

## Original research article

## A case-control study to assess whether giving zinc sulphate to children aged 2 to 59 months improved their clinical symptoms of pneumonia

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### Abstract

**Aim:** This study was investigating the effect of prescribing zinc sulphate on improving the clinical symptoms of pneumonia in 2 to 59 months of children's.

**Methods:** This Case-Control study was done the Department of Pediatrics, Darbhanga Medical College & Hospital, Laheriasarai, and Bihar, India for 2 years. Among 250 patients divided into two equal groups. 125 Children between the age of 2 months to 59 months and children with Acute Lower Respiratory Tract infection were included in case group and 125 children for control group. The details of blood investigations and imaging for confirmation of clinical diagnosis were also noted during the stay of the patient in the hospital. The serum zinc estimation was done by using colorimetric test. **Results:** The mean age of cases was  $1.73 \pm 1.54$  yrs and that of controls was  $1.87 \pm 1.75$  yrs. The Sex wise distribution of the cases and controls consisted of 74(59.20%) of cases being male and 51(40.80%) being female as compared to 69(55.20%) of controls being male and 56(44.80%) being female. The Mean serum zinc levels in the cases and controls, after comparison, were found to be significantly different [ $p=0.0001$ ], with mean value for the cases being  $57.78 \pm 11.73$  ug/dl as compared to  $84.22 \pm 12.89$  ug/dl for the controls. A total of 31 cases and controls (24.80%) were found to have deficiency of zinc, of which majority (88.80%) were cases (normal range of 60 to 150ug/dl). Severe Pneumonia group (Mean= $40.15 \pm 5.88$  ug/dl) having significantly lower value than that of Pneumonia group (Mean= $62.97 \pm 6.81$  ug/dl).

**Conclusion:** No overall effect on the duration of hospitalization or of clinical signs associated with severe infection in young children hospitalized for severe pneumonia in southern India.

**Keywords:** Zinc Level, Children, Lower Respiratory Tract Infection.

### Introduction

Worldwide, pneumonia is the leading cause of pediatric morbidity and mortality. It is estimated that pneumonia is responsible for >2 million deaths each year in children <5 y old, which represents 19% of the annual deaths in this age group.<sup>1</sup> Approximately 95% of the pneumonia-related deaths occur in developing countries, and the youngest age groups have the highest risk of death.<sup>2</sup> India has the largest number of deaths of children <5 y old in the world—an estimated 2 402 000, or >3 times the number in China. Pneumonia case management, which relies on early diagnosis and prompt empiric antibiotic therapy, has been effective, reducing pneumonia-related deaths by 47%.<sup>4</sup> However, the efficacy of this strategy

may be diminished by poor nutritional status.<sup>5</sup> Under nutrition is known to be associated with greater severity of pneumonia, a higher frequency of complications, longer episodes of infection, and greater case fatality rates.<sup>6</sup> In light of these issues, the evaluation of interventions that may improve the effectiveness of case management in children with poor nutritional status is warranted.

Zinc is an essential mineral that is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes<sup>7,8</sup> and it plays a role in immune function,<sup>9,10</sup> protein synthesis,<sup>10</sup> wound healing,<sup>11</sup> DNA synthesis,<sup>8,10</sup> and cell division.<sup>10</sup> It is required for maintaining intestinal cells, bone growth and immune function. It is second to iron as the most abundant trace element in the body. Zinc deficient children are at increased risk of restricted growth and developing diarrheal diseases and respiratory tract infections. Zinc is thought to decrease susceptibility to Acute Lower Respiratory Tract Infection (ALRTI) by regulating various immune functions including protecting the health and integrity of respiratory cells during lung inflammation and injury. Supplementation of zinc could reduce the risk of pneumonia and the risk and duration of diarrhea, dysentery and malaria deaths among all infectious diseases, and they accounted for 3.9 million deaths worldwide.<sup>11</sup> According WHO estimates respiratory infection cause about 987,000 deaths in India of which 969,000 are LRTI.<sup>12</sup> ALRTI are the leading cause of mortality and a common cause of morbidity in children below five years of age. Most of these deaths are caused by pneumonia and bronchiolitis. Pneumonia kills more children each year than AIDS, malaria or measles combined with more than 2 million deaths per year.<sup>13</sup> The need for the study was to establish that zinc deficiency may lead to LRTI. The study was Investigating the effect of prescribing zinc sulphate on improving the clinical symptoms of pneumonia in 2 to 59 months of children's.

### **Material and methods**

This Case-Control study was done the Department of Pediatrics, Darbhanga Medical College & Hospital, Laheriasarai, and Bihar, India for 2 years, Among 250 patients divided into two equal groups (125 cases and 125 controls) were included in the study. 125 Children between the age of 2 months to 59 months and children with Acute Lower Respiratory Tract infection were include in case group. Children suffering from Acute Gastroenteritis or diarrheal illness, reactive airway disease/asthma or with underlying chronic illnesses and congenital heart disease were exclude from this study.

### **Methodology**

The detailed demographic information, history, clinical findings, laboratory findings and details of clinical course of cases and controls included in the study were entered in predesigned and validated proforma. Socio-economic status was assessed according to the Modified Kuppaswamy scale updated in 2017.<sup>14</sup> Detailed General examination was carried out in the patients along with Respiratory system and other systemic examination and a clinical diagnosis was made and entered into the proforma. The details of blood investigations and imaging for confirmation of clinical diagnosis were also noted during the stay of the patient in the hospital. The serum zinc estimation was done by using colorimetric test. The kit used for this study was manufactured by Centromic GMBH, Germany. The Sample used was serum obtained by centrifugation of 2 ml of blood sample collected at 3000 rpm for 3 to 5 minutes. The blood sample was obtained at Day 1 of admission of cases and controls. In two different endoff tubes, 1000 ul of reagent in both along with 50 ul of serum in one tube and standard solution in other were mixed and incubated at 37<sup>0</sup> for 5 minutes. Absorption of the standard A (Standard) and the sample A (Sample) was measured against the reagent blank A (Blank) via the spectrophotometer at 560 nm wavelength, which was

directly proportional to the concentration of total zinc in the sample.<sup>15</sup> Apart from measuring the serum zinc levels, the details of clinical course of the cases were also documented in terms of the duration of stay, oxygen requirements, severity of disease according to WHO IMNCI grading 2014 and outcome of the cases.

### Statistical analysis

The data obtained from the cases and controls was compiled and entered into Microsoft Excel case sheet. Statistical analysis was done by using descriptive and inferential statistics using chi square test, students unpaired t test one way ANOVA and Pearson's correlation coefficient and software used in the analysis were SPSS 22. version and  $p < 0.05$  was considered as level of significance.

### Results

The mean age of cases was  $1.73 \pm 1.54$  yrs and that of controls was  $1.87 \pm 1.75$  yrs. The Sex wise distribution of the cases and controls consisted of 74(59.20%) of cases being male and 51(40.80%) being female as compared to 69(55.20%) of controls being male and 56(44.80%) being female. On comparison, the distribution of cases and controls in this study according to age, sex, nutritional status and socioeconomic status was statistically not significant.(table.1)

**Table 1: Demographic profile of children**

Parameter	Cases	Controls
Age(mean)	$1.73 \pm 1.54$ yrs	$1.87 \pm 1.75$ yrs
<b>Sex</b>		
Male	74(59.20%)	69(55.20%)
Female	51(40.80%)	56(44.80%)

The Mean serum zinc levels in the cases and controls, after comparison, were found to be significantly different [ $p = 0.0001$ ], with mean value for the cases being  $57.78 \pm 11.73$  ug/dl as compared to  $84.22 \pm 12.89$  ug/dl for the controls (Table 1). A total of 31 cases and controls (24.80%) were found to have deficiency of zinc, of which majority (88.80%) were cases (normal range of 60 to 150 ug/dl) .(Table 2)

**Table 2: Comparison of Zinc level in cases and controls**

Group	N	Mean(ug/ dl)	Std. Deviation(ug/ dl)	Std. Error Mean	t-value
Cases	125	57.78	11.73	1.77	11.09, $p = 0.0001, S$
Controls	125	84.22	12.89	2.63	

Table 3 shows comparison of serum zinc levels according to the clinical characteristics of cases. Here, the difference in mean serum zinc levels of cases according to WHO IMNCI grading was statistically significant ( $p$  value=0.0001) with cases belonging to Severe Pneumonia group (Mean= $40.15 \pm 5.88$  ug/dl) having significantly lower value than that of Pneumonia group (Mean= $62.97 \pm 6.81$  ug/dl). This is also reflected when we see serum zinc levels according to oxygen requirements, with cases managed on room air having mean of  $65.91 \pm 7.42$  ug/dl, cases requiring supplemental oxygen by nasal prongs having mean of  $62.93 \pm 10.32$  ug/dl and cases requiring mechanical ventilation having mean of  $40.91 \pm 7.69$  ug/dl (Table 3). The serum zinc analysis of patients according to outcome shows significantly lower zinc values ( $p$  value=0.0001) in cases who eventually died due to the ALRTI and its complications ( $n = 10$ ) as compared to those who got discharged after treatment ( $n = 115$ ) (Table 3)

**Table 3: Zinc level according to clinical characteristics in cases**

IMNCI Grading	No of cases	Mean(ug/dl)	SD	t-value
Pneumonia	91(72.80%)	62.97	6.81	9.27 p=0.0001,S
Severe Pneumonia	34(27.20%)	40.15	5.88	
Total	125(100%)	57.12	11.19	
<b>O2 Requirement</b>				
Room Air	50(40%)	65.91	7.42	35.27 p=0.0001,S
Supplemental Oxygen	39(21.20%)	62.93	10.32	
Mechanical Ventilation	34(27.20%)	40.91	7.69	
<b>Outcome</b>				
Discharge	115(92%)	60.83	11.77	37.12 p=0.0001,S
Death	10(8%)	41.08.	6.27	

The mean serum zinc level was found to have a negative correlation ( $r' = -0.048$ ) with the duration of stay of cases, however, this correlation was statistically not significant ( $p$  value = 0.678)

### Discussion

Zinc plays an important role in maintaining a normal immune function and participates in all major biochemical pathways. It plays multiple roles in the perpetuation of genetic material and cellular division. Studies have suggested that zinc deficiency impairs immunocompetence with reduced cell-mediated immune responses, decreased T-lymphocytes, abnormal T-helper and/or suppressor functions, impaired macrophage function, reduced killer cells and antibody dependent cytotoxicity.<sup>16</sup> Zinc supplementation in children causes an increase in the levels of complement in the blood that modulate the function of monocytes, macrophages and neutrophils polymorphs. It also helps in the development and activation of T-lymphocytes. When zinc supplements are given to individuals with low levels of zinc, the numbers of T-cell lymphocytes circulating in the blood increase and the ability of lymphocytes to fight against infection improves.<sup>17</sup> Meeks-Gardner J et al have shown a positive Zinc supplementation in these patients.<sup>18</sup> The mean serum zinc levels were comparable to that found in the study by Hussain et al.<sup>19</sup> A study in Bangladesh by Shakur et al. and a study in Egypt by Rady et al. showed mean serum zinc levels in cases to be higher than this study.<sup>20,21</sup> On the other hand, a study done by Ibraheem et al. in Nigeria showed mean serum zinc levels of cases to be lower than this study.<sup>22</sup> This variation of mean zinc values can be ascribed to the dietary habits of the country and nutritional status of the subjects of the study as a whole.<sup>20-22</sup> The difference in serum zinc levels of the cases and controls in this study, as well as in the above mentioned studies is statistically significant ( $p$  value=0.0001). Study by Kumar et al. in India and Arica et al in Greece also showed similar results.<sup>23,24</sup> These finding could be explained by the fact that serum zinc level is decreased by interleukins and tumour necrosis factor alfa as a part of a cute phase reaction in response to inflammatory stimulus.<sup>23</sup> The difference in mean serum zinc levels of cases according to WHO IMNCI grading was statistically significant ( $p$  value = 0.0001) with cases belonging to Severe Pneumonia group (Mean=39.95  $\pm$  5.98 ug/dl) having significantly lower value than that of Pneumonia group (Mean=63.78  $\pm$  6.76 ug/dl) and similar findings were seen in study by Rady et al., Hussain et al. and Brooks et.al.<sup>19,21,25</sup> This may be due to the fact that in zinc deficiency, there is loss of immunomodulatory effect of zinc causing unregulated immune response in the respiratory tract, leading to increased airway injury.<sup>25</sup> However, evidence to the contrary was found in studies by Bose et al. and Valentin - Branth et al.<sup>26,27</sup> Argument has been put by the above studies that as zinc is required to mount a better immune response

by the host against infection, there will be increased damage to the respiratory epithelium due to the increased immune response and thus leading to worsening of symptoms.<sup>27</sup> Regarding the duration of stay of cases, Basnet et al. also found lower duration of stay in zinc supplemented group as compared to placebo, but similar to our study, the difference was statistically not significant. However, Brooks et al., Singh et al. and Malik et al. found significant reduction in duration of stay of patients of ALRTI after supplementation of Zinc.<sup>25,28,29</sup> Meanwhile, Bose et al., Valentiner-Branth et al. and Yuan et al found the supplementation of zinc either had no benefit or increased the duration of stay of patients of ALRTI.<sup>26,27,30</sup> A similar trend is also seen while evaluating the patients in terms of oxygen requirement during treatment. In this study with cases managed on room air having mean of  $66.11 \pm 7.22$  ug/dl, cases requiring supplemental oxygen by nasal prongs having mean of  $63.23 \pm 10.29$  ug/dl and cases requiring mechanical ventilation having mean of  $41.17 \pm 7.88$  ug/dl. While studies by Rady et al. and Brooks et al. concur with the findings of our study, studies by Bose et al. and Valentiner-Branth et al. have found no significant reduction of oxygen requirement.<sup>21,25-27</sup> When comparing the outcome of cases according to serum zinc levels, the findings of our study were in concordance with Rady et al., Brooks et al. and Basnet et al.<sup>21,25,31</sup> Also, a large systematic review of zinc supplementation by Mayo- Wilson et al. found that giving children zinc supplements might reduce their risk of death in general, and their risk of death due to lower respiratory tract infection.<sup>32</sup>

### Conclusion

Zinc supplementation had no overall effect on the duration of hospitalisation or clinical signs associated with severe infection in young children hospitalised for severe pneumonia in southern India, according to the findings of this study. This contradicts the findings of two previous studies, which found that zinc supplementation was linked to a faster recovery from severe pneumonia. With the mixed findings, further study in a variety of settings is needed to better understand the function of zinc in the treatment of serious pneumonia.

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