

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Pregnancy anemia, child health and development: A cohort study in rural India
<b>AUTHORS</b>	Heesemann, Esther; Mähler, Claudia; Subramanyam, Malavika; Vollmer, Sebastian

### VERSION 1 – REVIEW

<b>REVIEWER</b>	Godoy Figueiredo, Ana Claudia Morais. State Department of Health of the Federal District, Epidemiological surveillance
<b>REVIEW RETURNED</b>	26-Feb-2021

<b>GENERAL COMMENTS</b>	<p>I would like to say that the topic is very important for public health. However, the article raises important concerns about the analytical inference of the data.</p> <ol style="list-style-type: none"><li>1) There are systematic reviews published on the topic that can help to provide a better basis on the hypothesis investigated. Systematic reviews help to clarify dissent from the literature.</li><li>2) There are concerns about calculating the sample size. The researchers did not demonstrate the minimum calculation, nor whether the sample has enough statistical power to answer the hypothesis. This reduces the credibility of the results. I recommend that the calculation of the power after completion of the research be carried out.</li><li>3) There are concerns about the statistical analysis performed. The significance levels of the tests were not described. The models chosen for categorical variables were not sufficiently justified. The measures that would be used have not yet been presented. The selection of confounders is not clear enough.</li><li>4) The article presents important problems regarding data analysis and inference of the findings.</li></ol>
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<b>REVIEWER</b>	McClure, Elizabeth Research Triangle Institute
<b>REVIEW RETURNED</b>	04-Mar-2021

<b>GENERAL COMMENTS</b>	<p>This is a very nice study describing the association of hemoglobin during pregnancy and child outcomes, using prospective measurement in a population identified through pregnancy registers. One important issue that is not described well is when during pregnancy (i.e., GA) the hemoglobin was measured (or if multiple measures were taken, which was used for the study). If 'trimester in pregnancy' (table 1) indicates the trimester of hb</p>
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	<p>assessment, there appears to be an association, as expected, with lower hb levels at later GA. There is potential for bias regarding this issue. The authors noted the large proportion of women who had no ANC, which is a strength of the study in terms of generalizability; however, also potential bias in terms of availability/quality of data. There was also substantial loss-to-follow-up as well as newborn mortality between enrollment and outcome ascertainment, which is of concern for bias. Finally, one issue that has been raised in prior studies is the U-shaped curve (i.e., adverse outcomes associated with both very low and high hb during pregnancy). It may be interesting to at least comment on the high hb levels (recognizing this is beyond the study objectives). Given the limitations of the data and methodology, the authors may want to temper their conclusions.</p>
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### VERSION 1 – AUTHOR RESPONSE

#### Reviewer 1

Comment 1: “1) There are systematic reviews published on the topic that can help to provide a better basis on the hypothesis investigated. Systematic reviews help to clarify dissent from the literature.”

Authors’ reply:

We thank the reviewer for this suggestion. We have added two additional systematic reviews to the introduction, in the second and third paragraph:

1. Georgieff MK. Iron deficiency in pregnancy. American Journal of Obstetrics and Gynecology. 2020;223:516–24. (reference 17 in the manuscript)

2. McCann S, Perapoch Amadó M, Moore SE. The Role of Iron in Brain Development: A Systematic Review. Nutrients. 2020;12:2001. (reference 22 in the manuscript)

Further we extended the discussion of the lasting association of pregnancy Hb and child Hb and argue that prolonged breastfeeding might inhibit catching up of iron deficient children at birth.

See section “Discussion”, subsection “Interpretation”, second paragraph:

„It is also possible that the high rate of prolonged breastfeeding (44% of the sample) hinders children with low iron stores at birth to catch up through an iron rich diet. The negative association between continued breastfeeding, anemia and iron deficiency of young children found in other studies supports this hypothesis. [4, 45]”

Comment 2: “2) There are concerns about calculating the sample size. The researchers did not demonstrate the minimum calculation, nor whether the sample has enough statistical power to answer the hypothesis. This reduces the credibility of the results. I recommend that the calculation of the power after completion of the research be carried out.”

Authors’ reply:

Thank you for this observation. We conducted an ex-post minimum detectable effect calculation, with an alpha of 10% and power of 80%. We concluded that with the given sample size, we are able to detect statistically significant differences between mild or moderate-to-severe pregnancy anemia and no pregnancy anemia on child Hb of 0.22 g/dl and on skills of 0.12 standard deviations. The results of the minimum detectable effect calculation have been added to the manuscript.

See section “Results, subsection “Sample description”, first paragraph:

“We calculated an unadjusted minimum detectable effect of 0.22 g/dl for child Hb and 0.12 SD for skill outcomes, statistically significant at the 10% level, comparing children of mothers with mild or moderate pregnancy anemia to children of mothers without pregnancy anemia.”.

Comment 3: “3) There are concerns about the statistical analysis performed. The significance levels of the tests were not described. The models chosen for categorical variables were not sufficiently justified. The measures that would be used have not yet been presented. The selection of confounders is not clear enough.

The significance levels of the tests are described below each table. We used conventional significance level for p-values of 0.1, 0.05 and 0.01.

We had chosen to transform the categorical explanatory variable “pregnancy anemia” to dummy variables indicating, i) no pregnancy anemia, ii) mild pregnancy anemia and, iii) moderate-to-severe pregnancy anemia. The dummy variables were then included in the estimation model simultaneously, while “no pregnancy anemia” was used as reference category and hence excluded. We have now revised the description of explanatory variables to give more clarity on the model.

See section “Methods”, subsection “Statistical analysis”, second paragraph:

“For the non-linear relationship, we used the expressions of anemia status as predictors: no pregnancy anemia mild pregnancy anemia and moderate-to-severe pregnancy anemia. Dummy variables were created for no, mild and moderate-to-severe anemia, and simultaneously added to the regression equation. No anemia served as the reference category.”

We extended the explanation of confounders in the manuscript to clarify our selection.

See section “Methods”, subsection “Statistical analysis”, third paragraph:

“Overall poor household nutrition during childhood is likely to correlate with a poor nutrition of women during pregnancy. As both factors are likely to adversely affect child health and development, ignoring the nutritional environment at the time of the outcome measure might lead to an overestimation of the correlation of pregnancy anemia and child wellbeing. We therefore adjusted for maternal Hb levels the household food diversity score in 2017, at the time of the outcome measurement. [...]. Additional covariates from the baseline data relate to the socio-economic status of the household (caste category, wealth quintile, maternal literacy) and pregnancy characteristics which might correlated with both the Hb levels during pregnancy and child outcomes (maternal age, pregnancy history (first birth dummy), trimester of gestation and take-up of antenatal care (ANC) services).”

Comment 4: “4) The article presents important problems regarding data analysis and inference of the findings.”

Authors’ reply:

We thank the reviewer for this observation. We addressed the mentioned concern with regard to the data analysis and inference of the findings in the previous comments and adjusted the manuscript accordingly.

## Reviewer 2

Comment 1: “One important issue that is not described well is when during pregnancy (i.e., GA) the hemoglobin was measured (or if multiple measures were taken, which was used for the study). If ‘trimester in pregnancy’ (table 1) indicates the trimester of hb assessment, there appears to be an association, as expected, with lower hb levels at later GA. There is potential for bias regarding this issue.”

### Authors’ reply:

We would like to thank the reviewer for the comment. The trimester of pregnancy indeed indicated the gestational trimester of Hb measurement. We have now adjusted the table to clarify this matter. It now reads “Gestational trimester of Hb (preg.) measurement” in table 1. We acknowledge the potential bias and therefore had included the gestational trimester during measurement as confounder to the estimations. We now explicitly raise this concern in the manuscript.

See section “Results”, subsection “Sample description”, fourth paragraph:

“Women with more severe pregnancy anemia are on average were on average in a higher gestational trimester at the time of measurement. This is unsurprising considering the elevated iron needs as pregnancy progresses. Yet as gestational trimester also correlates with child age, which might affect the development test outcomes and anemia status, we will control for gestational trimester at the time of pregnancy Hb measurement in all estimations, to avoid biases.”

Replacing gestational trimester during HB measurement with the gestational age in months or subsample analysis in each of the three gestational trimesters does not change the results. See section “Results”, subsection “Association of pregnancy anemia with childhood anemia and early skills”, fourth paragraph:

“Robustness checks including gestational month instead of gestational trimester, and the subgroup analysis by gestational trimester also confirmed our main results.”

Comment 2: “The authors noted the large proportion of women who had no ANC, which is a strength of the study in terms of generalizability; however, also potential bias in terms of availability/quality of data.”

### Authors’ reply:

We thank the reviewer for this comment. All data used in this study is primary data, collected from the authors in the villages in 2015 and 2017. Local data collectors were trained for blood sample collection and the analysis was done on the spot with HemoCue 301 machines. We acknowledge that point-of-care tests, such as the HemoCue, are slightly less accurate than laboratory tests. Yet, studies found that the bias of the HemoCue 301 lies with 0.25 g/dl well below the WHO defined threshold of 1 g/dl (Yadav et al 2020). Given the wide range of Hb values in our sample, we are confident that our findings are not driven by measurement error.

See section “Methods”, subsection “Outcome measures”, first paragraph, footnote 1:

“The bias of HemoCue 301 anemia assessments compared to laboratory tests is with 0.25 g/dl well below the recommended threshold for point-of-care machines according to the WHO.

[32]“

We further discuss the potential threat to the internal validity from missing Hb measurements of women and children in the limitations section.

See section “Discussion”, subsection “Limitations”, second paragraph:

“The loss of follow-up of around half of the study children is reason for concern about the external validity of our findings. Despite weighting the study sample according to their inverse probably of attrition, we are only able to correct for observable differences in the study population and the lost observations. If the unobserved characteristics which caused the loss in follow-up also correlated with the explanatory and outcome variables, the results of our analysis would be biased. “

Comment 3: “There was also substantial loss-to-follow-up as well as newborn mortality between enrollment and outcome ascertainment, which is of concern for bias.”

Authors’ reply:

We agree with the reviewer that the loss-to-follow up weakens our analysis. Yet we had weighted our sample with each observation’s probability to be lost between the waves and thereby avoided biases based on observable characteristics. We acknowledge however that this procedure does not correct for unobservable determinants of loss-to-follow up. We have now included this concern in the limitation section.

See section “Discussion”, subsection “Limitations”, second paragraph:

“The loss of follow-up of around half of the study children is reason for concern about the external validity of our findings. Despite weighting the study sample according to their inverse probably of attrition, we are only able to correct for observable differences in the study population and the lost observations. If the unobserved characteristics which caused the loss in follow-up also correlated with the explanatory and outcome variables, the results of our analysis would be biased. “

Comment 4: “Finally, one issue that has been raised in prior studies is the U-shaped curve (i.e., adverse outcomes associated with both very low and high hb during pregnancy). It may be interesting to at least comment on the high hb levels (recognizing this is beyond the study objectives).”

Authors’ reply:

Thank you very much for this comment. We agree with the reviewer that discussing the potential Ushaped relationship is of interest. For this reason, we have added a third estimation specification testing the U-shaped correlation of pregnancy Hb and child outcomes. The results, added to Table 2, showed a decrease in effect size on childhood Hb, the diminished effect is small and not able to full reverse the relationship in our sample.

See section “Introduction”, first paragraph:

“Yet, also high hemoglobin levels, especially in the first trimester, are associated with adverse birth outcomes, suggesting a U-shaped relationship [10].”

See section “Methods”, subsection “Statistical analysis, second paragraph:

“To test a possible U-shaped relationship between pregnancy Hb and the primary child outcomes, we include a quadratic term to the linear regression model.”

See section “Discussion”, subsection “Interpretation”, first paragraph:

“With increased pregnancy Hb level, the association with childhood Hb becomes weaker, yet in our sample it does not reach the tipping point to a full reversal. This might be due to the small sample size on the higher end of the Hb spectrum.”

See section “Discussion”, subsection “Interpretation”, third paragraph:

“The suggestive evidence for an inverse U-shaped relationship with childhood Hb is in line with findings on preterm birth and small-for-gestational age in a review of 19 studies across the world. [10]”

Comment 5: “Given the limitations of the data and methodology, the authors may want to temper their conclusions.”

Authors’ reply:

We thank the reviewer for their comment. We extended the limitation section, to address the concerns regarding loss of follow up.

In the section “Discussion”, subsection “Limitations”, second paragraph:

“The loss of follow-up of around half of the study children is reason for concern about the external validity of our findings. Despite weighting the study sample according to their inverse probably of attrition, we are only able to correct for observable differences in the study population and the lost observations. If the unobserved characteristics which caused the loss in follow-up also correlated with the explanatory and outcome variables, the results of our analysis would be biased. “

We also revised the concluding remarks in the main text and the abstract.

See section “Conclusion”:

“We find strong, yet not causal, evidence that pregnancy anemia is a risk factor for childhood anemia but not for any deficiency in the development of early skills, on average. [...] This study gives important insights into the consequences of pregnancy anemia for populations underserved by antenatal care services and with high rates of malnutrition. Nevertheless, using a singular Hb measurement during pregnancy and facing high rate of attrition between the waves might affect the external validity of our results. Nevertheless, the strong association between pregnancy anemia and childhood anemia we identified in this study should be further investigated to observe if it will affect later life outcomes, commonly associated with iron deficiency and anemia.”

See “Abstract”, paragraph “Conclusion”:

“While pregnancy anemia is a risk factor for anemia during childhood, we do not find evidence for an increased risk of infectious diseases or early childhood development delays.”