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The Effect of Serum Copper and Zinc Levels on the Immunity of Children

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Abstract Background: the proper zinc and copper levels seem very important to our immune system. Due to the association of copper and zinc in many enzymes structures, and also the important role of them in the immune system most of the evaluations have considered copper and zinc together. Objective: To evaluate serum copper and zinc levels in children suffering from pneumonia and its relation to CD34+ cell. Study design: 37 patients (21 males and 16 females)with proven diagnosis of pneumonia these patients were selected as one every three cases admitted to chest unit of Pediatric Department, Zagazig University Hospital during the year 2012,their ages ranging from 6 months to 5 years and 37 healthy children(19 males and 18 females) were studied as control group. Results: WBCs, platelets, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neutrophils and lymphocytes were highly significantly higher in patients than controls while hemoglobin, zinc, copper, CD34+ levels were highly significantly lower. There were highly significant positive correlation between CD34 and both copper and zinc levels.Conclusion: Low serum copper and zinc levels may be a contributing factor to the pathophysiology of community acquired pneumonia through its effect on the immune system.

Keywords Copper-Zinc-CD34+

1. Introduction

Several studies have been shown that malnutrition in children results in decrease in the serum levels of many micronutrients and vitamins[1]. Among micronutrients, Copper (Cu) and Zinc (Zn) are the most vital micronutrients under consideration[2]. Normally, the Copper enters the body orally[2]. It is mainly transported in the blood by binding to ceruloplasmin. Concentration of copper is highest in liver, brain, heart and kidney. Muscle contains a low level of copper, but because of its large mass, skeletal muscle contains almost 40% of all copper in the body[2]. The function of copper and its role in various body tissues are known. It presents in the structure of many vital enzymes and co-enzymes[2]. Similarly its role in internal antioxidants, mitochondrial energy production and melanin synthesis are clearly known[2]. In addition due to its role in the structure of enzymes, it is an essential micronutrient for microorganisms[3]. Similarly the role of zinc in the immune system, structure of enzymes and its effects in improving disease such as TB, pneumonia and diarrhea in children are apparent[4]. Because of association of copper and zinc in many enzymes structures, and the important role of them in the immune system most of the evaluations have considered both copper and zinc together[5]. There is significant difference exists in the proliferation and differentiation of $CD34^+$ cells from young and old subjects, zinc significantly increases the number of cells in culture and induces higher levels of cytotoxic activity in comparison with zinc unsupplemented cultures both in young and old subjects. The zinc-induced changes in CD34-derived NK cells [6]. As a result of limitation of the data determining the relationship between nutritional status and pneumonia and also the increasing incidence of pneumonia in children we decided to compare zinc and copper status in children suffering from pneumonia.

2. Aim of the Work

To evaluate serum copper and zinc levels in children suffering from pneumonia and its relation to CD34+ cell.

3. Methods

This case control study included 37 patients (21 males and 16 females)with proven diagnosis of pneumonia(8 had viral pneumonia with lymphocytosis and negative CRP and 29 had bacterial pneumonia with neutropenia and positive CRP)these patients were selected as one every three cases admitted to chest unit of Pediatric Department, Zagazig University Hospital during the year 2012,their ages ranging from 6 months to 5 years and 37 healthy children(19 males and 18 females) were studied as control group. Ethical approval was obtained from the local research ethics

committee and parents of all studied groups gave an informed written consent prior to the study.

Exclusion criteria

- -Patients diagnosed as protein energy malnutrition.
- -Patients diagnosed as immune deficiency.
- -Patients complained of any type of diarrhea.
- - Patients complained of malabsorption.
- -Patients had recurrent pneumonia, defined as two episodes or more in one year or more than three episodes of pneumonia in a child at any time, with radiological clearing between episodes7.

All patients were given intravenous (IV) gentamicin (in a dose of 6 mg/kg/day) in three divided dose and IV ampicillin (in a dose of 200-400 mg/kg/day) in 4 divided doses. Patients who failed to improve after 48h of antibiotics, had changed their antibiotic to ceftriaxone(50 mg/kg/day intravenously), these management according to Zagazig University Hospital's standard treatment guidelines[8].

All infants and children were subjected to the following:

1. History taking including socio-economic level, residence, nutritional history.
2. Clinical examination including body temperature, signs of respiratory distress, local chest examination to determine severity of pneumonia[9] and anthropometric measurements including body height, body weight, triceps skinfold thickness, and mid-arm circumference for each subject. Body height was estimated from the total standing height[10]. Body mass index BMI was calculated as weight (kg) divided by height squared (m2). Triceps skinfold thickness was measured by a Lange skinfold caliper (model 68902, USA) at the posterior midpoint between the acromion and the olecranon. Mid-arm circumference was measured midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. The mid-arm circumference was measured with a flexible plastic tape and recorded to the nearest 0.1 cm.
3. Laboratory investigations as complete blood count (CBC), CRP [11](positive above 6 mg/dl), ESR by Hitachi Model 736 automatic analyzer, Tokyo, Japan.
4. Chest X ray for patient group only.
5. Serum copper and zinc were determined by flame atomic absorption spectrophotometry.
6. 6- Flowcytometric measurement of expression of CD34+ cells[12]. 1 ml blood in EDTA tube for determination of CD34 cells. Incubated and centrifuged blood samples were analyzed on a FACS can flow cytometer (Becton-Dickinson San Jose California, USA), with auto COMP soft ware (Becton-Dickinson). Cells were excited with single 488nm argon laser. Fluorescein isothiocyanate green fluorescence was detected through 530nm filter. Phycoerythrin red fluorescence was detected through 585nm filter. Data were collected in list mode format to 10.000 events.

Table 1. Severity of pneumonia[9].

	Mild	Severe
Infants	Temperature <38.5°C RR <50 breaths/min Mild recession Taking full feeds	Temperature >38.5°C RR >70 breaths/min Moderate to severe recession Nasal flaring Cyanosis Intermittent apnea Grunting respiration Not feeding
Older children	Temperature <38.5°C RR <50 breaths/min Mild breathlessness No vomiting	Temperature >38.5°C RR >50 breaths/min Severe difficulty in breathing Nasal flaring Cyanosis Grunting respiration Signs of dehydration

Satistical Analysis

Data were presented as mean ± standard deviation (X±SD) or percentage (%).The means of two groups were compared using student "t" test. Linear correlation and regression were used to test the correlation between the measured parameters and the studied groups. Data were tabulated and statistically analyzed with the statistical package for Social Sciences (SPPS), version 10 software. P-values less than 0.05 were considered significant[13].

Table 2. Demographic characteristics of the studied groups.

	Cases		Control		t	p
Age						
X±SD	3.55±1.28		3.55±1.28		0.0	0.0
Range	2-6		2-6			
Gender	N	%	N	%	X^2	p
Male	21	56.8	19	51.4	0.22	0.64
Female	16	43.2	18	48.6		
Social						
Low	28	75.7	27	73.0	0.07	0.79
Middle	9	24.3	10	27.0		
Residence						
Rural	22	59.5	22	59.5	0.0	1.0
Urban	15	40.5	15	40.5		
Nutrition						
Good	6	16.2	37	100	53.3	<0.001
Poor	31	83.8	0	0.0		

5. Results

Analysis of demographic characteristics of the studied groups revealed that there were nonsignificant differences between patients and controls as regard age, sex,

socioeconomic state and residence but highly significant difference as regard nutritional history (Table 2). Table 3 showed that 81% of our patients had fever, 100% had tachypnea, 18.9% had effusion, 8% had unresolved pneumonia and hospital staying ranged from 3 to 16 days. Table 4 showed the laboratory data of 37 children with pneumonia versus 37 control children. WBCs, platelets, CRP, ESR, neutrophils and lymphocytes were highly significantly higher in patients than controls while hemoglobin, zinc, copper, CD34+ levels were highly significantly lower. Table 5 detected highly significant positive correlation between CD34 and both copper and zinc levels. Table 6 did not find any difference in zinc and copper levels in mild and sever pneumonia.

Table 3. Symptoms in patients group.

	N X±SD	% Range
Fever	30	81.1
Tachypnea	39.6±6.3	30-55
Unresolved pneumonia	3	8.1
Pneumonia and effusion	7	18.9
Hospital days	7.7±3.1	3-16

Table 4. Laboratory investigations of the studied groups.

	Cases X±SD or N-%	Controls X±SD or N-%	t	p
WBC	10.5±2.5 6-16	7.9±1.2 6-9.8	5.65	<o.oo1
HB	12.4±1.3 10-15	13.4±0.9 11.6-15	3.71	<o.oo1
PLT	246.2±65.9 183-475	318.6±53.8 203-420	5.17	<o.oo1
ESR	12.2±3.6 6-19	6.8±1.6 4-9	8.29	<o.oo1
CRP +ve	30 81.1%	0 0.0	50.4 X^2	<o.oo1
Neutrophils	5.9±1.85 2.4-9.8	4.0±1.3 2.4-6.3	5.08	<o.oo1
Lymphocytes	4.5±1.2 2.7-6.6	3.87±0.8 2.7-5.8	2.68	o.oo9
Zinc	70.9±2.6 65-78	95.6±7.1 79.9-107	19.8	<o.oo1
Copper	51.8±1.95 48.3-56	72.2±7.5 61-89	18.3	<o.oo1
CD34+	4.4±0.8 3.3-5.9	7.4±1.0 5.7-8.9	14.6	<o.oo1
Severity Mild Sever	16 43.2% 21 56.8%			<o.oo1

Table 5. Correlation between CD34 and others parameter.

	r	p	Sig
Zinc	0.83	<0.001	HS
Copper	0.81	<0.001	HS

Significant(Sig) Highly significant (HS)

Table 6. Zinc and copper levels according to the severity of pneumonia.

	Mild	Sever	t	P
Zinc X±SD Range	71±3.2 55-78	70.9±2.1 68-75	0.13	0.89
Copper X±SD Range	52.2±1.95 49.5-56	51.4±1.9 48.3±56	0.13	0.26

6. Discussion

Pneumonia is a leading cause of morbidity and mortality in children less than 5 years old. About 20% of deaths in such children are attributable to pneumonia (1·9 million deaths per year). Two-thirds of these deaths happen during infancy, and more than 90% are in developing countries[14]. Our study showed a highly significantly decrease in zinc, copper, CD34+ levels in patients than controls this can be explained by Bhandari N et al.,[15] who reported that zinc prevents pneumonia also prevents and treats diarrhoea[16]. It might act in the acute phase response to infection [17], helping to boost the body's immune response through a defense cascade, beginning with mobilization and sequestration of zinc to metallothionein-rich tissue, rapid upregulation of immune defense-specific protein synthesis, activation of immune defense activity such as macrophages, lymphocytes, and natural killer cells, and antibody-dependent cytotoxicity [18]. Children with good zinc status may have a more robust immune response than those with poor zinc status[19]. Also Brown KH et al., [20] added that zinc deficiency is one of the most common micronutrient deficiencies in children under the age of 5 years in developing countries. Deficiency of zinc is associated with dysfunction of the immune system, growth retardation, and a high risk of morbidities such as diarrhea and acute respiratory infections. Zinc (Zn) deficiency reduces immune responses and impairs the antioxidant defense system so zinc has a major role in immune responsiveness[21]. Zinc is one of the most important trace elements in the body for many biological functions, it is essential for cell growth and cell division, and it is required for the activities of a variety of enzyme systems in the body. Experiments in rodents, involving dietary zinc supplementation over the animals' whole life span, have demonstrated that many of the age-related immune modifications, including decreased NK cytotoxicity, can be prevented[22]. Zinc plays a crucial role in the development and maintenance of immune competence, including cells mediating non-specific immunity such as neutrophils and NK cells[23]. Our study declared that there was a highly significant positive correlation between CD34 and both copper and zinc levels. Smian OI., et[23] al observed in children with community-acquired pneumonia, imbalance of trace elements Zn, Cu and severity of such violations depended mainly on the severity of the disease and to a lesser extent on age. It has been suggested that Zn and Cu are required to maintain proper immune responses and Zn is crucial for development and expression of both T- and B-cell functions[24]. Copper deficiency in mice impairs immune

system function[25]. The ratio of copper to zinc is clinically more important than the concentration of either of these trace metals[26]. Muzzioli M ., et al[22] declared that significant difference exists in the proliferation and differentiation of $CD34^+$ cells and that zinc significantly increases the number of cells in culture and induces higher levels of cytotoxic activity in comparison with zinc unsupplemented cultures both in young and old subjects. The zinc-induced changes in CD34-derived NK cells is able to partially correct the impaired proliferation and differentiation of $CD34^+$ progenitors towards NK cells, and show that these effects may be related to the zinc-induced modulation of GATA-3 transcription factor[27]. The possibility to correct the zinc defect by the supplementation of the trace element could recover the immune frailty present and promote the differentiation of $CD34^+$ progenitors. It is clear that only subjects having an effective zinc deficiency will be susceptible of zinc supplementation, since zinc has potential toxic effects at high doses. The restoration of zinc levels might have an important biological and physiological impact in terms of regenerative medicine, increasing the effectiveness of stem cell-based therapies for age-related diseases [28]. The effect of zinc on increased differentiation and number of NK cells was associated with the zinc-induced increased expression of GATA-3 transcription factor. GATA-3 is one of the most important transcription factors providing for maturation and activity of NK and T-cell-related immunity. It depends by zinc for its function since it binds to the DNA consensus sequence by two characteristic zinc-finger motifs specific to the GATA family [29]. Zinc, taken with antibiotics, helps infants and young children with severe pneumonia to recover more quickly, and reduces the length of stay in hospital by about 1 day[30].

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