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Serum Zinc Levels in Children 1 - 59 Months of Age with Pneumonia: A Single-Center Surveillance in India from 2014 to 2016

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Abstract

Objectives: This study aimed to estimate serum zinc levels in children with pneumonia according to the WHO criteria and compare them with age-, sex-, and nutrition-matched controls.

Methods: The study was carried out in a pediatric tertiary care hospital from September 2014 to July 2016 after obtaining approval from the Institutional Ethics Committee. Children between 1 to 59 months of age admitted with pneumonia according to the WHO criteria were included as the case group (n = 50) and other non-pneumonia cases as the control group (n = 50). Various etiologies were analyzed, such as pneumonia, severe pneumonia, bronchiolitis, and viral infection-associated wheezing based on clinical and X-ray findings. Blood samples were collected for zinc estimation in both case and control groups. All children were investigated and treated as per our unit protocol for the particular condition.

Results: Serum zinc level was significantly low in the case group (n- 31; 62%) compared to the control group (n- 9; 18%). The mean zinc level in the case group was 60.36 $\pm$ 29.23, and that of the control group was 80.54 $\pm$ 25.70, with a significant P value of 0.001.

Conclusions: Serum zinc levels were significantly lower in children with pneumonia when compared to their age-, sex-, and nutrition-matched controls.

Keywords: Pneumonia, Bronchiolitis, Acute Respiratory Tract Infection, Zinc

1. Background

Zinc is an essential mineral that is involved in numerous aspects of cellular metabolism. Approximately 100 enzymes require zinc for their catalytic activity. It plays an important role in immune function, protein synthesis, wound healing, antioxidation, DNA synthesis, and cell division. It also protects the integrity of respiratory cells during lung inflammation and injury. Children with zinc deficiency are at increased risk of restricted growth, diarrheal diseases, and respiratory tract infections (1).

Zinc absorption happens predominantly in the jejunum through the specific transporter Zip4 (1, 2). It is absorbed either through passive diffusion or attaches to the apical membrane of enterocytes, where transport is aided by metallothionein and cysteine-rich proteins (3-5). This will be taken into the cell to release into the blood or back into the intestine (6, 7). According to the WHO estimates, respiratory infections cause about 987,000 deaths in India, of which 969,000 are lower respiratory tract infections (LRTI). Acute LRTI is the leading cause of mortality and morbidity in children less than five years of age. Most of these deaths are caused by pneumonia. Pneumonia incidence is 10-fold higher, and the number of childhood-related deaths due to pneumonia is approximately 2,000 fold higher in developing countries than developed ones. Although the implementation of safe, effective, and affordable interventions significantly reduced pneumonia mortality from 4 million in 1981 to one million in 2013, it still accounts for nearly one-fifth of childhood deaths worldwide (8). Many studies reported that routine supplementation of zinc lowers the risk of acute respiratory infections and pneumonia in children.

2. Objectives

To know the difference in blood levels of zinc in pediatric pneumonia, this study was conducted with the appropriate control group.

3. Methods

This was a prospective descriptive study conducted in a tertiary health care center from September 2014 to July 2016. Children between 1 month and 59 months of age, admitted as in-patients for pneumonia according to the WHO criteria, were taken as cases and age-, sex- and nutrition-matched controls were taken from non-pneumonia patients. The cases were later categorized as pneumonia, bronchiolitis, or viral-associated wheezing based on clinical and X-ray chest findings. Age and sex were matched between the case and control groups as the following groups 1 to < 12 months, 12 to < 36 months, and 36 to < 59 months. Nutritional status was matched using weight for height z scores in 2 groups as undernutrition (weight/height less than -2 Z score) and normal nutrition (weight/height greater than -2 Z score) (9). Children with a history of diarrhea in the previous month, febrile seizures, history of zinc supplementation in the past three months, chronic illness, metabolic diseases, and severe acute malnutrition were excluded. Informed consent from the child's parent or guardian was obtained before enrolment in the study. Based on the study reported by Panneerselam et al., the mean serum zinc level in pediatric pneumonia was 60.98 (standard deviation 18.89) and 73.12 (standard deviation 17.14) in control (10). To pick up this difference for 5% alpha error and 90% power, the minimum sample size needed was 40. Considering 20% refusal to prick the blood sample, the sample size was increased to 48 and rounded to 50. Hence, 50 cases and 50 controls were chosen for the present study. According to the WHO criteria, pneumonia is defined as cough or difficulty in breathing with fast breathing $\geq$ 60/min in < 2 months, $\geq$ 50/min in 2 months to < 1 year, and $\geq$ 40/min in children between 1 to 5 years of age with or without chest in-drawing (11). Severe pneumonia is defined as cough or breathing difficult and any danger sign (unable to drink or breastfeed, vomiting, convulsions, lethargy or unconscious) or chest indrawing and stridor in a calm child. Bronchiolitis was defined as clinical symptoms and signs, including a viral prodrome followed by increased respiratory effort, wheezing, and diffuse bilateral crackles in children < 24 months. Viral infection-associated wheezing is defined as febrile episodes, with respiratory symptoms, without a personal or family history of atopy or asthma, and with variable response to bronchodilators (12, 13).

Data on age, sex, environmental exposures, maternal education, socioeconomic status (modified Kuppuswamy scale (14)), admission symptoms, anthropometry, and examination findings were collected. Various etiological groups of cases such as pneumonia, bronchiolitis, and viral infection-associated wheezing were analyzed, and the final diagnosis was categorized based on clinical and chest X-ray findings. Apart from baseline investigations, blood samples were collected for zinc estimation in both the cases and controls. Under aseptic precaution, using a 22-gauge sterile needle, 2 mL of venous blood was collected within 24 hours of admission. It was then centrifuged for 4 minutes at 3000 – 4000 rpm and preserved in sterile deionized plain vials at 2 - 8°C. Estimation of Zinc was carried out within seven days of collection. Serum zinc was estimated by spectrophotometry. Nitro-paps 2-[5-Nitro-2-pyridxylazo]-5[N-n Propyl-N-(3-Sulfopropyl) amino] phenol disodium salt reacts with zinc in alkaline solution and form a purple-colored complex, which was measured at 575 nm. Interference from copper and iron were eliminated by pH and chelating agents. The normal range of serum zinc was considered 70 - 110 μg/dL (15). All statistical procedures were performed using SPSS V. 17.0 software. All results were expressed in number (percentage) or Mean $\pm$ Standard Deviation (SD)/median (range) as appropriate. The results were measured in terms of the significance of association at a 95% confidence level with a "P value" of less than 0.05.

4. Results

Fifty children with pneumonia as defined by WHO criteria and 50 children as controls matched as per age, sex, and nutrition status were enrolled in the study over 23 months from September 2014 to July 2016. Twenty (40%) children were in the age group of 12 to 16 months, 17 (34%) in 1 to 12 months of age, and 13 (26%) in 36 to 59 months of age. The mean age of our patients was 26 $\pm$ 17 months. Among 50 children with pneumonia, 27 (54%) were male, and 23 (46%) were female. Thirty-nine (78%) children were normally nourished with a Z-score of > -2, and 11 (22%) were undernourished with the Z score was < -2. Children with < -3 Z-score or severe malnutrition were excluded from the study.

Baseline characteristics among cases and controls were compared. There was no significant difference observed among cases and controls in parental smoking and the use of biomass fuels (Table 1). The significant difference was observed in the socioeconomic status with the P value of < 0.05. The majority of the cases belonged to III, IV, and V (n-13, 15, and 11; 26%, 30%, and 22%, respectively). Maternal education status was compared between cases and controls revealed 11 (22%) cases, and 7 (14%) controls had no formal education, but it was not significant statistically.

The clinical profile of children with pneumonia is shown in Table 2. Cough was the predominant symptom observed in 47 (94%) cases. Crepitations in 43 (86%) on auscultation was the predominant sign followed by chest in-

Table 1. Characteristics of the Study Population

Characteristics	Cases, No. (%) (N = 50)	Controls, No. (%) (N = 50)	P Value
Age group, mo			
1 - < 12	17 (34)	17 (34)	
12 - < 36	20 (40)	20 (40)	
36 - 59	13 (26)	13 (26)	
Sex distribution			
Male	27	27	
Female	23	23	
Nutritional status			
Z-score > -2	39 (78)	39 (78)	
Z-score ≤ -2	11 (22)	11 (22)	
Smoking			
Parental smoking	9 (18)	8 (16)	0.05
Use of biomass fuels	6 (12)	4 (8)	0.05
Maternal education status			0.05
No formal education	11 (22)	7 (14)	
Primary	16 (32)	12 (24)	
Secondary	14 (28)	18 (36)	
Postsecondary (college)	9 (18)	13 (26)	
Socioeconomic status			0.05
I	5 (10)	7 (14)	
II	6 (12)	12 (24)	
III	13 (26)	12 (24)	
IV	15 (30)	13 (26)	
V	11 (22)	6 (12)	

Table 2. Clinical Profile of Children with Pneumonia

Characteristics	Frequency (%)
Fever	50 (100)
Cough	47 (94)
Refusal to feed	32 (64)
Lethargy	13 (26)
Sleep disturbance	19 (38)
Altered sensorium	5 (10)
Tachypnea	50 (100)
Hypoxia (SpO_2 < 93% in room air)	21 (42)
Cyanosis	6 (12)
Chest indrawing	38 (76)
Stridor/grunting	14 (28)
Reduced air entry	14 (28)
Wheeze	37 (74)
Crackles	43 (86)
Ventilation requirement	
Non-invasive	7 (14)
Invasive	5 (10)

Table 3. Distribution of Mean Zinc Values Among Cases and Controls, Also with Respect to Age and Sex

Characteristics	Mean ± SD	P Value
Zinc level in cases and controls		0.001 (< 0.005)
Cases	60.363 ± 29.23	
Controls	80.545 ± 25.70	
Zinc level distribution age-wise, m		0.946 (> 0.05)
1 - < 12	60.0 ± 26.7	
12 - < 36	63.6 ± 30.1	
36 - 59	52.6 ± 32.0	
Zinc levels distribution sex-wise		0.987 (> 0.05)
Male	60.4 ± 27.8	
Female	60.3 ± 30.7	

drawing in 38 (76%) and wheeze in 37 (74%) children. The mean duration of illness was 9.6 ± 2.3 days. Mean white blood cell counts were 15.7 ± 2.9 X 109/L. Overall, 31 cases (62%) and nine controls (18%) had low serum zinc levels. The mean serum zinc level in cases was 60.363 ± 29.23, whereas in control was 80.545 ± 25.70 μg/dL. Zinc level was significantly low in children with pneumonia compared to the age-, sex- and nutrition-matched controls with a P value of < 0.001 (Table 3). Compared with controls both in normal nutrition as well as malnutrition groups, zinc levels were lower in the case group.

Of 50 children, 7 (14%) required non-invasive ventilation and 5 (10%) were put on invasive ventilation. Pulmonary infiltrate (n- 33; 66%) was the most common radiological findings on X-ray chest, followed by collapse (n- 6; 12%), consolidation (n- 4; 8%) and pleural effusion (n- 4; 8%). Three children (6%) did not show any radiological abnormality. The final diagnosis and classification were based on clinical examination and chest X-ray findings. Thirty-three children (66%) were diagnosed as having pneumonia (19 as pneumonia (38%) and 14 as severe pneumonia (28%)). Others were bronchiolitis (n- 11, 22%) and viral-associated wheezing (n- 6, 12%). All of them received treatment as per the institutional protocol. The majority of the children

(n- 47; 94%) recovered from the illness and 2 (4%) were discharged against medical advice due to some social reasons. One child had H1N1 pneumonia, developed severe acute respiratory distress syndrome, and succumbed.

5. Discussions

Zinc plays a crucial role in the development and maintenance of host defense mechanisms against infections (16). In the present study, pneumonia was diagnosed according to the WHO criteria. Similar criteria were used in a study conducted by Kumar et al. (17). However, we also used chest X-ray findings to confirm the final diagnosis that showed 33 children had pneumonia, 11 children were diagnosed with bronchiolitis, and six were categorized as having viral-associated wheezing. Our study population consists of the cases and controls between 1 month to 59 months, similar to the study conducted by Shah et al. (18), and Kumar et al. (17). We observed that male children were affected more by pneumonia than females. Male gender predominance was also recorded by other studies (19, 20). But mean serum zinc level between male and female children recruited in this study did not show any significant difference statistically ($P > 0.05$) similar to other published data (10, 17, 21).

The prevalence of malnutrition varied from 25% to 40% in previous studies. Similarly, we observed 11 patients (22%) in each category, a total of 44% were classified as moderate acute malnutrition, with Z-scores between -3 and -2, and children with severe acute malnutrition were excluded from the study (Z-score < -3), as they often have coexisting zinc deficiency (16, 22). Previous studies that were done by Smith et al. and Awasthi et al. reported that a large proportion of cases presented with pneumonia had an association with the use of biomass fuels (coal, wood, dung, and kerosene) (23, 24). But the association of pneumonia with biomass fuels was not statistically significant in the present study.

The epidemiological characteristics were compared among cases and controls, and it was observed that children with pneumonia had significantly lower socioeconomic status in comparison to the control group ($P < 0.05$). The educational background of mothers of cases in our study was comparatively lower, though it was not significant statistically. A study reported by Arica et al. (21) showed that the educational background of mothers of children with pneumonia was significantly low compared to the control group. This highlights the need for the proper education of prospective mothers so that proper nutrition and care will be given to the children. The average number of days in which the cases were reported being ill before the admission in the current study was double than that in the study conducted in Bangladesh by Brooks et al., which were 4.5, and 2.5, respectively (25). The proportion of children with wheezing at the time of admission was significantly higher in our study population than in the study reported by him (74%, and 37%, respectively). This may suggest a difference in the etiology of "Pneumonia" between various population groups.

In our study, the mean serum zinc level in children with pneumonia was 60.363 ± 29.23, whereas in the control group was 80.545 ± 25.70 μg/dL. The blood zinc level was lower in the case group compared to the control group ($P < 0.001$). Analysis of the zinc level in the cases was significantly low irrespective of the nutritional status. Compared with the controls, in both normal nutrition as well as malnutrition groups, zinc levels were lower in cases, similar to studies conducted earlier (10, 26). Though Rady et al. reported normal zinc levels in children with pneumonia, zinc levels were low in children admitted to intensive care units compared to inpatient ward admissions (27).

Some studies have reported significant positive benefits in terms of morbidity and mortality associated with zinc supplementation in children with pneumonia (28, 29). Bose et al. study showed no difference in early recovery or outcome in pediatric with pneumonia who received zinc in comparison to the placebo group (30). But we have not studied the benefits of zinc supplementation in our patients. In the current study of 50 cases, 47 patients improved, 2 of them went against medical advice, and one death was reported. In our study, the cases were recruited as per the WHO criteria to diagnose pneumonia and it does not include the chest X-ray findings for case diagnosis; hence, our study case population includes even Bronchiolitis and WALRI giving rise to lower specificity of pneumonia cases. We did not include the assessment of dietary composition and intake of the child, which is an important confounding factor. The follow-up with zinc supplementation and its effectiveness is not demonstrated.

5.1. Conclusion

We conclude that serum zinc levels were significantly lower in children with pneumonia compared with age-, sex-, and nutrition-matched controls. The role of zinc in the management of pneumonia should be investigated. Education of girls who turn out to be prospective mothers is very important to provide appropriate nutrition for their younger generation with knowledge and awareness.

Footnotes

Authors' Contribution: Study concept and design: RCP, SG, and JR; analysis and interpretation of data: RCP, SG, JR

and DJ; drafting the manuscript: RCP, SG, and JR; critical revision of the manuscript for important intellectual content: RCP, SG, and JR; statistical analysis: JR.

Conflict of Interests: None.

Ethical Approval: Institutional Ethics Committee approved the study (CSP-MED/14/SEP/18/161).

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Informed Consent: Written informed consent was obtained from the children's parents after explanation of the study.

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