asthmatic children **Razi C.H. et al 2009**(32) studied serum heavy metals ,trace elements in children with recurrent wheezes .They found significant higher level of level of lead and mercury in wheezy children compared to control while increase in copper was not significant . The difference from our results may be attributed to method of measurements, age difference and environmental factors, and type of patients .They used ICP-MS method while we used colorimetric methods. Their age ranged from 1-6y and they had recurrent wheezes and selected from area with average socioeconomic classes in urban area. Our patient randomly selected from rural and urban and below average socioeconomic level with different exposure to different toxicants and pollutants Contrarily to our results, **Kociquit A. et al 2004**(33) found no significant differences between asthmatic children and control the difference from our study may be due to difference in patients selection and socioeconomic level **Elkoly MS et al 1990( 34) found** significant increase in serum cupper in asthmatic and atopic children No single reports described level of copper in non-recurrent wheezy infants in literature In our study we included both recurrent and non-recurrent In our study. We found little higher level of cupper in recurrent wheezes compared to those with non-recurrent cases but the rise in non-significant this results apparently different from previous study **(Elkoly MS et al 1990)** but this may be attributed small sample size in our recurrent wheezy cases Similar results recorded by **Di Tori et al 1987**(35) Contrary to our results **, Magboula et al 2008** founded significant lower level of copper in cases with bronchial asthma cases compared to control , however that study was done in adult cases(36)

Increases level of cupper in wheezy infants has different explanations Although some literatures founded lower level of copper in asthmatics and assumed that cupper is protective to lung owing to possible antioxidant activity through contribution in superoxide dismutase activity(26).Also our hypothesis that is copper is protective to lung through contribution in antioxidant system ceruloplasmin (23)superoxide dismutase( 24)and prevention of inflammation(25) and colds(26), however our result reveled opposite Little reports support our results ,one report was available in wheezy infants(32),others in asthmatic and allergic children(34,35) **Vural H. et al 2000(37**) hypothesized increase cupper decrease zinc in plasma and hypozincemia mediates allergy and inflammation through decrease antioxidant system capacity They founded negative correlation between zinc and copper, however in our study zinc was not low in patients and no significant correlation was founded so another explanation must be applied copper has the potential to have a pro-oxidative effect in vivo by catalyzing hydroxyl radical formation (38) accelerating auto-oxidation reactions and lipid peroxidation, and promoting the pro-oxidative activity of vitamin C(39) Recent reports demonstrated hazardous effect of copper on lung .Copper is redox-active metal undergoes redox active cycling and cause an increase in the production of reactive oxygen species such as .hydroxyl radical, superoxide radical or hydrogen peroxide. Enhanced generation of reactive oxygen species can surpass cells' intrinsic antioxidant defenses, resulting in a condition known as ''oxidative stress''(.40) Copper oxide nanoparticles induced greatest oxidative stress and cytotoxicity in airway epithelial cells and compared to silicone and ferric oxides (41) Genetic damage included DNA damage repair proteins Rad51 and MSH2 expression was also demonstrated copper oxide nanoparticles on lung epithelial cells(42) the source of cupper exposure may through water supplied via copper pipes (43) inhalation nanoparticle in dust area around factories and industry( 44)

In this study we found significant rise in magnesium in wheezy cases compared to control (p 0.006) .No available reports about serum magnesium in wheezy infants to dates ,may be this is 1st report related to magnesium in wheezy infants our results looks surprising because expected level that is low or normal most reports about magnesium described in) bronchial asthma Vural et al 2000(37) founded no significant differences in serum magnesium between asthmatic patients and control the same rsults also recorded by **Magboula et al 2008(36)** However Ermis B et al 2004 found significant lower level of magnesium in asthmatic children these study were done in pure asthmatic children with different age group ( 46) This apparent surprising results in our study needs further and repeated study especially no available study with similar diagnosis and age group The possible explanation for these results that hypermagnesaemia is results and not a cause of clinical situation . Magnesium is the 2nd intracellular anion and serum level subjective to change secondary to acid base disturbance medications Hypoxemia ,acidosis, rhabdomyolysis ,muscle fatigue causes hypermagnesaemia(45) Possible hypoxemia acidosis respiratory distress accompanied wheezy cases and medication used in treatment of such cases contributed to elevated magnesium level.

In our study no significant difference in serum zinc was found between cases and control Only one report described decreased zinc in infant wheezes (45)described decreased zinc in wheezy infants the difference from our reports may be attributed to age difference and environmental factors .It is expected to find zinc low in our study based on previous reports described negative correlation between copper and zinc( 34&36&37) This negative correlation was explained by their competition either for the same absorptive binding sites on the intestinal mucosal cells or for similar functional protein systems(47) however this correlation was not found in other repots(32&33)similar to our study. Possible explanation that environmental factors not identified is the cause of this disturbed correlation Some reports described elevated ambient air zinc contributed to, pulmonary toxicities in rodents, worsening pulmonary function ,occupational asthma in adults (48&89) High ambient air fine particulate matter [PM ≤ 2.5 μm in aerodynamic diameter ($PM_{2.5}$)] zinc levels was associated with an increase in ED visits/hospital admissions for asthma on the following day among children living in an urban area.(50)

` In this study copper was lower in wheezes associated due to infection (bronchopneumonia) compared to wheezes without associated infection .Also serum zinc was higher in wheezy bronchopneumonia cases than non-infection cases. this can be explained by anti-infectious properties of copper (51&52&55) Copper ions interfere with several microbial metabolic activities and interrupt the integrity of the cellular DNA, the cytoplasmic membrane, and the cell wall.(53&54) Also higher zinc level in wheezy bronchopneumonia cases than non-infection may need further investigation ,It is expected that zinc is low in infection cases(56&57&58) ,However our results can be explained on basis on decreases sample size .another possible explanation higher zinc level may related to lower copper level .We found weak non significant –ve correlation between copper and zinc in wheezy cases .previous reports described significant –ve correlation between both as discussed before (34&36&37)

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