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"SERUM ZINC LEVELS AS A PREDICTOR OF MORTALITY IN UNDER 5 CHILDREN WITH PNEUMONIA"

Paediatric Medicine

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ABSTRACT

Objectives: 1.To assess the reliability of serum Zinc levels as predictor of mortality in children with Pneumonia; 2. and to compare serum Zinc levels in pneumonia and severe pneumonia. **Method:** It is a prospective observational study that included all children aged 2 months to 59 months with features of pneumonia or severe pneumonia as per the Revised WHO guidelines 2014 for paediatric pneumonia. Serum Zinc levels were measured in all children included in the study, whereas the PRISM 3 scoring system was applied only to children with Severe disease who were admitted to the PICU. Serum Zinc levels of < 80mcg/dl suggested Zinc deficiency. All children were followed up for the predefined primary outcomes – Discharge/ Death. The association of low serum Zinc levels in severe pneumonia and the accuracy of Serum Zinc levels in predicting mortality was assessed using appropriate statistical methods. **Results:** Of the 100 children enrolled in the study, 54% were <1 year of age, with a slight male preponderance in incidence of pneumonia. 54% of included children had features suggestive of severe pneumonia at admission. Case fatality rates were 0% in the pneumonia group and 22.4% in the severe pneumonia group. The mean Serum Zinc levels were 82.81 +/- 19.989 mcg/dl in the pneumonia group and 71.98 +/- 26.605 mcg/dl in the severe pneumonia group($p = 0.029$). The mean PRISM 3 score was 25.92(SD 3.715) in the mortality group whereas it was 1.56 (SD 7.216) in the discharge group ($p = <0.001$).There was a strong negative correlation (r of -0.708) between Serum Zinc levels and the PRISM 3 score. PRISM 3 had an Area Under Curve (AUC) of 0.993 (95% CI of 0.925 to 1.000) whereas Serum Zinc had AUC of 0.958 (95% CI of 0.870 to 0.993) with a sensitivity of 92.31%, specificity of 91.95%, PPV of 63.2% and NPV of 98.8% in predicting mortality in childhood pneumonia. **Conclusion:** Serum Zinc levels can be a simple test to predict mortality in children with pneumonia .

KEYWORDS

Pneumonia; Mortality; Serum Zinc levels; PRISM 3 scoring system

INTRODUCTION

Pneumonia is defined as an infection or inflammation of the lung parenchyma [1] .The etiology of paediatric pneumonia varies according to age. Bacterial pathogens such group B streptococci, Klebsiella, Escherichia coli, and Listeria monocytogenes may be present in the birth canal and pose a risk to newborns. Late-onset newborn pneumonia is caused by Streptococcus pneumoniae, Streptococcus pyogenes, and Staphylococcus aureus. In older infants and toddlers between the ages of 30 days and 2 years, viruses are the primary cause of pneumonia. Respiratory viruses are also most prevalent in youngsters between the ages of 2 and 5.

Acute respiratory infection(ARI) is a leading cause of morbidity and mortality among under-5 children in the low and middle income countries. Pneumonia is the leading cause of mortality in children worldwide, with India accounting for 20% of those deaths and a higher burden of childhood pneumonia than any other country[5] .

Under-5 mortality in India is 32 per 1000 LB(SRS 2020) [3]. NFHS 4 states that the prevalence of ARI in India is 2.7% of the general population and 73.2% among hospitalised children in India [4]. SDG 3 has set a target of Under-5 mortality rate of 25 per 1000 LB to be achieved by 2030[5].

Zinc is an essential trace element which has a role in mucosal barrier function, inate and adaptive immunity, oxidative stress response and a cofactor for enzymes [6] .Zinc deficiency has been responsible for up-to 4.4% of deaths attributable to infection in developing countries. WHO states that up to 25% of the Indian population is Zinc deficient. Several studies have established low serum Zn levels in children with severe pneumonia. Zinc supplementation in treatment of severe childhood pneumonia is recommended by the WHO and standard textbooks in developing countries.

To the best of our knowledge and publication search, there are no studies with respect to the role of Serum Zinc in predicting mortality in childhood pneumonia in Indian population. Hence, the relevence of this study.

Zinc: Zinc is a crucial trace element in humans [7] It is the only metal that can be found in all kinds of enzymes and is the second most common trace metal in people after iron. Zinc, a crucial cofactor for numerous enzymes.

Role Of Zinc In Immunity :

Zn deficiency leads to a high susceptibility to many infections. Zn supplementation efficiently reduces chronic dysfunctional inflammatory responses while also significantly boosting immunity. These results strongly imply that Zn is necessary for immune cells to maintain appropriate homeostasis and function.

METHODS

It was a Prospective observational study done from February 2021 to July 2022 (18 months) in ramaiah medical college.Children aged between 2 months and 59 months presenting to Ramaiah medical college with features suggestive of pneumonia or severe pneumonia as per the Revised WHO classification of childhood pneumonia were enrolled in this study.

Children on Zinc supplements in the past 15 days, Children with diarrhoea in the past 15 days, Children with Aspiration pneumonitis and Children on steroid therapy (in the past 2 years) and immunocompromised children were excluded from this study.

Informed consent was taken from the parents/guardian of the children involved in the study. The presenting complaints of the child were noted. They were examined for respiratory rate, chest retractions and the danger signs of Pneumonia ;and categorized as either pneumonia or severe pneumonia as per the Revised WHO classification of Childhood Pneumonia – 2014. The PRISM 3 scoring system was applied to children with severe pneumonia admitted in the PICU, within 24 hours of admission.Investigations 5ml of venous blood was collected under all aseptic precautions within 24 hours of admission and Stored at -20 degrees until analysis.

The name of the testing kit was "CORAL Clinical systems" Zinc kit The Principle of the testing kit being Colorimetric assay – by using absorptiometry. The Normal reference values for zn – 60 to 120 microgram/dl .All children in the study were followed up for the outcome – either recovery and discharge OR death.

Statistical Analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data. Continuous data was represented as mean and

standard deviation. **Independent t test** was used as test of significance to identify the mean difference between two quantitative variables.

Correlations were performed with **Pearson Correlation coefficient** Receiver operating characteristic curves (ROCs) was constructed for Zinc levels and mortality. Comparison of Zinc levels with PRISM score was done. Receiver operating characteristic (ROC) and optimal cut-off points was chosen for the calculation of sensitivity, specificity, positive and negative predictive values. A test that predicts an outcome no better than chance has an area under the ROC curve of 0.5. An area under the ROC curve above 0.8 indicated fairly good prediction.

P value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical Software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data

RESULTS.

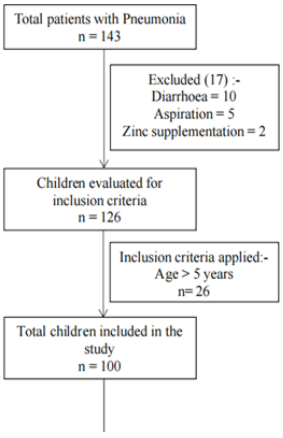
During the study period, 143 children were admitted to the hospital with pneumonia, out of which 17 children were excluded as per the exclusion criteria. After the inclusion criteria was applied, a total number of 100 children were deemed eligible for the study. Out of the 100 included children, 42 children had Pneumonia and were admitted in the wards, whereas 58 children had Severe pneumonia ,requiring admission to the PICU. Serum Zinc levels were measured in all 100 patients (both groups), while PRISM 3 scoring system was applied only to the Severe pneumonia group. Children of both groups were followed up for the primary outcome –Discharge or death.

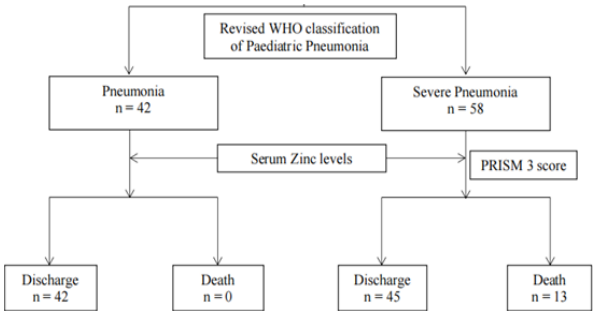
Among the 100 children included in the study, 54% were infants, while 46% were between 1-5 years of age.The gender distribution among all the included children was almost similar, boys comprised of 54%, whereas girls were 46%.All the children included in the study had tachypnea, which followed a prodrome of cough and cold. Chest indrawing/ retractions was observed only in 56% of the included children. Persistant vomiting was the most commonly observed danger sign (22%) , followed by lethargy(15%) and convulsions (1%).

During the study period, we found that more children presented to our institute with severe pneumonia (58%), when compared to children with pneumonia (42%) At admission, Serum Zinc levels was measured in all included children in both the groups. The predefined primary outcome in the study was either Death or Discharge from the hospital. The mortality rate secondary to Under 5 Pneumonia in our centre was found to be 13%.In our study, deaths due to severe pneumonia occurred more frequently in the age group <1 year (infants) .Mortality secondary to paediatric pneumonia was found to be slightly more common in girls when compared to boys, in our study ; although it was not statistically significant.

Among the 2 study groups, mortality was observed in only children who presented to our centre with severe pneumonia. All children who presented with pneumonia, did not progress to severe pneumonia, nor did any child in the pneumonia group suffer Mortality. Mortality secondary to paediatric pneumonia was found to be significantly associated with lower S.Zn levels , when compared to the children who survived, with a p value of <0.001

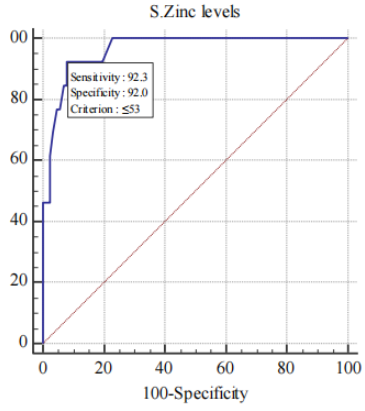
Table 1 Shows The Flowchart Of Patients Enrolled In The Study.





The mean PRISM 3 score among the included children who were discharged was 1.56, whereas it was 25.92 among children who suffered mortality. Our study demonstrated a statistically significant association between the PRISM 3 scoring system and the primary outcome(discharge/death), with a p value of <0.001.

The study also demonstrated a strong negative correlation between S.Zn levels and PRISM 3 score , with a correlation coefficient value (r) of -0.708 and a p value of <0.001.A ROC curve was drawn to test the hypothesis of the study. It showed that S.Zn levels < 53 mcg/dl predicted mortality in children with Pneumonia , with a sensitivity of 92.31%, specificity of 91.95%, PPV of 63.2% and NPV of 98.8% (AUC – 0.964; p value <0.0001)



ROC for Serum Zinc Levels in predicting mortality

Table ROC for serum zinc levels in predicting mortality

Area under the ROC curve (AUC)	0.964
95% Confidence interval	0.906 to 0.991
P value	<0.0001

Demographic Data:

Among the 100 children included in the study, 54% were infants, while 46% were between 1-5 years of age.The gender distribution among all the included children was almost similar, boys comprised of 54%, whereas girls were 46%.

All the children included in the study had tachypnea, which followed a prodrome of cough and cold. Chest indrawing/ retractions was observed only in 56% of the included children. Persistant vomiting was the most commonly observed danger sign (22%) , followed by lethargy(15%) and convulsions (1%).

All children presenting to our hospital (study centre), were grouped into two groups :-

a) Pneumonia group (children fitting into the diagnosis of pneumonia as per Revised WHO guidelines for paediatric pneumonia) – admitted to the wards
b) Severe Pneumonia group (children fitting into the diagnosis of Severe pneumonia as per Revised WHO guidelines for paediatric pneumonia) – admitted to the PICU.

During the study period, we found that more children presented to our institute with severe pneumonia (58%), when compared to children with pneumonia (42%)

At admission, Serum Zinc levels was measured in all included children in both the groups. The predefined primary outcome in the study was either Death or Discharge from the hospital.

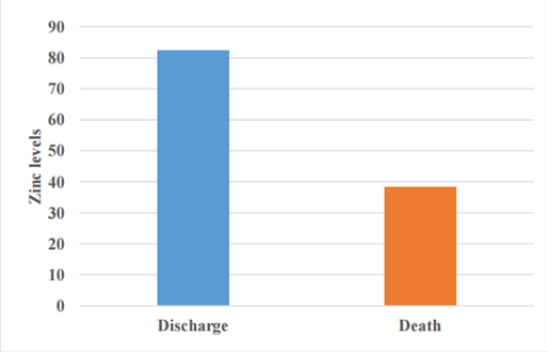
Primary outcome : Death /Discharge

The mortality rate secondary to Under 5 Pneumonia in our centre was found to be 13% . In our study, deaths due to severe pneumonia occurred more frequently in the age group <1 year (infants).

Mortality secondary to paediatric pneumonia was found to be slightly more common in girls when compared to boys, in our study ; although it was not statistically significant.

Primary study variable :- Serum Zinc(S.Zn) levels

The mean S.Zn levels in the Pneumonia group was 82.82 +/- 19.989 mcg/dl, whereas it was 71.98 +/- 26.605 mcg/dl in the Severe pneumonia group, which is statistically significant with a p value of 0.029.Mortality secondary to paediatric pneumonia was found to be significantly associated with lower S.Zn levels , when compared to the children who survived, with a p value of <0.001.



Graph Showing Comparison Of Mean Zn Levels According To Outcome.

Table Showing-comparison Of Mean Zinc Levels According To Outcome

	Mean	SD	P Value
Discharge	1.56	3.715	<0.001
Death	25.92	7.216	

Secondary Study Variable : PRISM 3 scoring system

The mean PRISM 3 score among the included children who were discharged was 1.56, whereas it was 25.92 among children who suffered mortality.

Our study demonstrated a statistically significant association between the PRISM 3 scoring system and the primary outcome (discharge/ death), with a p value of <0.001.

Table 2: Comparison of mean PRISM 3 score according to Outcome

	Mean	SD	P Value
Discharge	1.56	3.715	<0.001
Death	25.92	7.216	

The study also demonstrated a strong negative correlation between S.Zn levels and PRISM 3 score , with a correlation coefficient value (r) of -0.708 and a p value of <0.001. A ROC curve was drawn to test the hypothesis of the study.

It showed that S.Zn levels < 53 mcg/dl predicted mortality in children with Pneumonia , with a sensitivity of 92.31%, specificity of 91.95%, PPV of 63.2% and NPV of 98.8% (AUC – 0.964; p value <0.0001).

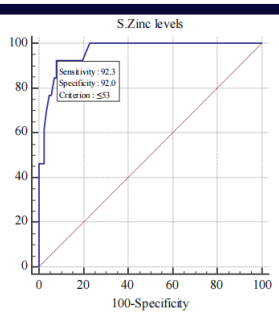


Figure 26: ROC for Serum Zinc Levels in predicting mortality

Table ROC for Serum Zinc levels in predicting mortality

Area under the ROC curve (AUC)	0.964
95% Confidence interval	0.906 to 0.991
P value	<0.0001

Table Characteristics Of S.zinc Levels As A Test To Predict Mortality

Cut off ≤53	Value	95% CI
Sensitivity	92.31	64.0 - 99.8
Specificity	91.95	84.1 - 96.7
PPV	63.2	38.4 - 83.7
NPV	98.8	93.3 - 100.0

Serum Zinc levels had AUC 0.958 and PRISM 3 score had AUC 0.993, suggesting good accuracy as a test in predicting mortality in children with severe pneumonia.

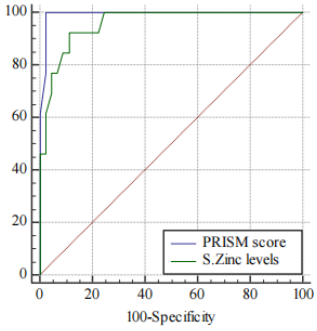


Figure: ROC comparison for Serum Zinc Levels and PRISM 3 score in predicting Mortality.

Table: Comparison of AUC of S.Zinc levels and PRISM 3 score

	AUC	95% CI
PRISM 3 score	0.993	0.925 to 1.000
S.Zinc levels	0.958	0.870 to 0.993

DISCUSSION

Pneumonia is the leading infectious cause of mortality in children under the age of 5 years, with over 7 lakh deaths annually or about 2000 deaths per day. The approximate incidence in South east Asia is 2500 cases per 1 lakh children [8]. The burden of the disease has warranted attention from national and international policy makers towards its prevention and therapy.

Zinc has been identified as one of the key trace elements in the human physiology, with a major role in the function of the Innate and adaptive immune systems. The role of Zinc in childhood pneumonia has particularly been the focus of research in the past 1-2 decades. A double blinded Randomized controlled trial by Abdullah et.al demonstrated early recovery in children hospitalized with Pneumonia, under the age of 2 years and suggested Zinc supplementation, along with

conventional antimicrobial therapy in paediatric pneumonia[9]

Several studies have also established low serum Zinc levels in children suffering from pneumonia . In a hospital based case control study performed in a tertiary care hospital in Egypt, Hamed et.al. demonstrated significantly lower Serum Zinc levels in children with Pneumonia, when compared to age matched healthy controls[10] . Our study aims to primarily assess whether Serum Zinc levels can be used as a prognosticating tool to predict mortality in childhood pneumonia.

We observed in our study, that 54 % of the study population were infants , suggesting that the infantile age group was more susceptible to pneumonia, compared to children aged 2-5 years. A hospital based case control study done by Kumar et.al in North India also demonstrated that 74% of the Under 5 children who were admitted for pneumonia were <1 year of age[11], suggesting that age could be a non modifiable risk factor for developing Pneumonia.

With respect to genderwise predisposition for developing pneumonia, there has been no definitive consensus among various studies. Srinivasan et.al. conducted a double blinded RCT to investigate the role of zinc supplementation in paediatric pneumonia and they have identified a slight male preponderance with respect to the incidence of the disease, although not statistically significant [12] .Our study also demonstrates a non significant male preponderance(54%) among all included children.

In this study, we have used the Revised WHO guidelines 2014 , for diagnosing and categorizing children clinically as Pneumonia and Severe Pneumonia. Radiological diagnosis was not considered for diagnosis as neither the WHO or the ARI programme by the Government of India suggest Chest Xray as a diagnostic tool in paediatric pneumonia. The latest recommendations by the British Thoracic Society also recommend against performing an Xray at admission, in non severe cases of paediatric pneumonia[12.]

The predefined primary outcomes in our study were Discharge from the hospital or Death. The Case fatality rate of paediatric pneumonia in our study was found to be 0% for Pneumonia and 22% for Severe pneumonia, which is in line with contemporary Indian standards. A prospective observational study done by Bokade et.al. in a Tertiary care centre in India demonstrated a case fatality rate of 6.33% for Pneumonia and 15.94% for Severe Pneumonia[13.] Our study also noted that mortality was more common in the infantile age group, showing 34.1% mortality , whereas mortality in >1 year old children was 8.7%, which was statistically significant.

In the past decade, Serum Zinc levels in pneumonia has been a topic of interest among researchers. A single centre surveillance study performed in Indian by Rajasekaran et.al. in 2014 , among children with pneumonia, concluded that Serum Zinc levels were significantly lower in children with Pneumonia, when compared to age matched healthy controls[14] . An RCT performed by Basnet et.al in 2012, to test the role of supplemental Zinc as an adjuvant in the treatment of paediatric pneumonia, concluded that children who received Zinc supplementation had faster recovery and had lower duration of hospital stay[15.]

Our study compared Serum Zinc levels in children with Pneumonia and children with severe pneumonia. We demonstrated that mean serum Zinc levels in children with severe pneumonia was 71.98 +/- 26.605 mcg/dl, whereas it was 82.81 +/- 19.989 mcg/dl in children with pneumonia, which was statistically significant with a p value of 0.029. A significant highlight was that children who suffered mortality had a mean serum Zinc levels of 38.23 +/-14.001 mcg/dl, when compared to 82.25 +/- 20.264 mcg/dl in children who recovered and were discharged with the hospital (p value = <0.01). Hence, our study was in complete support of previous studies, demonstrating a significantly lower Serum zinc levels in children with severe pneumonia. However, a new finding was a significant statistical association of low serum Zinc levels with mortality in children with severe pneumonia.

Mortality indicators in the Paediatric intensive care unit have been extensively used in various Tertiary care centres. The PRISM 3 score (24 hours) has established itself as a simple but yet reliable indicator of mortality in children admitted to the intensive care units. A prospective cohort study done by De León et.al. To assess the performance of PRISM 3 scoring system in a tertiary care centre in France showed that the PRISM 3 score performed well in predicting mortality in children admitted to the PICU, with a sensitivity of 71% and a specificity of 64%[16].

A cross sectional study carried out by Kumar et.al to assess the association between mortality in paediatric pneumonia and the PRISM 3 scoring system, demonstrated a significant association between mortality and the PRISM 3 scoring system142. Our study also demonstrated a strong performance of the PRISM 3 scoring system in predicting mortality in children admitted with severe pneumonia, with an Area under curve (AUC) of 0.993 (95% confidence interval of 0.925 to 1.000) and a significant p value.

Our study also assessed the corelation between Serum Zinc and the PRISM 3 score in all children with severe pneumonia, which was a statistically significant strong negative corelation with a corelation coefficient (r) of -0.708.

CONCLUSIONS.

The novel finding in our study was that, Serum Zinc levels of < 53 mcg/dl(done at admission to the unit), predicted mortality in children with pneumonia, with a sensitivity of 92.31%, specificity of 91.95%, PPV of 63.2%, NPV of 98.8% and hence demonstrated a strong performance as a marker to predict mortality in childhood pneumonia. Pediatric pneumonia is a high burden disease with severe diseases demonstrating high case fatality rates. Infants are at a higher risk for developing severe pneumonia and also prone for mortality. Serum Zinc is a good predictor of mortality in under 5 children with pneumonia. The PRISM 3 scoring system demonstrates a good performance as mortality predictor in children with severe pneumonia. Serum Zinc levels , along with the PRISM 3 scoring system can accurately serve as mortality indicators in children with severe pneumonia

Limitations.

The most important limitation of the study is that it is a single centre study. Multicentric studies would help in reducing Institutional protocol based bias. A small sample size of 100 was another minor point of concern. The study was designed to compare Serum Zinc in the mortality group (children with mortality) with Serum Zinc levels in children who recovered .i.e use of a Internal control. An external age matched control group, with baseline Serum Zinc assessment would have provided a better picture of the association. Diagnosis and categorization of children solely based on the revised WHO guidelines for Paediatric pneumonia 2014, allowed for under 5 wheezers such as Viral episodic wheeze and multiple trigger wheeze to be included in the study, which may have partly confounded the results.

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