

## REVIEW REPORT

### Title: Serum zinc level in children with acute lower respiratory tract infection: A hospital-based case-control study

Authors: Taskina Mosleh, Nitin Das, Nilanjon Ghose

Reviewer A: Kazi Ali Aftab, ORCID: 0009-0001-5221-0021, COI: None, AI disclosure: None

1. Comment Appropriateness of the Title.

The title is good, But I suggest as The effect of serum zinc level in children with acute lower respiratory tract infection: a hospital based case-control study.

**Response:** Thank you for the valuable suggestion. However, we respectfully prefer to retain the original title: "Serum zinc level in children with acute lower respiratory tract infection: A hospital-based case-control study." Since the present study was observational in nature, the term "effect" may imply a causal relationship or interventional design, which was not the objective of this study. Our study aimed to assess the association between serum zinc levels and ALRTI rather than determining causality. Therefore, we believe the current title more accurately reflects the study design and findings.

2. Comment The Methods are described in sufficient details so that the study can be reproduced. Whether ethical concerns have been well described. Pre-valance of ALRTI in children in hospital was not written Actual calculated sample size of this study was not written. Sample size is so small to reach for concluding remarks.

**Response:** Thank you for these important observations. The proportion of ALRTI among screened children has been clarified in the Methods section by mentioning that among 36,298 screened children, 2,148 were diagnosed with ALRTI during the study period (*Lines 98-103*).

Regarding sample size, this study was conducted within a fixed study duration and resource limitations. All eligible participants fulfilling the inclusion criteria during the study period were considered for enrollment. We agree that the sample size was relatively small, and this limitation has now been acknowledged in both the Discussion and Limitations sections (*Lines 215-216*).

However, despite the relatively small sample size, statistically significant differences in serum zinc levels were observed between cases and controls, and multivariable analysis also demonstrated a significant association after adjustment for important confounders. Therefore, we believe the study still provides valuable preliminary local evidence from Bangladesh where updated data remain limited.

3. Comment Appropriate and thorough description of the Statistical methods.

Statistical methods were appropriate. Analysis was done with only zinc level. other risk factors were not present in regression analysis.

**Response:** Thank you for the comment. We would like to respectfully clarify that the multivariable logistic regression model included several potential confounding variables in addition to serum zinc level, including age, sex, maternal education, and smoking exposure, as described in the Statistical Analysis and Results sections. Smoking exposure was also found to be independently associated with ALRTI (*Line 144-145 and 173-174*).

We acknowledge that due to limitations in sample size and study scope, all possible variables could not be incorporated into the regression model. This limitation has now been further clarified in the revised manuscript (*Lines 212-213*).

4. Comment Pertinence of the Discussion section whether it justify the main message of the manuscript without repeating the results. Discussion section was well written but the other factors that may cause ALRTI was not discussed .

**Response:** Thank you for this important suggestion. Additional discussion regarding other established risk factors for ALRTI has now been further elaborated in the revised Discussion section to provide broader context for the findings (*Lines 206-217*).

5. Comment Whether Strength(s) and Limitation(s) are well described. Confounding risk factors were very important and which was not ruled out in this study was not mentioned in limitations.

**Response:** Thank you for this valuable observation. We agree that some residual confounding factors could not be fully evaluated in the present study. The limitation section has now been revised to mention those factors (*Lines 212-216*).

6. Comment Whether the Conclusion of the manuscript is supported by the data. Conclusion was supported by the data but the sample size was so small for making any final remarks.

**Response:** Thank you for this important comment. We agree that the relatively small sample size limits the precision and generalizability of the findings. However, despite the modest sample size, statistically significant differences in serum zinc levels were observed between cases and controls, and the association remained significant after multivariable adjustment. Therefore, we believe the findings provide important preliminary evidence regarding the possible association between severe zinc deficiency and ALRTI among Bangladeshi children.

- 7. Comment** Whether the manuscript is supported by appropriate and up-to-date References.  
Nine references were from more than 10 years.
- Response:** We respectfully note that several older references were intentionally retained because they represent landmark or foundational studies relevant to ALRTI epidemiology, zinc physiology, and WHO diagnostic criteria. However, to improve the overall contemporariness of the manuscript, additional recent references (Reference no-1, 24, 26, 27) have also been incorporated.
- 8. Comment** Straightforward, clear, and logical Storytelling.  
Storytelling is clear and straight forward but if other risk factors were assed it would be more logical.
- Response:** We agree that inclusion of additional risk factors could further strengthen the analysis and interpretation. However, the primary objective of the present study was specifically to evaluate the association between serum zinc levels and ALRTI. Due to limitations related to study scope and sample size, comprehensive assessment of all established ALRTI risk factors was not feasible. We have acknowledged this limitation more clearly in the revised manuscript and discussed its potential implications (*Lines 212-216*).

**Reviewer B: Amina Akter, ORCID: 0000-0001-9375-5805, COI: None, AI disclosure: None**

- 9. Comment** Appropriateness of the Title.  
Title is appropriate and scientifically clear but from my side methodology resembles cross sectional study rather than case control.
- Response:** We respectfully clarify that the present study was designed as a hospital-based case-control study because participants were selected based on disease status (children with ALRTI as cases and children without ALRTI as controls), and serum zinc levels were compared between these two groups. In a cross-sectional study, exposure and outcome are usually assessed simultaneously within a single population without predefined case and control groups. Since our methodology involved separate recruitment of cases and controls and estimation of odds ratios for association, we believe the study design is appropriately classified as case-control.
- 10. Comment** Completeness and accuracy of the Abstract.  
While the abstract adequately summarizes the study, it exceeds the desirable length.
- Response:** The abstract has been revised and shortened to improve conciseness while keeping the essential methodological details and principal findings (*Lines 25-47*).
- 11. Comment** Appropriate and thorough description of the Statistical methods.  
The statistical methods are generally appropriate for the study objectives. Multivariable analysis may strengthen the interpretation by controlling potential confounders. The analytical approach may not fully account for confounding variables. The regression model requires clarification regarding variable selection strategy.
- Response:** We would like to clarify that multivariable logistic regression analysis was already performed in the study to control for selected potential confounders including age, sex, maternal education, and smoking exposure. This has now been described more clearly in the Statistical Analysis section (*Lines 144-245 and 172-176*).
- 12. Comment** Quality, clarity and appropriateness of the Table(s).  
Tables are informative; however, formatting could be improved for clarity. In table 1, smoker characteristics, active or passive as age below 5 yrs should be defined in footnotes. Nutritional status of child, feeding history is important as age range 2 months to 60 months some were breast feed baby. other variables like economic status , maternal nutrition . how maternal education related this study clarification recommended. another one is in case of SD no upper and lower limit value and number percent not clearly showed.
- Response:** The tables have been reformatted to improve clarity and presentation. Fo clarity “smoker” variable is replaced with “passive smoking” in Table 1.  
We agree that nutritional status, breastfeeding history, socioeconomic condition, and maternal nutritional status are clinically relevant variables in ALRTI research. However, the primary objective of this study was specifically to evaluate the association between serum zinc level and ALRTI. Due to limitations in study scope and sample size, detailed assessment of all potential risk factors was not feasible. This limitation has now been acknowledged more clearly (*Lines 212-213*).  
Maternal education was included as ,previous literature has shown that parental education may influence child nutrition, healthcare-seeking behavior, hygiene practices, and exposure to infections. The presentation of mean  $\pm$  SD, percentages, and categorical distributions has also been revised for better readability and consistency. (Table 1 and table 2)
- 13. Comment** Quality, clarity and appropriateness of the Figure(s), if any.  
The figure is clear and visually informative.  
Inclusion criteria includes age group 2-60 months but figure shows initially enrolled 16497 patients then excluded according to age so clarification needed regarding this.
- Response:** The flowchart has now been revised (Figure-1) to clarify that the initial screened population included all pediatric respiratory cases attending the hospital during the study period, after which age-based and other eligibility criteria were applied to identify eligible ALRTI cases within the target age range of 2–60 months.

- 14. Comment** Pertinence of the Discussion section whether it justify the main message of the manuscript without repeating the results. While the discussion adequately summarizes the principal findings, substantial improvement is needed in contextualizing the results within current literature and addressing methodological limitations.
- Response:** The Discussion section has been revised. Additional emphasis has also been given to methodological limitations, including small sample size, possible residual confounding, and limited assessment of nutritional and environmental variables (*Lines 206-217*).
- 15. Comment** Whether the manuscript is supported by appropriate and up-to-date References. The references are relevant and appropriately cited. Several references are outdated and should be updated with more recent literature ( reference number 11,12,13 etc).
- Response:** Some older references were retained intentionally because they represent foundational studies relevant to zinc physiology and ALRTI epidemiology. However, additional recent references have now been incorporated to improve the contemporariness and balance of the literature review.( Reference no-1,24,26,27)
- Responsible editor: M Mostafa Zaman, ORCID: 0000-0002-1736-1342**
- 16. Comment** The role of zinc in lower respiratory tract infection is well established. Therefore, the justification for conducting the study is not strong. Kindly clarify why the readers should read it. You may have a specific gap to bridge.
- Response:** We agree that the association between zinc deficiency and lower respiratory tract infection has been explored previously. However, findings across studies remain heterogeneous due to differences in population characteristics, nutritional status, socioeconomic conditions, and study methodology. In Bangladesh, updated data evaluating serum zinc status in children with ALRTI remain limited.
- In contrast to several earlier local studies, our study evaluated the association between severe zinc deficiency and ALRTI using multivariable logistic regression while adjusting for selected potential confounders including age, sex, maternal education, and passive smoking exposure. The study also quantified the magnitude of association between severe zinc deficiency and ALRTI and highlighted the additional contribution of household smoke exposure among Bangladeshi children. We believe this study provides updated context-specific evidence from a resource-limited setting that may contribute to future nutritional and preventive public health strategies. The study rationale and knowledge gap have now been clarified further in the Introduction section (*Lines 76-89*).
- 17. Comment** The Abstract is lengthy; its word count should be <250. The Background could easily be reduced: one sentence for the background and one sentence for the objective.
- Response:** The abstract has been revised and shortened to fit with the journal's preferred word limit. The Background and Objective sections have been condensed while preserving the essential scientific information.
- 18. Comment** The abstract indicates a recruitment of matched controls, which is different from the Methods section of the main text. Please clarify which one is correct. For the matched case-control study, the analysis should have been different.
- Response:** We sincerely apologize for the lack of clarity. The controls were not individually matched to cases. Therefore, the study should be considered an unmatched case-control study. It has been now corrected in abstract section (*Line 35*).
- 19. Comment** The word count of the manuscript should be reduced to 2000 or fewer.
- Response:** The manuscript has been carefully edited to improve conciseness. Several sections, particularly the Introduction, Abstract, and Discussion, have been shortened to reduce the overall word count while preserving the scientific content and clarity.
- 20. Comment** Table two could have crude OR in addition to the adjusted OR.
- Response:** Crude odds ratios have now been added to Table 2 along with adjusted odds ratios to improve clarity and allow better interpretation.
- 21. Comment** Figure 1 should have another exclusion box for the controls (because you have excluded 2031), similar to the cases.
- Response:** Figure 1 has now been revised to include a separate exclusion box for controls, similar to the cases to improve transparency and methodological clarity.
- 22. Comment** The Abstract is not formatted according to the Journal style. The background statement does not justify the gap in the established knowledge.
- Response:** The abstract has been revised and reformatted according to the Journal guidelines. The background section has also been modified to specify the existing knowledge gap better (*Line 26-30*).
- 23. Comment** The key message still has an age-matched case-control study; no matching was done.
- Response:** The term "age-matched" has been replaced by "unmatched" in the key message (*Line 48*).

- 24. Comment** The knowledge gap given in lines 76-80 is too general. It should be specified.
- Response:** The knowledge gap has been revised and specified with appropriate references (18,19,20,21) for clarity (Lines 73-79).
- 25. Comment** The exclusion of subjects described in lines 114-116 does not match the flowchart's exclusion box.
- Response:** Flow chart (Figure1) has been revised for the consistency of exclusion criteria with methods in (Line 108-111).
- 26. Comment** Validity of serum zinc measurement should be addressed. Was it done using the same machine and the same batch of the reagent? Inter- and intr-observer variations should be described. What was the coefficient of variation?
- Response:** The laboratory assessment section has been revised to clarify that all serum zinc measurements were performed in the biochemistry laboratory, BMU using the same spectrophotometer and standardized colorimetric method throughout the study period (Line 126-140).
- 27. Comment** How were the variables selected for the statistical model described in line 144?
- Response:** The Methods section has been revised to clarify that variables for the multivariable logistic regression model were selected based on previous research, clinical relevance, and their known association with ALRTI and zinc status (Line 150-152).
- 28. Comment** Avoid +/- signs before the SDs; we use SDs in the parenthesis.
- Response:** All results throughout the manuscript have been revised to present standard deviations within parentheses
- 29. Comment** Begin the Discussion by reiterating your key findings. Do not use expressions like "so" to begin a sentence. Use "therefore."
- Response:** The Discussion section has been revised. Informal transitional expressions such as "so" have been replaced with "therefore."
- 30. Comment** Table 1: Reduce the number of educational categories to have a meaningful number in each category. Table 2 has P for the adjusted OR but not for the crude OR. Either use it for both or just remove it. We do get it from the 95% CIs.
- Response:** In table 1, Maternal education categories have been regrouped into "up to primary education" and "above primary education" to ensure more meaningful sample sizes within each category. In Table 2, the p-value column is removed
- 31. Comment** The Abstract must align with the changes made.
- Response:** The Abstract has been revised to ensure consistency with the updated manuscript.
- 32. Comment** The Methods section does not include anthropometric indices. It has not been presented in the Results section; it has not been discussed either. Its negative association deserve discussion. Make sure that the finding is not refuted by others.
- Response:**
1. Anthropometric assessment has now been described in detail in the Methods section (Lines 115–120)
  2. The findings have been reported in the Results section (Lines 156–159, Table 1).
  3. These variables have also been included in the multivariable logistic regression analysis (Table 2).
  4. The discussion has been expanded to address the lack of a significant association between anthropometric indices and ALRTI. We discuss possible explanations for this finding, including the exclusion of children with overt malnutrition, the relatively homogeneous nutritional status of the study population, and the possibility that anthropometric indicators may not adequately reflect micronutrient deficiencies such as zinc deficiency. (Lines 209–221).
- 33. Comment** Passive smoking has been highlighted in this study, but not discussed with emphasis. Studies in favour and against must be brought in.
- Response:** The Discussion section has been expanded to provide a more detailed discussion of passive smoking as a risk factor for ALRTI (Lines 223–231).
- 34. Comment** Table 2 needs revision to include ORs for the third category of anthropometric variables
- Response:** The table has been revised to include odds ratios and adjusted odds ratios for all categories of the anthropometric variables, using the designated reference category.