Original research article

Assessment of the serum zinc levels in children aged 2 months to 5 years admitted with lower respiratory tract infections: A Case-Control study

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Abstract

Aim and objective: The study was planned to assess the serum zinc levels in children aged 2 months to 5 years admitted with lower respiratory tract infections and to study the association between low zinc levels and other known risk factors for lower respiratory tract infections.
Material and Methods: This Case-Control study was done the Department of Pediatric, Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India for 1 year. Total of 80 cases and 80 controls were enrolled in the study. 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.
Results: The mean age of cases was 1.61±1.32 yrs and that of controls was 1.88±1.71 yrs. The Sex wise distribution of the cases and controls consisted of 51(63.75%) of cases being male and 29(36.25%) being female as compared to 49(61.25%) of controls being male and 31(38.75%) 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 59.64 ± 11.82 ug/dl as compared to 84.94 ± 15.83 ug/dl for the controls. A total of 45 cases and controls (28.12%) were found to have deficiency of zinc, of which majority (91.11%) were cases (normal range of 60 to 150 ug/dl). 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 = 43.23 ± 6.06 ug/dl) having significantly lower value than that of Pneumonia group (Mean = 66.24 ±7.77 ug/dl). we see serum zinc levels according to oxygen requirements, with cases managed on room air having mean of 65.93 ± 7.73 ug/dl, cases requiring supplemental oxygen by nasal prongs having mean of 61.31 ±10.12 ug/dl and cases requiring mechanical ventilation having mean of 40.88 ±7.07 ug/dl.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 = 13) as compared to those who got discharged after treatment (n=67).
Conclusion: The deficiency of zinc causes the patient to have a more serious form of the disease, requiring patient to be hospitalized for longer duration as well as requiring higher oxygen demands and increasing the chances of mortality by it.

Introduction

The incidence, severity and case fatality of ALRI, especially pneumonia is adversely affected by malnutrition which has been highlighted in earlier reports.[1-3] Indeed, malnutrition has been

identified as an important determinant of ALRI-related mortality.[3] The vulnerability of malnourished children to ALRI in developing countries has been ascribed to a reduction in cellular immunity and possibly zinc deficiency.[4,5] Zinc is a trace element with a direct anti-viral activity and a demonstrable effect on immune-mediated production of interferon.[6,7] Also, zinc prevents pathogens from gaining entry into cells and hinders their intra-cellular multiplication.[6,7] Zinc deficiency decreases the ability of the body to respond to infection, and also adversely affects both cell-mediated and humoral immune responses.[6] The impaired immunocompetence due to low zinc states would not only enhance the establishment of a particular infection but is also associated with a reduction in the clearance of infectious agents.[6] The zinc concentration in plasma, hair and urine can be assessed in detecting zinc deficient states but measuring the serum zinc level has been recommended as an appropriate biomarker.[8] The serum concentration is affected by factors such as age, dietary intake and infections.[8] Children with pneumonia have been found to have lower blood zinc levels as compared to uninfected children.[5,9,10] Even in well nourished children with ALRI, serum zinc levels have been found to be lower compared with the uninfected.[5,10] Trials of zinc supplements also constitute a reliable method of assessing the health consequences of zinc deficiency.[7] Zinc is an essential antioxidant mineral that is involved in numerous aspects of cellular metabolism. A potent antioxidant can act against inflammation and prevent the resulting tissue injury. In 2009, a systematic review of studies evaluating preventive effects of zinc supplementation on the morbidity burden of ALRI notedan overall reduction of 15-21% in the incidence of ALRI among zinc-supplemented preschool children.[11] 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.[12] The need for the study was to establish that zinc deficiency may lead to LRTI. The study was planned to assess the serum zinc levels in children aged 2 months to 5 years admitted with lower respiratory tract infections and to study the association between low zinc levels and other known risk factors for lower respiratory tract infections.

Material and methods

This Case-Control study was done the Department of Pediatric, Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India for one year.

Inclusion criteria

- Children between the age of 2 months to 5 years
- Children with Acute Lower Respiratory Tract infection

Exclusion criteria

- Children suffering from Acute Gastroenteritis or diarrheal illness
- Reactive airway disease/asthma or with underlying chronic illnesses
- Congenital heart disease

Methodology

Age, sex and nutritionally matched patients admitted from OPD or casualty for indications other than inclusion and exclusion criteria were enrolled as Controls. Age of the controls was matched within 6 months. Total 160 children were include in this study. Thus a total of 80 cases and 80 controls were enrolled in the study. 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 Kuppuswamy scale updated in

2017[13]. 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 ependoff 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 degree Celsius 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 560nm wavelength, which was directly proportional to the concentration of total zinc in the sample.[14] 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 and student's un-paired t-test 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 1.61±1.32 yrs and that of controls was 1.88±1.71 yrs. The Sex wise distribution of the cases and controls consisted of 51(63.75%) of cases being male and 29(36.25%) being female as compared to 49(61.25%) of controls being male and 31(38.75%) 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)	1.61±1.32 yrs	1.88±1.71 yrs
Gender		
Male	51(63.75%)	49(61.25%)
Female	29(36.25%)	31(38.75%)

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 59.64 ± 11.82 ug/dl as compared to 84.94 ± 15.83 ug/dl for the controls (Table 1).A total of 45 cases and controls (28.12%) were found to have deficiency of zinc, of which majority (91.11%) 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	80	59.64	11.82	1.49	10.21,
Controls	80	84.94	15.83	2.12	p=0.0001,S

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 = 43.23 ± 6.06 ug/dl) having significantly lower value than that of Pneumonia group (Mean = 66.24 ±7.77 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 65.93 ± 7.73 ug/dl, cases requiring supplemental oxygen by nasal prongs having mean of 61.31 ±10.12 ug/dl and cases requiring mechanical ventilation having mean of 40.88 ±7.07 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 = 13) as compared to those who got discharged after treatment (n=67) (Table 3)

Table 3: Zinc level according to clinical characteristics in cases

IMNCI Grading	**No of cases**	**Mean(ug/dl)**	**SD**	**t-value**
Pneumonia	57(71.25%)	66.24	7.77	10.32 p=0.0001,S
Severe Pneumonia	23(28.75%)	43.23	6.06	
Total	80(100%)	59.51	12.31	
O2 Requirement				
Room Air	36(45%)	65.93	7.73	34.92 p=0.0001,S
Supplemental Oxygen	28(35%)	61.31	10.12	
Mechanical Ventilation	16(20%)	40.88	7.07	
Outcome				
Discharge	67(83.75%)	61.98	11.21	38.61 p=0.0001,S
Death	13(16.25%)	41.15	6.27	

The mean serum zinc level was found to have a negative correlation ('r' = -0.052) with the duration of stay of cases, however, this correlation was statistically not significant (p value = 0.682)

Discussion

The mean serum zinc levels were comparable to that found in the study by Hussain et al.[15] 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.[16,17] 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[18] 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.[16-18] 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.[19,20] 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.[19] 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 = 43.23 ± 6.06 ug/dl) having significantly lower value than that of Pneumonia group (Mean = 66.24 ±7.77 ug/dl) and similar findings were seen in study by Rady et al., Hussain et al. and Brooks et.al.[15,17,21] 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.[21] However, evidence to the contrary was found in studies by Bose et al. and Valentiner - Branth et al.[22,23] 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.[23] 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.[21,24,25] 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.[22,23,26] 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 65.93±7.73 ug/dl, cases requiring supplemental oxygen by nasal prongs having mean of 61.31±10.12 ug/dl and cases requiring mechanical ventilation having mean of 40.88±7.07 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.[17,21-23] 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.[17,21,]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.[28]

Conclusion

We concluded that the zinc has a significant role to play in the acquiring and progression of ALRTI in children aged 2 months to 5 years, with patients of ALRTI having significantly lower zinc levels as compared to age, sex and nutritionally matched controls. Also, the deficiency of zinc causes the patient to have a more serious form of the disease, requiring patient to be hospitalized for longer duration as well as requiring higher oxygen demands and increasing the chances of mortality by it.

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