

SERUM ZINC LEVELS IN CHILDREN HOSPITALIZED WITH PNEUMONIA: A CASE CONTROL STUDY

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INTRODUCTION: Pneumonia is the leading cause of morbidity and mortality in children aged below 5 years. Pneumonia is responsible for about 19% of all deaths in this age group.¹ As per the data published by World Health Organization, 10.5 million children under the age of 2 years across the world lose their lives due to 5 preventable and curable diseases every year. Respiratory tract infections are responsible for 28% of all these deaths.^{2,3}

Of the total 156 million new episodes each year worldwide, approximately 43(24%) million cases take place in India. Approximately 95% of pneumonia related deaths occur in developing countries and the youngest age group has the highest risk of death.⁴

Pneumonia and Diarrhoea are major causes of death in children of this age group. Zinc administration as a therapeutic agent has been well documented in acute or persistent pediatric diarrhea which reduces the duration of the diarrhea and is associated with a lower rate of treatment failure or death.⁵

Zinc is known to protect children from respiratory tract infections by its role in regulation of immunomodulators, immunoregulators, protection of the epithelium of the respiratory tract from infections and improvement of T-lymphocytes.⁶ Zinc is also an important antioxidant and a cytoprotective agent which acts against toxins and inflammatory mediators which damage the respiratory epithelium.⁷ Even a mild and moderate deficiency of Zinc impairs the function of the immune system, thus resistance against the infections is reduced and T-lymphocytes could not exhibit sufficient effectiveness.⁸

Recent works have provided conflicting evidence on the role of zinc against pneumonia. While some studies, report that there are no significant difference in blood zinc levels in pediatric pneumonia, some other studies have shown significant reduction in the blood zinc levels in pediatric pneumonia when compared to controls.⁴ Hence the present study was conducted with an intent to estimate serum zinc levels in pediatric Pneumonia.

MATERIALS AND METHODS: This study was conducted at Department of Biochemistry, Mysore Medical College and Research Institute (MMC & RI), Mysore and Department of Pediatrics, Cheluvamba hospital Mysore, which is attached to Mysore Medical College and Research Institute (MMC & RI), Mysore district, Karnataka, India.

The sample size was 60. This sample size was arrived at by keeping 5% significance and allowable error of 20%. Out of them, 30 subjects who were included as cases were children who were in the age group of 2 months to 5 years and diagnosed as per WHO criteria and hospitalized for pneumonia. 24 cases were of bacterial pneumonia and rest were caused by other pathogens (Viral and fungal).

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30 normal, age, sex and Nutritionally matched subjects were considered as controls. Children diagnosed with diabetes (Type 1), persistent diarrhea, asthma, drug allergy and children on zinc supplementation were excluded from the study. Controls were chosen among children who came to department of pediatrics, MMC and RI for pediatric surgery for hernia repair/circumcision after considering the inclusion and exclusion criteria. Thorough History taken from the attendants of the study subjects regarding immunization history revealed that they were appropriately immunized for their age according to IAP (Indian Academy of Pediatrics) schedule.

After obtaining Institutional Ethical Committee Clearance and Informed consent from parents of subjects, 5 ml of venous blood was collected from cubital vein after taking aseptic precautions. The sample was centrifuged for 10 minutes at 3000 rpm and serum was separated. All the serum samples were stored under -20°C until estimation. 250 μL of serum sample was diluted to 3 mL in 6% n-butanol solution. Aliquot of the diluted solution was aspirated to the instrument and calibration was done using the working standards.

Serum zinc was assayed using Atomic Absorption Spectrophotometry method, which is the method of choice for Zinc estimation. It uses the absorption of light to measure the concentration of gas-phase atoms. It is based on Beer-Lambert's Law, which says that the absorption of light is directly proportional to the number of atoms absorbing it. The more concentrated the sample solution is, the higher absorbance is measured. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.⁹

STATISTICAL METHODS: The results were tabulated, data was analysed, mean, standard deviation was calculated and the results were compared by using student t test using instatstatistical software. $P < 0.05$ was taken as statistically significant. Odds ratio was calculated to know the odds of developing pneumonia in zinc deficient children.

RESULTS:

AGE DISTRIBUTION AMONG STUDY SUBJECTS: In the current study a total of 60 subjects were analysed. Out of this, 30 subjects are children hospitalized with pneumonia and 30 subjects are normal healthy individuals. More number of pneumonia cases were found in age group of 2 months to 2 years (79.9%). It has been shown in Table 1.

GENDER DISTRIBUTION AMONG STUDY SUBJECTS: Current study included 30 subjects hospitalized for pediatric pneumonia. Among them, 18 subjects were males (60%) and 12 subjects were females (40%). Hence, proportionate numbers of male and female subjects were chosen as control. The same has been depicted in Table 1.

SERUM ZINC LEVEL IN PEDIATRIC PNEUMONIA CASES AND CONTROLS: The mean serum zinc value in pediatric pneumonia patients is $39.390 \pm 13.16 \mu\text{g/dl}$ and in controls the mean value is $62.811 \pm 13.20 \mu\text{g/dl}$. The difference between mean serum zinc values of controls and cases was $23.51 \mu\text{g/dl}$ which was a statistically significant difference. The two-tailed P value was found to be < 0.0001 , considered extremely significant. The same has been shown in Table-1.

SERUM ZINC LEVEL AMONG SUBJECTS IN THE AGE GROUP OF 2 MONTHS TO 2 YEARS:

Present study showed significant number of cases in the age group of 2 months to 2 years (24 out of 30 which is approximately 80% of total cases). Hence, mean and standard deviation was computed for the above subgroup due to the significant scattering of cases in the above subgroup. The mean and standard deviation for serum zinc values for the control group was 58.87 ± 11.53 $\mu\text{g/dl}$ and for the cases it was 40.48 ± 13.68 $\mu\text{g/dl}$. The difference between the means was found to be statistically significant ($p < 0.05$). It has been depicted in Table 2.

Odds ratio was found to be 19. It shows that the odds of developing Pneumonia in zinc deficient children (2 months – 5 years) is 19 times more than children having normal zinc levels. The normal serum zinc levels for the present age group was taken as $65 \mu\text{g/dl}$.¹⁰ This shows that deficiency of zinc acts as a risk factor for developing pneumonia. It has been shown in Table-3.

DISCUSSION: Extremely significant reduction in serum zinc levels were seen in pediatric pneumonia cases compared to controls. (Mean value in controls was 62.811 ± 13.20 $\mu\text{g/dl}$ and in cases was 39.39 ± 13.16 $\mu\text{g/dl}$, $p < 0.0001$). The result obtained in the study is an obvious evidence of decrease Zn levels in pneumonia affected children compared to their normal counterparts

24 out of 30 (80% of cases) cases were found in the age group of 2 months to 2 years. Significant reduction in serum zinc levels of pneumonia patients in this age group. (Mean serum zinc level in controls -58.87 ± 11.53 $\mu\text{g/dl}$ and cases -40.48 ± 13.68 $\mu\text{g/dl}$, $p < 0.05$). Remaining cases belonged to the age group of 2 years to 5 years.

Odds ratio is 19 which shows that odds of developing Pneumonia in zinc deficient children (2 months-5 years) is 19 times more than children having normal zinc levels. The result of the present study was concordant with that of a study⁴ conducted by Kumar S, Awasthi S, Jain A, Srivastava RC who have concluded that cases of severe pneumonia have a significantly lower blood zinc level as compared to age, sex and nutritional status matched controls. The reasons stated for decrease in the zinc levels in pneumonia cases are:

- Pre-existing zinc deficiency, making the child susceptible to pneumonia due to impaired immunity.
- respiratory tract infections which are known to result in lowered zinc level due to its role in acute phase reaction mediated by interleukins and tumor necrosis factor alpha (TNF- α).

The limitations of this study is that it is a case control study where definitely lowered and statistically significant zinc changes were seen in cases but the exact mechanism of decrease in Zn level in the study subjects could not be assessed.

More number of cases (approx 80%) were found in the age group of 2 months to 2 years. Bose, Coles and Gunavathi have also found in their study that there is increased number of pneumonia cases in 2 months to 2 years of age group.¹ The quantity of zinc derived from breast milk by 6 months is very limited, and zinc-unfortified, plant-based complementary foods do not provide adequate zinc.¹¹ Thus, typically low dietary zinc intake of older infants suggests that zinc supplements for the treatment of pneumonia are more likely to be effective in infants aged greater than 6 months.

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Mean serum Zinc levels in controls have been found to be slightly decreased in the present study, which resembles the results of the study by mahalanobis et al.¹² The reason might be mild to moderate deficiency is common in several developing countries because of commonly consumed staple food having low zinc content and rich in phytates, which inhibit the absorption and utilization of zinc.

Adjuvant treatment of zinc reduces administration of multiple antibiotics and decreases the development of antibiotic resistance. It also speeds up the recovery from severe pneumonia in children, thereby reducing the complications and mortality associated with such infections. In most of the studies conducted, no adverse effect was observed when zinc was administered in therapeutic doses. This is significant for the reliable administration and application of the zinc in the studies.⁸

Limitation of present study was small sample size. Correlation between nutritional status and serum zinc levels could not be done. Studies on Nutritional behavior of zinc, correlation of antioxidant enzymes with zinc level, correlation of zinc levels with duration of illness and correlation with maternal zinc levels are required. Moreover the prior nutritional status of the cases (Height and weight and skin fold thickness) could not be established from the questionnaire since already diagnosed and hospitalized cases were chosen. Further research is needed to establish sustainable ways of supplementing large numbers of apparently well-nourished children in the general population. This might help in planning intervention studies for possible benefits of zinc supplementation for prevention, treatment and prognosis of pediatric pneumonia.

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TABLE 1: DEMOGRAPHIC STUDY AND SERUM ZINC LEVELS AMONG STUDY SUBJECTS

Parameter:	Column A (CONTROLS)	Column B (CASES)
Number of subjects	30	30
Age:		
2 months to 2 yrs	22(73.3%)	24(79.9%)
2 yrs to 5 yrs	8(26.7%)	6(20.1%)
Gender:		
Male	22(73.3%)	18(60%)
Female	8(26.7%)	12(40%)
Serum Zinc level (µg/dl) (Mean±Std deviation)	62.811± 13.20	39.390 ± 13.158
Minimum:	40.800	21.900
Lower 95% CI:	55.498	34.478
Upper 95% CI:	70.124	44.303

TABLE 2: SERUM ZINC LEVELS AMONG STUDY SUBJECTS BETWEEN 2 months -2 years

Zinc (µg/dl)	Controls Mean±SD	Cases Mean±SD	t-value
	58.87±11.53	40.48 ±13.68	3.865*
*significant p<0.05			

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TABLE 3: CALCULATION OF ODDS RATIO

	Controls	Cases	Total
Normal zinc(≥ 65 $\mu\text{g}/\text{dl}$)	12	1	13
Low zinc(< 65 $\mu\text{g}/\text{dl}$)	18	29	47
Total	30	30	

$$\begin{aligned}\text{Odd's ratio} &= 12 \times 29 / 18 \times 1 \\ &= 348 / 18 \\ &= 19.\end{aligned}$$

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