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# Pregnancy anemia, child health and development: A cohort study in rural India



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# Pregnancy anemia, child health and development: A cohort study in rural India

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## Abstract

**Objective:** To assess how pregnancy anemia affects the offspring's early childhood development, child hemoglobin (Hb) levels, growth and diseases incidence outcomes two years after birth in a low-income setting. Further we investigate the mediating role of childhood Hb levels with disease incidences and skills.

**Design:** Prospective cohort study.

**Setting and participants:** The study participants are 999 mother-child dyads from rural Madhepura in Bihar, India. In 2015, the women were recruited during pregnancy from registers in mother-child centers of 140 villages for the first wave of data collection. At the time of the second wave in 2017, the children were 22-32 months old.

**Primary and Secondary Outcome Measures:** The recruited women were visited at home for a household survey and the measurement of the women's and child's hemoglobin level, child weight and height. Data on the incidence of diarrhea and fever were collected from interviews with the mothers. To test motor, cognitive, language and socio-emotional skills of the children, we used an adapted version of the child development assessment FREDI.

**Results:** The average Hb during pregnancy was 10.2 g/dl and 69% of the women had pregnancy anemia. At the age of 22-32 months, a 1 g/dl increase in Hb during pregnancy was associated with a 0.17 g/dl (95% CI: 0.11- 0.23) increase in Hb levels of the child. Children of moderately or severely anemic women during pregnancy showed 0.57 g/dl (95% CI: -0.78 - -0.36) lower Hb than children of not anemic women. We find no association between the maternal Hb during pregnancy and early skills, stunting, wasting, underweight, or disease incidence. Childhood Hb correlates positively with skills (marginal effect 0.04, 95% CI: 0.01 - 0.08).

**Conclusions:** Pregnancy anemia is a risk factor for anemia during childhood, but does not increase the risk of infectious diseases or affect early childhood development.


Keywords: Pregnancy, anemia, hemoglobin, early childhood development, motor skills, cognitive skills, language skills, socio-emotional skills, child health, India

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- To reduce endogeneity, we controlled for maternal Hb and household food security after pregnancy, together with other relevant confounders factors.
- The study used a unique sample of women with little contact to antenatal health services and high rate of anemia.
- Inverse probability weighting was used to account for missing data and attrition.
- The data contains only one measure of Hb during pregnancy.

## INTRODUCTION

Sufficient intake of macro- and micronutrients during pregnancy is a prerequisite for healthy child development.[1] Yet, the high rates of pregnancy anemia, i.e. having a low level of hemoglobin level in the blood, document the precarious state of nutrition among many pregnant women worldwide. In 2011, 32 million women were estimated to be anemic, the vast majority living in South Asia.[2] One of the most common causes of anemia in low- and middle-income countries, such as India, is iron deficiency.[3, 4]. Anemia can however also result from other micronutrient deficiencies such as folic acid, vitamin B12, and vitamin A, as well as infectious diseases and genetic disorders. A focus on anemia is imperative because hemoglobin (Hb) is a crucial ingredient of red blood cells and thereby responsible for the transport of oxygen to the body tissues. Low hemoglobin during pregnancy is a known risk factor for premature birth, low birth weight, and in extreme cases leads to death.[5–9]

In this paper, we investigate the consequences of pregnancy anemia on child Hb levels, early skills and other health indicators in the first 1,000 days. Most of the iron requirement in the first year of life is met by the body iron a child is born with, either in the form of hemoglobin or in iron stores (ferritin). The amount of ferritin and hemoglobin at birth depends heavily on the iron transfer from mother to child in-utero, which occurs in the second and third trimester of pregnancy.[10] Studies have shown that anemia during pregnancy correlates with low cord hemoglobin levels and anemia during infancy.[11–15] To our knowledge, there are no studies investigating the relationship of pregnancy anemia and anemia of children older than 18 months. Yet, with a prevalence of 58%, anemia of children below the age of five remains a significant global health challenge in South Asia and its causes are worth investigating.[16] The

known adverse consequences of childhood anemia on human development add importance to this matter.[17, 18]

While several experimental and non-experimental studies have examined the impact of pregnancy anemia on early skills, the empirical findings are inconclusive.[19] This might be a result of the different study design, age variation of the study participants, the dimensions of skills measured, or geographical diversity.[20–27] Furthermore, as pregnancy anemia is a risk for adverse birth outcomes, the existing experimental studies exclude moderately and severely anemic women from the trials. Hence, the external validity of their findings for the general population of pregnant women is unclear. Observational studies without the appropriate quasi-experimental methods are unable to identify a causal impact of anemia during pregnancy on child outcomes due to omitted, endogenous variables. For instance, ignoring time-invariant environmental factors is likely to upwardly bias the results of non-experimental studies.

We contribute to the existing literature by analyzing the consequences of pregnancy anemia on child outcomes in three essential ways. First, we eliminate an important confounder in the analysis by controlling for the maternal hemoglobin levels and the food diversity of the household after birth. While not being able to fully capture the unobservable differences between children exposed to pregnancy anemia and without, our set of covariates will omit the bias emerging from any differences in micronutrient diversity in the post-natal period. Second, by following the children more than two years after birth, we can observe if potential initial disadvantages persisted over time. Lastly, in addition to cognitive and non-cognitive functions, and anemia, we also assess the influence of pregnancy anemia on secondary health outcomes, namely child growth and disease incidence. This analysis will help to get a deeper understanding of the adverse consequences of anemia during pregnancy.

## METHODS

### Data and procedures

Our dataset is a panel of two waves, consisting of household surveys, anthropometric and blood sample collections, and child development tests conducted in Madhepura in the North-Eastern state Bihar. Bihar is one of the poorest states of India and Madhepura belongs to its socio-economically most deprived districts. Our study sample was taken from pregnancy registers in local mother-child-centers (*Anganwadi* centers) in 140 villages in six sub-districts (blocks) of Madhepura. It should hence be noted that our sample is not representative of the full population

of pregnant women in that area, but only for those who registered in the centers. In 2015/16, this covered 76% of pregnant women.[28]

All women listed in the registries in March/April 2015 were visited and invited to participate in the baseline survey and medical tests. During the follow-up in 2017, we attempted to revisit the households of all formerly pregnant women. In addition to the survey and medical measures, a development test was administered to the child that resulted from the pregnancy. **Supplementary Figure S1** visualizes the data collection timeline and the age of the children.

**Outcome measures**

Anemia is defined over the hemoglobin level in the blood, which in our case was obtained from a finger prick in the field collected by trained local enumerators. Using HemoCue® machines for capillary blood, the hemoglobin level can be determined immediately and communicated to the tested individual or its caregivers on the spot. According to the WHO and the Indian Council of Medical Research definitions, a pregnant woman is anemic if her hemoglobin concentration falls below 11.0 g/dl.[29, 30] Pregnancy anemia is further distinguished into mild anemia (10.0-10.9 g/dl), moderate anemia (7.0-9.9 g/dl) and severe anemia (<7.0 g/dl). The anemia thresholds for children between 6 and 59 months are the same as for pregnant women.[30]

During data collections, the field teams followed a strict protocol upon detection of anemia. In case of mild or moderate anemia, the women / caregivers were advised to go to the nearest primary health care center soon to seek treatment for anemia. In case of severe anemia, the household was alerted that immediate attention was needed. In the follow-up survey, we also offered to cover the treatment costs and transport to a health facility for all severely anemic children.

Child development was measured with a variation of the FREDI 0-3, a German development test similar in structure to the Bayles Scales of Infant and Toddler Development.[31–33] Due to the different home environments of the children, certain items of the original FREDI 0-3 were adjusted to the Bihari context. The development test consists of a parent questionnaire and a child assessment, and covers four areas: fine and gross motor development, receptive and expressive language development, cognition, and socio-emotional development. Two age-specific tests were administered, each covering skills over an age range of five months. Each

test consisted of around 40 items. All raw scores have been standard normalized and are hence presented as z-scores. In addition to the four individual test scores, we calculate the total FREDI z-score over all 40 test items. A brief validation of the FREDI with regard to physical growth and maternal education is presented in **Supplemental Figure S2 and S3**.

Stunting (i.e. being too short for their age), wasting (i.e. being too light for their height), and underweight (i.e. being too light for their age) are used as secondary health outcomes. Children's height and weight were measured during the field visits by the blood testing team. We age-standardized the raw height and weight values following the WHO Growth Standards.[34] With a respective z-score of two standard deviations (SD) below the median of the WHO references population, a child is defined as being either stunted, wasted or underweight. Any value above six standard deviations or below – six SD was coded as measurement error and dropped from the analyses.[1] The information on diarrhea and respiratory disease incidences in the two weeks before the survey were collected from maternal reports and coded as binary variables.

## Patient and public involvement

The aims and the survey design were shared at a meeting of state-and district-level government functionaries who provided services in Madhepura through the Women and Child Development ministry, village-level leaders of women's groups prior to the baseline. At this meeting, there was a detailed discussion of the types of questions that needed to be asked during the data collection. Several of these suggestions were incorporated in the baseline questionnaire. Residents of Madhepura were involved to the extent that they participated in the pre-testing of the baseline questionnaire and the FREDI tool. Patients had not been involved in the interpretation of results, writing or editing of the final document.

## Statistical analysis

In our main analysis, we estimated the association between pregnancy anemia and the child hemoglobin level and child development approximately two years after birth using an ordinary

[1] This was the case for 21 weight-for-height z-scores, seven weight-for-age z-scores and eleven height-for-age z-scores.

least square regression model. Our secondary outcomes of interest, - being stunted, wasted, or underweight, incidence of diarrhea and respiratory diseases in the two weeks before the survey, were analyzed with a logistical regression model.

We considered both continuous and discrete hemoglobin levels to allow for linear and non-linear relationships between pregnancy anemia and the child outcomes. For the linear relationship, our explanatory variable of interest was the Hb levels of the women during pregnancy. For the non-linear relationship, we used the categorical variables anemia status: no pregnancy anemia, mild pregnancy anemia and moderate-to-severe pregnancy anemia.

All estimations controlled for the maternal Hb levels at the time of the follow-up and the household food diversity scores. This was done to avoid an overestimation of the relationship between pregnancy and child outcomes due to an overall poor food environment or chronic diseases of the mothers. We further add age, sex and current breast-feeding status of the child, as well as development test facilitator or HemoCue machine as control variables to obtain more precise estimates. Additional covariates from the baseline data collected are: caste category, wealth quintile, maternal literacy, maternal age, pregnancy history (first birth dummy), trimester of gestation and take-up of antenatal care (ANC) services. Lastly, we added sub-district (block) fixed effects and clustered the standard errors on village level to take spatial correlation of the outcome variables into account. For the sensitivity analysis, we include birth spacing, macronutrient deficiency and postnatal depression to the estimation, and replaced block with *panchayat* fixed effects.[2]

In light of strong son preferences in the study region [35, 36], we considered heterogeneous effects for boys and girls. Further, we investigated heterogeneous effects by ANC take-up, as a proxy for health preferences, caste categories, maternal literacy levels and gestational trimester at the time of the baseline survey.

As child anemia could be a result as well as a mediator for pregnancy anemia, we tested the association between childhood Hb levels and early childhood development and infectious diseases in separate analyses. Using the same set of covariates as described above, we

[2] We did not include those variables in the main specification due to missing information, which would have further reduced the sample size. A *panchayat* is a subdivision of a block and comprises several villages.

controlled for household level and child specific characteristics that might affect both child Hb levels and the outcome variables of interest.

Finally, given the existing evidence of maternal anemia for adverse pregnancy outcomes, we conducted a survival analysis that assessed whether the Hb level of pregnant women correlates with child loss. Such a correlation would downward biased the estimates of our main analysis, as worst of children would systematically be missing in the group with higher exposure. We tested this hypothesis by estimating a probit model for non-survival on the pregnancy anemia and hemoglobin levels.

All estimations were weighted with inverse probability weight, as proposed by Fitzgerald, Gottschalk and Moffitt (1998) adjusting the sample for selective attrition.[37] The statistical analyses were conducted with the statistical software Stata 16 (StataCorp LP).

## RESULTS

### Sample description

The final sample consisted of 941 to 1000 mother-child-dyads, depending on the outcome variable. In 2017, the age of the children lied between 22 and 32 months. **Table 1** presents the explanatory variable, covariates and the outcome variables of interest of the estimation sample separately for three anemia categories: no pregnancy anemia (column 1-3), mild pregnancy anemia (column 4-6) and moderate-to-severe pregnancy anemia (column 7-9).

The distribution shown in the table is suggestive of a gradient in child Hb levels and growth indicators across pregnancy anemia levels. We do not observe a clear trend for the skill outcomes or disease incidences. Interestingly, the distribution of Hb levels of women after pregnancy across the anemia groups mirrors the Hb levels during pregnancy suggesting that suboptimal micronutrient intake during pregnancy continued after delivery. This is however not the case for the household food diversity scores, which is the highest for the mild pregnancy anemia group.

**Table 1 Summary statistics across exposure categories in the Hb sample**

| | No preg. anemia | | | Mild preg. anemia | | | Moderate or severe preg. anemia | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | N | Mean | SD | N | Mean | SD | N |
| *Outcome variables* | | | | | | | | | |
| Hb (child) in 2017 | 10.97 | (1.35) | 292 | 10.71 | (1.23) | 305 | 10.26 | (1.39) | 344 |
| Motor skills z-score | -0.02 | (1.04) | 284 | -0.04 | (1.00) | 298 | 0.07 | (0.93) | 335 |
| Language skills z-score | 0.01 | (0.96) | 291 | -0.01 | (1.00) | 304 | 0.00 | (1.02) | 344 |
| Cognition skills z-score | -0.01 | (0.99) | 290 | -0.04 | (1.02) | 304 | 0.07 | (0.97) | 340 |
| Socio-emo. skills z-score | 0.01 | (0.98) | 291 | -0.02 | (1.00) | 304 | -0.02 | (1.02) | 342 |
| Height-for-age z-score | -2.36 | (1.39) | 287 | -2.40 | (1.27) | 300 | -2.54 | (1.34) | 338 |
| Weight-for-age z-score | -1.91 | (1.10) | 286 | -1.94 | (1.05) | 294 | -2.07 | (1.08) | 339 |
| Weight-for-height z-score | -0.98 | (1.47) | 288 | -0.97 | (1.71) | 300 | -0.98 | (1.60) | 338 |
| Respiratory disease or fever | 0.20 | (0.40) | 291 | 0.21 | (0.41) | 305 | 0.20 | (0.40) | 344 |
| Diarrhea incidence | 0.24 | (0.43) | 287 | 0.25 | (0.43) | 304 | 0.26 | (0.44) | 344 |
| *Other child characteristics* | | | | | | | | | |
| Age of child (months) | 27.27 | (2.43) | 292 | 27.26 | (2.41) | 305 | 27.54 | (2.16) | 344 |
| Currently breastfed | 0.44 | (0.50) | 292 | 0.46 | (0.50) | 305 | 0.44 | (0.50) | 344 |
| Sex (Male=1) | 0.53 | (0.50) | 292 | 0.50 | (0.50) | 305 | 0.53 | (0.50) | 344 |
| *Pregnancy characteristics* | | | | | | | | | |
| Hb (preg.) | 11.76 | (0.72) | 292 | 10.44 | (0.29) | 305 | 8.68 | (1.07) | 344 |
| Trimester of pregnancy | 1.89 | (0.76) | 292 | 2.01 | (0.69) | 305 | 2.16 | (0.69) | 344 |
| First pregnancy | 0.22 | (0.42) | 292 | 0.22 | (0.41) | 305 | 0.19 | (0.40) | 344 |
| Any ANC visits | 0.53 | (0.50) | 292 | 0.48 | (0.50) | 305 | 0.55 | (0.50) | 344 |
| Supplementary iron intake during pregnancy | 0.61 | (0.49) | 292 | 0.60 | (0.49) | 305 | 0.57 | (0.50) | 344 |
| Other micronutrient intake during pregnancy | 0.43 | (0.50) | 259 | 0.40 | (0.49) | 265 | 0.44 | (0.50) | 306 |
| *Mother characteristics* | | | | | | | | | |
| Hb (mother) in 2017 | 12.09 | (1.32) | 292 | 11.86 | (1.35) | 305 | 11.14 | (1.62) | 344 |
| Mother can read | 0.25 | (0.43) | 292 | 0.27 | (0.44) | 305 | 0.24 | (0.43) | 344 |
| Age of mother (years) | 24.65 | (3.66) | 292 | 24.90 | (3.89) | 305 | 24.67 | (3.93) | 344 |
| *Household characteristics* | | | | | | | | | |
| Food diversity index | 7.01 | (1.50) | 292 | 7.30 | (1.46) | 305 | 7.10 | (1.52) | 344 |
| Scheduled case or tribe | 0.29 | (0.46) | 284 | 0.29 | (0.46) | 295 | 0.33 | (0.47) | 331 |
| Improved sanitation facility | 0.14 | (0.34) | 292 | 0.12 | (0.33) | 305 | 0.12 | (0.32) | 344 |
| Asset index quintile | 3.09 | (1.44) | 292 | 3.01 | (1.42) | 305 | 2.85 | (1.45) | 344 |

The majority of women in our sample (69%) were at least mildly or moderately anemic during pregnancy, with an average Hb level of 10.2 g/dl. Of all anemic women, 48% showed signs of moderate anemia, and only 4% severe anemia. Overall, the literacy level of the study population was low with a quarter of the women being able to read and write, similar in all three presented anemia groups. Such low levels of literacy are comparable with district-wide statistics of a nationally representative survey from 2015-16.[28] Importantly, half of the women reported not having received any ANC during pregnancy. This figure sets our sample strongly apart from most existing studies, many of which recruited their participants during ANC visits [20, 26, 38] or delivered ANC visits as part of the study [25]. The uptake of ANC services is the highest in

the group of moderate or severe pregnancy anemia and lowest for women of mild pregnancy anemia.

The sample size during follow-up data collection reduced considerably from the baseline (N=1,918) due to the unavailability of the women or children at the time follow-up visits, inaccurate location information, refusal to provide a blood sample or to participate in the child testing, and child death (**Figure 1**). We account for this loss in sample size by using inverse probability weights, assigning higher weights to those households that had a higher probability of dropping out.[3]

*- Figure 1 here -*

**Association of pregnancy anemia with childhood anemia and early skills**

We found a strong association of Hb during pregnancy, mild, and moderate or severe pregnancy anemia with the child's hemoglobin levels (**Table 2)**. An increase of 1 g/dl in Hb during pregnancy was associated with 0.17 g/dl higher Hb levels in the offspring. Children born to women with mild anemia had 0.20 g/dl lower Hb level than their non-anemic peers did. The coefficient was more than twice the size for children born to mothers with moderate or severe pregnancy anemia. Current HB of the mothers are consistently positively correlated with the child Hb levels, at a statistical significance level of 1%.

[3] Relevant weighting variables were used despite missing information, which reduces the estimation samples by two more observations.

**Table 2 Weighted regression results on the association between maternal hemoglobin (Hb) levels and anemia during pregnancy and early childhood development and childhood hemoglobin levels**

| | Hb (child) | Motor skills | Language skills | Cognition skills | Socio-emo. skills |
|---|---|---|---|---|---|
| *Panel A* | | | | | |
| Hb (preg.) | 0.17*** | -0.01 | -0.01 | -0.03* | -0.02 |
| | [0.11,0.23] | [-0.05,0.03] | [-0.05,0.04] | [-0.07,0.00] | [-0.05,0.02] |
| Hb (mother) | 0.13*** | 0.01 | 0.04 | 0.06*** | 0.05** |
| | [0.07,0.20] | [-0.03,0.05] | [-0.01,0.08] | [0.02,0.10] | [0.01,0.10] |
| $R^2$ | 0.174 | 0.246 | 0.218 | 0.303 | 0.321 |
| *Panel B* | | | | | |
| Mild preg. anemia | -0.20* | 0.03 | 0.01 | 0.06 | 0.05 |
| | [-0.41,0.00] | [-0.13,0.19] | [-0.13,0.15] | [-0.09,0.22] | [-0.08,0.17] |
| Moderate/severe preg. anemia | -0.57*** | 0.06 | 0.03 | 0.12* | 0.01 |
| | [-0.78,-0.36] | [-0.10,0.22] | [-0.12,0.19] | [-0.02,0.26] | [-0.12,0.15] |
| Hb (mother) | 0.15*** | 0.01 | 0.04* | 0.06*** | 0.05** |
| | [0.08,0.22] | [-0.03,0.05] | [-0.01,0.08] | [0.02,0.10] | [0.00,0.09] |
| $R^2$ | 0.172 | 0.246 | 0.218 | 0.303 | 0.321 |
| Controls | Yes | Yes | Yes | Yes | Yes |
| Tester fixed effects | Yes | No | No | No | No |
| FREDI fixed effects | No | Yes | Yes | Yes | Yes |
| N | 939 | 972 | 996 | 990 | 994 |

*Notes*: Outcome variables in column (2)-(5) are standardized test scores and the coefficients are shown in standard deviations. *Panel A* uses pregnancy Hb level of the mother as main explanatory variable. In *Panel B*, the two explanatory variables of interest are mild pregnancy anemia and moderate/severe pregnancy anemia. The omitted category in Panel B is no pregnancy anemia. 95% Confidence intervals using standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), trimester of pregnancy, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in skill estimation: test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

We did not find a statistically significant relationship of Hb or any type of pregnancy anemia with the child development in general, or any specific dimension of development. The coefficients were small in magnitude, indicating indeed zero-effects, rather than an imprecise estimation. For cognitive development, we found a small, negative and weakly statistically significant association with Hb level during pregnancy and moderate or severe pregnancy anemia.

The heterogeneity analyses showed that there were only small differences in the estimates of pregnancy Hb on child Hb and skills on by gestational trimester, caste category, maternal literacy, sex and ANC take-up (**Supplemental Table S1**). In the case of language skills, we found a small, weakly statistically significant, negative interaction effect with ANC visits and a statistically significant, but positive interaction term for households belonging to a scheduled caste or tribe. Hence, for scheduled castes and tribes, we find evidence for a positive correlation of pregnancy anemia and early language skills.

Our findings were robust to the inclusion of additional, potentially confounding, covariates, namely birth spacing, body-mass-index and postnatal depression, panchayat fixed effects, and the exclusion of all covariates and fixed effects (**Supplemental Table S2 and S3**). We found no evidence for survival bias, i.e. that the surviving children were exposed to higher maternal Hb levels in-utero (**Supplemental Table S4).**

**Association of pregnancy anemia and child health indicators**

We found no indication that suboptimal Hb levels during pregnancy were associated with stunting, wasting or underweight of children, or increased the incidence of respiratory diseases or diarrhea (**Figure 2**).

*- Figure 2 here -*

### Association of childhood anemia with skill and child health

The lack of association between pregnancy anemia, early skills and infectious diseases, despite the strong correlation with early Hb levels, might be an indication that *childhood* anemia is not a risk factor for early childhood development or disease incidence in our study population. The cross-sectional analysis confirmed this hypothesis for diarrheal and respiratory diseases (**Table 3**). Yet, for the cumulative development scores, we found a small, but positive and statistically significant coefficient.

**Table 3. Association of child's hemoglobin (Hb) with their early skills and infectious disease incidence**

| | Cum. development z-score | Respiratory disease | Diarrhea |
|---|---|---|---|
| | Marginal effects | Odds ratios | Odd ratios |
| Hb (child) | 0.04*** | 1.02 | 0.99 |
| | [0.01,0.08] | [0.89,1.16] | [0.88,1.11] |
| Hb (mother) | 0.02 | 1.00 | 0.98 |
| | [-0.01,0.05] | [0.89,1.12] | [0.88,1.08] |
| (Adjusted) $R^2$ | 0.334 | 0.061 | 0.047 |
| Controls | Yes | Yes | Yes |
| Tester fixed effects | Yes | Yes | Yes |
| FREDI fixed effects | Yes | No | No |
| N | 915 | 938 | 933 |

*Notes*: The outcome variable in column (1) is the standardized total test score and the coefficients are shown in standard deviations. Outcomes in column (2) and (3) are binary variables and the coefficient are shown as odds ratios. 95% Confidence intervals using standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), trimester of pregnancy, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in column (1) and (2): test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl.. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

## DISCUSSION

### Interpretation

Our cohort study from rural Bihar, India, shows a strong association between maternal hemoglobin levels during pregnancy and the hemoglobin levels of the offspring between 22 and 32 months after birth. The association is strongly statistically significant and robust to the inclusion of several, potentially confounding, variables, such as current nutritional status of the mother or child age. The relationship does not differ by child sex, caste category, gestational trimester, ANC take-up or maternal literacy. Moderate and severe pregnancy anemia is associated with lower Hb levels of children than mild pregnancy anemia.

Our analysis extends the current literature that connects pregnancy anemia with direct birth outcomes in India, such as prematurity birth weight and size. [39, 40] The biological link between pregnancy anemia and child anemia more than two years after birth is unclear as the iron transferred from mother to child in-utero is typically consumed by the child's needs within the first year of life, before complementary feeding starts. [10] Our findings might be explained by a lower Hb trajectory since birth, initiated by the low hemoglobin environment in-utero as has been shown in Benin.[11]

Overall, our findings on childhood anemia are in line with a recent study from the US, that showed an elevated risk ratio of infant anemia from anemia during pregnancy, in particular for moderate and severe anemic condition.[14] Similarly, an analysis with data from China showed reduced Hb levels of infants for maternal anemia during the 24 - 28 gestational week, the authors find however no association for the first gestational trimester.[15]

We did not find any correlation between low Hb level during pregnancy and other child health outcomes, such disease incidence or growth indicators. The disadvantage of children born to anemic mothers, compared to their peers, hence seems to be limited to the low Hb levels. Our study does also not show any association between *childhood* Hb and infectious diseases, even though iron deficiency, a major cause of anemia, is known to weaken the immune functions.[41] In this regard, our findings contradict a study on Bedouin children in Israel, presenting a strong linkage between anemia, diarrhea and respiratory illnesses of children.[42]

Lastly, we find no evidence that pregnancy anemia is associated with lower early childhood development, even though children's Hb levels do correlate with early skills in our sample. Our results thereby go against the outcomes of several observational studies, most prominently Chang et al.'s analysis in rural China, with children as a similar age. While they did not find an association of pregnancy anemia and psychomotor development, children of anemic mothers showed lower scores in language and cognitive development when 18 and 24 months old.[25]

Differences in the socio-economic characteristics of two study populations might explain this difference. Not only did the mothers in Chang et al.'s study have higher levels of education, but the prevalence of childhood undernutrition among them was about a fifth as large as in our study population. The poor nutritional status of children in Madhepura is likely not only a result of the insufficient intake of macro- and micronutrients but also of frequent gastrointestinal infections. The low coverage of improved toilet facilities and high prevalence of unsafe disposal

of children's stool possibly led to greater exposure to fecal bacteria, thus facilitating the spread of diarrheal diseases and parasites. The constant exposure to fecal bacteria could also cause environmental enteropathy which hampers the absorption of nutrients and worsens malnutrition.[43–45]

Furthermore, it is important to note that the human brain development is largely driven by experience.[46] A lack of adequate learning opportunities and stimulation in the early years can have long lasting consequences for the functioning of the brain.[47, 48] The stimulation environment created by the caregivers for their children is limited in our study area. Only about half of the caregivers told stories, sung songs or read a book to the children during the three days prior to the survey. About a quarter of the mothers reported that no household member had played with the child in that time.

Taken together, all of these lead us to argue that the additional, possible adverse impact of pregnancy anemia on child development in our study setting was not large enough to be detectable in our estimations.

**Limitations**

Our analysis is based on the assumption that the single hemoglobin measure taken at baseline is informative of the hemoglobin status during the full course of pregnancy. Most Hb measurements of our participants were taken in the second trimester, allowing sufficient time for any improvement in the Hb levels during the remaining months. Such improvements after the baseline data collection would hence weaken the relationship of Hb that we measured with child outcomes. Given that half of the mothers in our sample reported not having consumed any iron supplements during pregnancy or received any antenatal care, we believe that the extent of such attenuation bias is limited.

Any systematic error in Hb measurement, depending on the Hb level, could be a bigger concern. This could have been induced by the differential recommendations our field team gave based on the blood test results. However, our data do not support this hypothesis. We did not observe a particular behavior change in more severely anemic women: even after delivery only 57% of the women with moderate or severe pregnancy anemia reported consuming iron supplements during pregnancy, compared to 60-61% in the mildly and non-anemic group. During pregnancy, the reported supplementation rates across the groups were as well similar (16% of

the moderately or severely anemic women vs. 19-20% of the mildly or non-anemic women). This suggests that our advice did not alter the women's behavior.

Two additional points should be kept in mind when interpreting our findings. First, we collected Hb levels but not ferritin levels, which leaves room for speculation on the origin of anemia in the study sample. Although iron deficiency is commonly believed to be the major reason for anemia, to our knowledge, no study has documented the actual share of iron deficiency anemia among the anemic pregnant women in rural Bihar. Second, it should be noted that the share of severely anemic pregnant women in our study is very small. Our estimated association of moderate or severe anemia and child development and Hb is hence mainly attributable to the group of women who were moderately anemic during pregnancy.

## Conclusion

We find strong evidence that pregnancy anemia is a risk factor for childhood anemia but not for any deficiency in the development of early skills, on average. The relationship between pregnancy anemia and childhood Hb grows stronger with lower levels of Hb during pregnancy. Yet, diarrhea or respiratory disease incidence or child growth is not affected by either childhood Hb or pregnancy Hb. The strong association between pregnancy anemia and childhood anemia should be further investigated to observe if it will affect later life outcomes, commonly associated with iron deficiency and anemia.

**Author contribution:** EH, MAS and SV designed research and the survey instruments, CM designed the child skill assessment. SV and MAS coordinated the first wave data collection, EH coordinated and supervised the second wave data collection, with the support by CM for the child testing. EH analyzed the data, wrote the paper, had primary responsibility for final content. All authors have read and approved the final manuscript.

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**Competing interests:** None declared.

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**Ethics approval:** The study was approved by the ethics board of the IIT Gandhinagar (IEC/2014-15/2/MS/006, IEC/2016-17/2/MS/025) and the University of Goettingen (no IRB number available). We obtained written informed consent for the interviews, growth measurements, blood sample collections and the child development testing during each wave.

**Data availability statement:** No additional data available.

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## Figures

Figure 1 Attrition between 2015 (wave 1) and 2017 (wave 2) in childhood hemoglobin and skills sample

Figure 2 Weighted regression results on the association between maternal hemoglobin (Hb) levels during pregnancy and growth and disease incidence indicators (odds ratios)

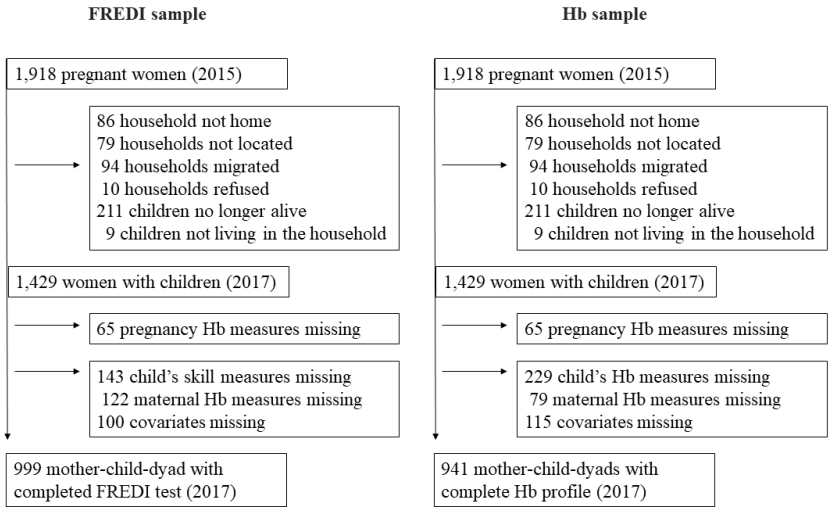



Figure 1
Attrition between 2015 (wave 1) and 2017 (wave 2) in childhood hemoglobin and skills sample

338x190mm (96 x 96 DPI)

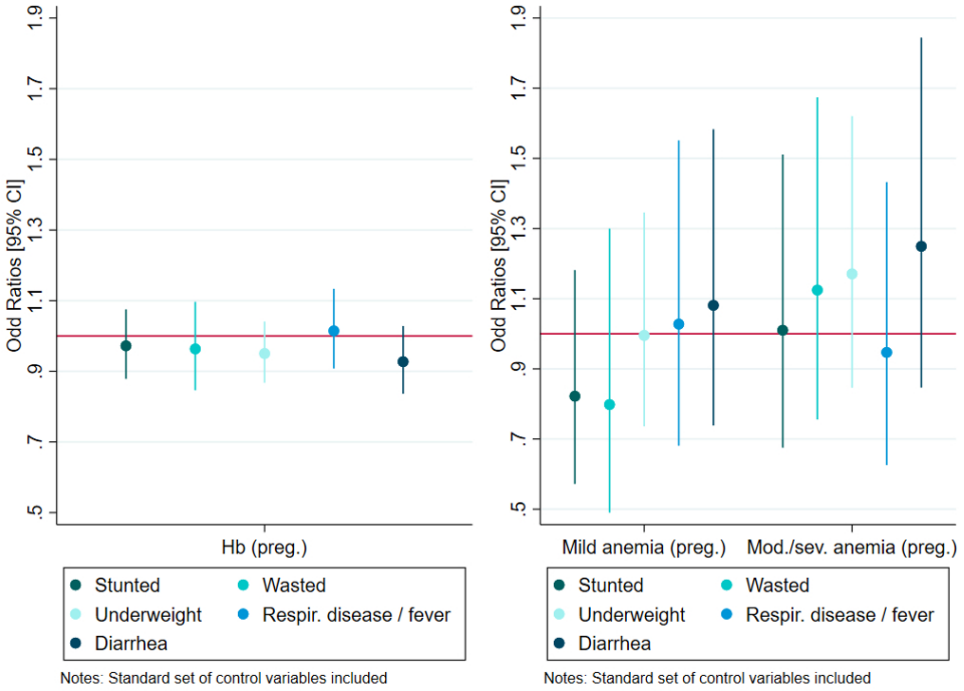
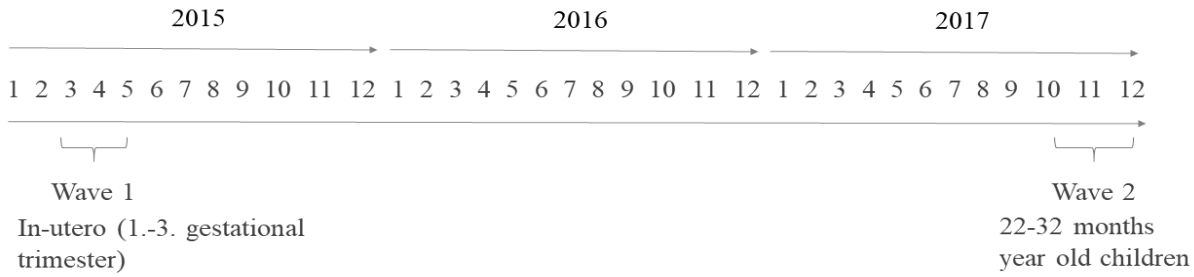


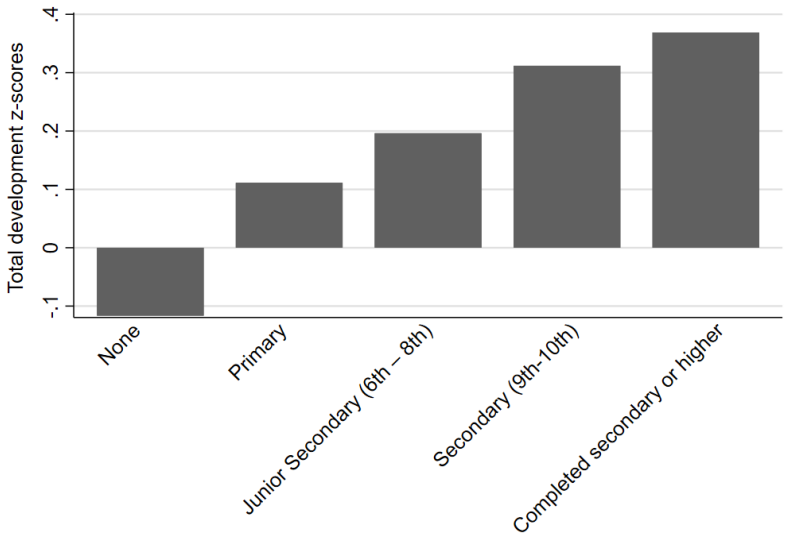

Figure 2
Weighted regression results on the association between maternal hemoglobin (Hb) levels during pregnancy and growth and disease incidence indicators (odds ratios)

207x151mm (129 x 129 DPI)

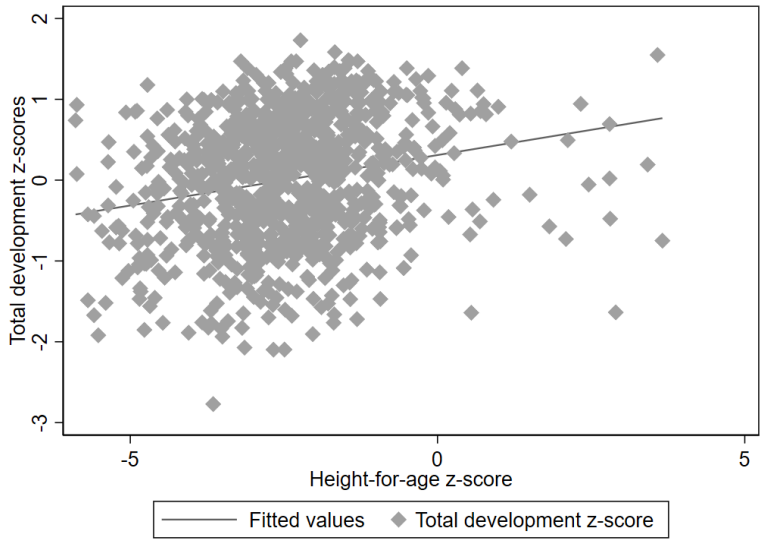
## Online Supporting Material




**Supplemental Figure S1 Timeline of data collections and the respective age of the children**




**Supplemental Figure S2 Average cumulative development test scores by maternal education category**




**Supplemental Figure S3 Average cumulative development test scores by of FREDI by child growth**

**Supplemental Table S1 Heterogeneous treatment effects using a linear regression model on child hemoglobin levels and skills dimensions**

| | Hb (child) | Motor skills | Language skills | Cognition skills | Socio-emo. skill |
|---|---|---|---|---|---|
| **Panel A**: By antenatal care uptake | | | | | |
| Hb (preg.) | 0.162*** | -0.006 | 0.034 | -0.029 | 0.013 |
| | (0.042) | (0.029) | (0.030) | (0.025) | (0.025) |
| Any ANC # Hb (preg.) | 0.014 | -0.006 | -0.079* | -0.007 | -0.059* |
| | (0.058) | (0.035) | (0.041) | (0.034) | (0.035) |
| $R^2$ | 0.174 | 0.248 | 0.221 | 0.304 | 0.332 |
| N | 938 | 972 | 996 | 990 | 994 |
| **Panel B:** By child's sex | | | | | |
| Hb (preg.) | 0.208*** | 0.009 | -0.018 | -0.027 | -0.025 |
| | (0.041) | (0.028) | (0.025) | (0.027) | (0.024) |
| Male # Hb (preg.) | -0.077 | -0.037 | 0.024 | -0.009 | 0.015 |
| | (0.064) | (0.037) | (0.038) | (0.034) | (0.036) |
| $R^2$ | 0.176 | 0.247 | 0.218 | 0.303 | 0.321 |
| N | 939 | 972 | 996 | 990 | 994 |
| **Panel C:** By caste category | | | | | |
| Hb (preg.) | 0.164*** | -0.016 | -0.030 | -0.035* | -0.019 |
| | (0.033) | (0.023) | (0.026) | (0.019) | (0.022) |
| Scheduled caste or tribe # Hb (preg.) | 0.033 | 0.032 | 0.088** | 0.005 | -0.008 |
| | (0.079) | (0.039) | (0.044) | (0.041) | (0.039) |
| $R^2$ | 0.177 | 0.245 | 0.220 | 0.306 | 0.318 |
| N | 908 | 941 | 964 | 958 | 962 |
| **Panel D:** By maternal literacy | | | | | |
| Hb (preg.) | 0.166*** | -0.011 | -0.007 | -0.021 | -0.015 |
| | (0.035) | (0.022) | (0.023) | (0.020) | (0.021) |
| Maternal literacy # Hb (preg.) | 0.014 | 0.010 | 0.003 | -0.047 | -0.013 |
| | (0.072) | (0.050) | (0.047) | (0.047) | (0.038) |
| $R^2$ | 0.174 | 0.246 | 0.218 | 0.304 | 0.321 |
| N | 939 | 972 | 996 | 990 | 994 |
| **Panel E:** By gestational trimester | | | | | |
| Hb (preg.) | 0.173*** | -0.029 | -0.029 | -0.051 | -0.026 |
| | (0.054) | (0.038) | (0.040) | (0.037) | (0.034) |
| Second gestational trimester # Hb (preg.) | -0.031 | 0.039 | 0.061 | 0.029 | 0.041 |
| | (0.077) | (0.045) | (0.047) | (0.044) | (0.046) |
| Third gestational trimester # Hb (preg.) | 0.027 | 0.011 | -0.010 | 0.020 | -0.031 |
| | (0.073) | (0.052) | (0.053) | (0.050) | (0.040) |
| $R^2$ | 0.175 | 0.246 | 0.220 | 0.303 | 0.323 |
| N | 939 | 972 | 996 | 990 | 994 |
| Controls | Yes | Yes | Yes | Yes | Yes |
| Tester fixed effects | Yes | No | No | No | No |
| FREDI fixed effects | No | Yes | Yes | Yes | Yes |

Notes: Outcome variables in column (2)-(5) are standardized test scores and the coefficients are shown in standard deviations. Standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), trimester of pregnancy, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in column (2)-(5): test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S2 Linear regression model results of haemoglobin level of children on haemoglobin levels during pregnancy with additional covariates**

| | Hb (child) | Hb (child) | Hb (child) | Hb (child) | Hb (child) |
|---|---|---|---|---|---|
| Hb (preg.) | 0.176*** | 0.163*** | 0.165*** | 0.154*** | 0.213*** |
| | (0.029) | (0.030) | (0.029) | (0.030) | (0.033) |
| Hb (mother) | 0.127*** | 0.133*** | 0.128*** | 0.134*** | |
| | (0.034) | (0.034) | (0.036) | (0.035) | |
| Mother gave birth in past 2 years | 0.013 | | | | |
| | (0.111) | | | | |
| BMI (preg.) | | 0.009 | | | |
| | | (0.016) | | | |
| Postnatal depression | | | 0.087 | | |
| | | | (0.118) | | |
| Controls | Yes | Yes | Yes | Yes | No |
| Panchayat dummies | No | No | No | Yes | No |
| Block dummies | Yes | Yes | Yes | No | No |
| N | 896 | 933 | 807 | 939 | 939 |
| $R^2$ | 0.173 | 0.174 | 0.161 | 0.236 | 0.054 |

Notes: Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), trimester of pregnancy, ANC visit (dummy), child's sex and age, tester fixed effects. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S3 Linear regression model results of cumulative test scores of children on haemoglobin levels during pregnancy with additional covariates**

| | Cum. development score | Cum. development score | Cum. development score | Cum. development score | Cum. development score |
|---|---|---|---|---|---|
| Hb (preg.) | -0.023 | -0.015 | -0.009 | -0.015 | -0.008 |
| | (0.015) | (0.014) | (0.016) | (0.016) | (0.017) |
| Hb (mother) | 0.042*** | 0.041** | 0.036** | 0.042** | |
| | (0.016) | (0.016) | (0.017) | (0.017) | |
| Mother gave birth in past 2 years | -0.017 | | | | |
| | (0.050) | | | | |
| BMI (preg.) | | 0.004 | | | |
| | | (0.008) | | | |
| Postnatal depression | | | 0.045 | | |
| | | | (0.051) | | |
| Controls | Yes | Yes | Yes | Yes | No |
| Panchayat dummies | No | No | No | Yes | No |
| Block dummies | Yes | Yes | Yes | No | No |
| N | 926 | 966 | 836 | 972 | 972 |
| $R^2$ | 0.335 | 0.325 | 0.325 | 0.375 | 0.000 |

Notes: Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), trimester of pregnancy, ANC visit (dummy), child's sex and age, FREDI fixed effects. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S4 Logit estimation results of child survival until wave 2 on hemoglobin levels and anemia status during pregnancy**

| | Not alive | Not alive | Not alive | Not alive |
|---|---|---|---|---|
| Hb (preg.) | 1.017 | 0.991 | | |
| | (0.047) | (0.052) | | |
| Mild anemia (preg.) | | | 0.995 | 1.012 |
| | | | (0.183) | (0.205) |
| Moderate/severe anemia (preg.) | | | 1.080 | 1.170 |
| | | | (0.195) | (0.230) |
| Controls | No | Yes | No | Yes |
| N | 1821 | 1622 | 1821 | 1622 |

*Notes:* Standard errors clustered in village level are in in parentheses. The binary outcome variable equals 1 if a child had not survived from pregnancy to wave 2. The coefficients are reported in odds rations. Column (1) and (2) uses pregnancy Hb level of the mother as main explanatory variable. In Column (3) and (4), the two explanatory variables of interest are mild pregnancy anemia and moderate/severe pregnancy anemia. The reference category in column (3) and (4) is "no pregnancy anemia" Control variables include maternal literacy, ANC visit (dummy), first pregnancy (dummy), gestational trimester, caste category and block dummies. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

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# Pregnancy anemia, child health and development: A cohort study in rural India



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# Pregnancy anemia, child health and development: A cohort study in rural India

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## Abstract

**Objective:** To assess how pregnancy anemia affects the offspring's early childhood development, child hemoglobin (Hb) levels, growth and diseases incidence outcomes two years after birth in a low-income setting. Further we investigate the mediating role of childhood Hb levels with disease incidences and skills.

**Design:** Prospective cohort study.

**Setting and participants:** The study participants are 999 mother-child dyads from rural Madhepura in Bihar, India. In 2015, the women were recruited during pregnancy from registers in mother-child centers of 140 villages for the first wave of data collection. At the time of the second wave in 2017, the children were 22-32 months old.

**Primary and Secondary Outcome Measures:** The recruited women were visited at home for a household survey and the measurement of the women's and child's hemoglobin level, child weight and height. Data on the incidence of diarrhea and fever were collected from interviews with the mothers. To test motor, cognitive, language and socio-emotional skills of the children, we used an adapted version of the child development assessment FREDI.

**Results:** The average Hb during pregnancy was 10.2 g/dl and 69% of the women had pregnancy anemia. At the age of 22-32 months, a 1 g/dl increase in Hb during pregnancy was associated with a 0.17 g/dl (95% CI: 0.11- 0.23) increase in Hb levels of the child. Children of moderately or severely anemic women during pregnancy showed 0.57 g/dl (95% CI: -0.78 - -0.36) lower Hb than children of not anemic women. We find no association between the maternal Hb during pregnancy and early skills, stunting, wasting, underweight, or disease incidence.

**Conclusions:** 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.


Keywords: Pregnancy, anemia, hemoglobin, early childhood development, motor skills, cognitive skills, language skills, socio-emotional skills, child health, India

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study used a unique cohort of women and their newborn children.
- We controlled for maternal Hb and household food security after pregnancy, together with other relevant confounders factors.
- The data contains only one measure of Hb during pregnancy and the attrition between the waves is high.

## INTRODUCTION

Sufficient intake of macro- and micronutrients during pregnancy is a prerequisite for healthy child development.[1] Yet, the high rates of pregnancy anemia, i.e. having a low level of hemoglobin level in the blood, document the precarious state of nutrition among many pregnant women worldwide. In 2011, 32 million women were estimated to be anemic, the vast majority living in South Asia.[2] One of the most common causes of anemia in low- and middle-income countries, such as India, is iron deficiency.[3, 4] Anemia can however also result from other micronutrient deficiencies such as folic acid, vitamin B12, and vitamin A, as well as infectious diseases and genetic disorders. A focus on anemia is imperative because hemoglobin (Hb) is a crucial ingredient of red blood cells and thereby responsible for the transport of oxygen to the body tissues. Low hemoglobin during pregnancy is a known risk factor for premature birth, low birth weight, and in extreme cases leads to death.[5–9] Yet, also high hemoglobin levels, especially in the first trimester, are associated with adverse birth outcomes, suggesting a U-shaped relationship. [10]

In this paper, we investigate the consequences of pregnancy anemia on child Hb levels, early skills and other health indicators in the first 1,000 days. Most of the iron requirement in the first year of life is met by the body iron a child is born with, either in the form of hemoglobin or in iron stores (ferritin). The amount of ferritin and hemoglobin at birth depends heavily on the iron transfer from mother to child in-utero, which occurs in the second and third trimester of pregnancy.[11] Studies have shown that anemia during pregnancy correlates with low cord hemoglobin levels and anemia during infancy.[12–17] To our knowledge, there are no studies investigating the relationship of pregnancy anemia and anemia of children older than 18 months. Yet, with a prevalence of 58%, anemia of children below the age of five remains a significant global health challenge in South Asia and its causes are worth investigating.[18] The

known adverse consequences of childhood anemia on human development, in particular cognitive health, add importance to this matter.[17, 19, 20]

While several experimental and non-experimental studies have examined the impact of pregnancy anemia or iron deficiency on early skills, the empirical findings are inconclusive. [21, 22] This might be a result of the different study design, age variation of the study participants, the dimensions of skills measured, or geographical diversity.[23–30] Furthermore, as pregnancy anemia is a risk for adverse birth outcomes, the existing experimental studies exclude moderately and severely anemic women from the trials. Hence, the external validity of their findings for the general population of pregnant women is unclear. Observational studies without the appropriate quasi-experimental methods are unable to identify a causal impact of anemia during pregnancy on child outcomes due to omitted, endogenous variables. For instance, ignoring time-invariant environmental factors is likely to upwardly bias the results of non-experimental studies.

We contribute to the existing literature by analyzing the consequences of pregnancy anemia on child outcomes in three essential ways. First, we eliminate an important confounder in the analysis by controlling for the maternal hemoglobin levels and the food diversity of the household after birth. While not being able to fully capture the unobservable differences between children exposed to pregnancy anemia and without, our set of covariates will omit the bias emerging from any differences in micronutrient diversity in the post-natal period. Second, by following the children more than two years after birth, we can observe if potential initial disadvantages persisted over time. Lastly, in addition to cognitive and non-cognitive functions, and anemia, we also assess the influence of pregnancy anemia on secondary health outcomes, namely child growth and disease incidence. This analysis will help to get a deeper understanding of the adverse consequences of anemia during pregnancy.

## METHODS

### Data and procedures

Our dataset is a panel of two waves, consisting of household surveys, anthropometric and blood sample collections, and child development tests conducted in Madhepura in the North-Eastern state Bihar. Bihar is one of the poorest states of India and Madhepura belongs to its socio-economically most deprived districts. Our study sample was taken from pregnancy registers in local mother-child-centers (*Anganwadi* centers) in 140 villages in six sub-districts (blocks) of

Madhepura. It should hence be noted that our sample is not representative of the full population of pregnant women in that area, but only for those who registered in the centers. In 2015/16, this covered 76% of pregnant women.[31]

All women listed in the registries in March/April 2015 were visited by teams of trained, local survey enumerators, medical data collectors and child development testers, and invited to participate in the baseline survey and medical tests. During the follow-up in November/December 2017, we attempted to revisit the households of all formerly pregnant women. In addition to the survey and medical measures for mothers and children, a development test was administered to the child that resulted from the pregnancy. The household survey, the medical measurements and the child development test were conducted within one weeks' time for each household. **Supplementary Figure S1** visualizes the data collection timeline, the age of the children and the gestational stage of the women during the data collections.

## Outcome measures

Anemia is defined over the hemoglobin level in the blood, which in our case was obtained from a finger prick in the respondents' homes, collected by trained local enumerators. Using point-of-care HemoCue 301® machines for capillary blood, the hemoglobin level can be determined immediately and communicated to the tested individual or its caregivers on the spot.[1][32] According to the WHO and the Indian Council of Medical Research definitions, a pregnant woman is anemic if her hemoglobin concentration falls below 11.0 g/dl.[33, 34] Pregnancy anemia is further distinguished into mild anemia (10.0-10.9 g/dl), moderate anemia (7.0-9.9 g/dl) and severe anemia (<7.0 g/dl). The anemia thresholds for children between 6 and 59 months are the same as for pregnant women.[34]

During data collections, the field teams followed a strict protocol upon detection of anemia. In case of mild or moderate anemia, the women / caregivers were advised to go to the nearest primary health care center soon to seek treatment for anemia. In case of severe anemia, the

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

household was alerted that immediate attention was needed. In the follow-up survey, we also offered to cover the treatment costs and transport to a health facility for all severely anemic children.

Child development was measured with a variation of the FREDI 0-3, a German development test similar in structure to the Bayles Scales of Infant and Toddler Development.[35–37] Due to the different home environments of the children, certain items of the original FREDI 0-3 were adjusted to the Bihari context. The development test consists of a parent questionnaire and a child assessment, and covers four areas: fine and gross motor development, receptive and expressive language development, cognition, and socio-emotional development. Two age-specific tests were administered, each covering skills over an age range of five months. Each test consisted of around 40 items. All raw scores have been standard normalized and are hence presented as z-scores. In addition to the four individual test scores, we calculate the total FREDI z-score over all 40 test items. A brief validation of the FREDI with regard to physical growth and maternal education is presented in **Supplemental Figure S2 and S3**.

Stunting (i.e. being too short for their age), wasting (i.e. being too light for their height), and underweight (i.e. being too light for their age) are used as secondary health outcomes. Children's height and weight were measured during the field visits by the medical testing team. We age-standardized the raw height and weight values following the WHO Growth Standards.[38] With a respective z-score of two standard deviations (SD) below the median of the WHO references population, a child is defined as being either stunted, wasted or underweight. Any value above six SD or below six SD was coded as measurement error and dropped from the analyses.[2] The information on diarrhea and respiratory disease incidences in the two weeks before the survey were collected from maternal reports during the household survey and coded as binary variables.

## Patient and public involvement

The aims and the survey design were shared at a meeting of state-and district-level government functionaries who provided services in Madhepura through the Women and Child Development ministry, village-level leaders of women's groups prior to the baseline. At this meeting, there

[2] This was the case for 21 weight-for-height z-scores, 7 weight-for-age z-scores and 11 height-for-age z-scores.

was a detailed discussion of the types of questions that needed to be asked during the data collection. Several of these suggestions were incorporated in the baseline questionnaire. Residents of Madhepura were involved to the extent that they participated in the pre-testing of the baseline questionnaire and the FREDI tool. Patients had not been involved in the interpretation of results, writing or editing of the final document.

**Statistical analysis**

In our main analysis, we estimated the association between pregnancy anemia and the child hemoglobin level and child development approximately two years after birth using an ordinary least square regression model. Our secondary outcomes of interest, - being stunted, wasted, or underweight, incidence of diarrhea and respiratory diseases in the two weeks before the survey, were analyzed with a logistical regression model.

We considered both continuous and discrete hemoglobin levels to allow for linear and non-linear relationships between pregnancy anemia and the child outcomes. For the linear relationship, our explanatory variable of interest was the Hb levels of the women during pregnancy, measured at the time of the baseline data collection. 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. 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.

All estimations controlled for the maternal Hb levels at the time of the follow-up and the household food diversity scores. This was done to avoid an overestimation of the relationship between pregnancy and child outcomes due to an overall poor food environment or chronic diseases of the mothers. 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. We further add age, sex and current breast-feeding status of the child, as well as development test facilitator (FREDI fixed effects) or

HemoCue machine/medical tester fixed effects as control variables to obtain more precise estimates. 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 at the time of the Hb measurement and take-up of antenatal care (ANC) services).[3] Lastly, we added sub-district (block) fixed effects and clustered the standard errors on village level to take spatial correlation of the outcome variables into account. For the sensitivity analysis, we include birth spacing, macronutrient deficiency and postnatal depression to the estimation, and replaced block with *panchayat* fixed effects.[4]

In light of strong son preferences in the study region [39, 40], we investigated heterogeneous effects for boys and girls. Further, we tested for heterogeneous effects by ANC take-up, as a proxy for health preferences, caste categories, maternal literacy levels and gestational trimester at the time of the baseline survey.

As child anemia could be a result as well as a mediator for pregnancy anemia, we tested the association between childhood Hb levels and early childhood development and infectious diseases in separate analyses. Using the same set of covariates as described above, we controlled for household level and child specific characteristics that might affect both child Hb levels and the outcome variables of interest.

Finally, given the existing evidence of maternal anemia for adverse pregnancy outcomes, we conducted a survival analysis that assessed whether the Hb level of pregnant women correlates with child loss. Such a correlation would downward biased the estimates of our main analysis, as worst of children would systematically be missing in the group with higher exposure. We tested this hypothesis by estimating a probit model for non-survival on the pregnancy anemia and hemoglobin levels.

[3] To test the robustness of our findings, we also conducted an analysis in which we replaced gestational trimester during pregnancy Hb measurement with the gestational months, and a subsample analysis for each of the three gestational trimesters during the pregnancy Hb measurements.

[4] We did not include those variables in the main specification due to missing information, which would have further reduced the sample size. A *panchayat* is a subdivision of a block and comprises several villages.

All estimations were weighted with inverse probability weight, as proposed by Fitzgerald, Gottschalk and Moffitt (1998) adjusting the sample for selective attrition between the waves.[41] The statistical analyses were conducted with the statistical software Stata 16 (StataCorp LP).

## RESULTS

### Sample description

The final sample consisted of 941 to 1000 mother-child-dyads, depending on the outcome variable. 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. At the time of the endline data collection in 2017, the age of the children lied between 22 and 32 months.

**Table 1** presents the explanatory variable, covariates and the outcome variables of interest of the estimation sample separately for three anemia categories: no pregnancy anemia (column 1-3), mild pregnancy anemia (column 4-6) and moderate-to-severe pregnancy anemia (column 7-9).

The distribution shown in the table is suggestive of a gradient in child Hb levels and growth indicators across pregnancy anemia levels. We do not observe a clear trend for the skill outcomes or disease incidences. Interestingly, the distribution of Hb levels of women after pregnancy across the anemia groups mirrors the Hb levels during pregnancy suggesting that suboptimal micronutrient intake during pregnancy continued after delivery. This is however not the case for the household food diversity scores, which is the highest for the mild pregnancy anemia group.

**Table 1 Summary statistics across exposure categories in the Hb sample**

| | No preg. anemia | | | Mild preg. anemia | | | Moderate or severe preg. anemia | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | N | Mean | SD | N | Mean | SD | N |
| *Outcome variables in 2017* | | | | | | | | | |
| Hb (child) | 10.97 | (1.35) | 292 | 10.71 | (1.23) | 305 | 10.26 | (1.39) | 344 |
| Motor skills z-score | -0.02 | (1.04) | 284 | -0.04 | (1.00) | 298 | 0.07 | (0.93) | 335 |
| Language skills z-score | 0.01 | (0.96) | 291 | -0.01 | (1.00) | 304 | 0.00 | (1.02) | 344 |
| Cognition skills z-score | -0.01 | (0.99) | 290 | -0.04 | (1.02) | 304 | 0.07 | (0.97) | 340 |
| Socio-emo. skills z-score | 0.01 | (0.98) | 291 | -0.02 | (1.00) | 304 | -0.02 | (1.02) | 342 |
| Height-for-age z-score | -2.36 | (1.39) | 287 | -2.40 | (1.27) | 300 | -2.54 | (1.34) | 338 |
| Weight-for-age z-score | -1.91 | (1.10) | 286 | -1.94 | (1.05) | 294 | -2.07 | (1.08) | 339 |
| Weight-for-height z-score | -0.98 | (1.47) | 288 | -0.97 | (1.71) | 300 | -0.98 | (1.60) | 338 |
| Respiratory disease or fever | 0.20 | (0.40) | 291 | 0.21 | (0.41) | 305 | 0.20 | (0.40) | 344 |
| Diarrhea incidence | 0.24 | (0.43) | 287 | 0.25 | (0.43) | 304 | 0.26 | (0.44) | 344 |
| *Other child characteristics in 2017* | | | | | | | | | |
| Age of child (months) | 27.27 | (2.43) | 292 | 27.26 | (2.41) | 305 | 27.54 | (2.16) | 344 |
| Currently breastfed | 0.44 | (0.50) | 292 | 0.46 | (0.50) | 305 | 0.44 | (0.50) | 344 |
| Sex (Male=1) | 0.53 | (0.50) | 292 | 0.50 | (0.50) | 305 | 0.53 | (0.50) | 344 |
| *Pregnancy characteristics at baseline* | | | | | | | | | |
| Hb (preg.) | 11.76 | (0.72) | 292 | 10.44 | (0.29) | 305 | 8.68 | (1.07) | 344 |
| Gestational trimester during Hb (preg.) measurement | 1.89 | (0.76) | 292 | 2.01 | (0.69) | 305 | 2.16 | (0.69) | 344 |
| First pregnancy | 0.22 | (0.42) | 292 | 0.22 | (0.41) | 305 | 0.19 | (0.40) | 344 |
| Any ANC visits | 0.53 | (0.50) | 292 | 0.48 | (0.50) | 305 | 0.55 | (0.50) | 344 |
| Supplementary iron intake during pregnancy | 0.61 | (0.49) | 292 | 0.60 | (0.49) | 305 | 0.57 | (0.50) | 344 |
| Other micronutrient intake during pregnancy | 0.43 | (0.50) | 259 | 0.40 | (0.49) | 265 | 0.44 | (0.50) | 306 |
| *Mother characteristics* | | | | | | | | | |
| Hb (mother) in 2017 | 12.09 | (1.32) | 292 | 11.86 | (1.35) | 305 | 11.14 | (1.62) | 344 |
| Mother can read at baseline | 0.25 | (0.43) | 292 | 0.27 | (0.44) | 305 | 0.24 | (0.43) | 344 |
| Age of mother (years) at baseline | 24.65 | (3.66) | 292 | 24.90 | (3.89) | 305 | 24.67 | (3.93) | 344 |
| *Household characteristics* | | | | | | | | | |
| Food diversity index in 2017 | 7.01 | (1.50) | 292 | 7.30 | (1.46) | 305 | 7.10 | (1.52) | 344 |
| Scheduled case or tribe at baseline | 0.29 | (0.46) | 284 | 0.29 | (0.46) | 295 | 0.33 | (0.47) | 331 |
| Improved sanitation facility at baseline | 0.14 | (0.34) | 292 | 0.12 | (0.33) | 305 | 0.12 | (0.32) | 344 |
| Asset index quintile at baseline | 3.09 | (1.44) | 292 | 3.01 | (1.42) | 305 | 2.85 | (1.45) | 344 |

The majority of women in our sample (69%) were at least mildly or moderately anemic during pregnancy, with an average Hb level of 10.2 g/dl. Of all anemic women, 48% showed signs of moderate anemia, and only 4% severe anemia. Less than 2% of women had Hb level above 13 g/dl, which can be considered as high. Overall, the literacy level of the study population was low with a quarter of the women being able to read and write, similar in all three presented anemia groups. Such low levels of literacy are comparable with district-wide statistics of a nationally representative survey from 2015-16.[31] 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. Importantly, half of the women reported not having received any ANC during pregnancy. This figure sets our sample strongly apart from most existing studies, many of which recruited their participants during ANC visits [23, 29, 42] or delivered ANC visits as part of the study [28]. The uptake of ANC services is the highest in the group of moderate or severe pregnancy anemia and lowest for women of mild pregnancy anemia.

The sample size during follow-up data collection reduced considerably from the baseline (N=1,918) due to the unavailability of the women or children at the time follow-up visits, inaccurate location information, refusal to provide a blood sample or to participate in the child testing, and child death (**Figure 1**). We account for this loss in sample size by using inverse probability weights in all estimations, assigning higher weights to those households that had a higher probability of dropping out.[5]

*- Figure 1 here -*

**Association of pregnancy anemia with childhood anemia and early skills**

We found a strong association of Hb during pregnancy, mild, and moderate or severe pregnancy anemia with the child's hemoglobin levels (**Table 2)**. An increase of 1 g/dl in Hb during pregnancy was associated with 0.17 g/dl higher Hb levels in the offspring. Children born to women with mild anemia had 0.20 g/dl lower Hb level than their non-anemic peers did. The coefficient was more than twice the size for children born to mothers with moderate or severe pregnancy anemia. Current HB of the mothers are consistently positively correlated with the child Hb levels, at a statistical significance level of 1%. Testing for a U-shape relationship showed a positive, but decreasing correlation of pregnancy Hb and child Hb, statistically significant at the 5% level (*Panel C*, **Table 2**).

[5] Relevant weighting variables were used despite missing information, which reduces the estimation samples by two more observations.

**Table 2 Weighted regression results on the association between maternal hemoglobin (Hb) levels and anemia during pregnancy and early childhood development and childhood Hb levels**

| | Hb (child) | Motor skills | Language skills | Cognition skills | Socio-emo. skills |
|---|---|---|---|---|---|
| *Panel A* | | | | | |
| Hb (preg.) | 0.17*** | -0.01 | -0.01 | -0.03* | -0.02 |
| | [0.11,0.23] | [-0.05,0.03] | [-0.05,0.04] | [-0.07,0.00] | [-0.05,0.02] |
| Hb (mother) | 0.13*** | 0.01 | 0.04 | 0.06*** | 0.05** |
| | [0.07,0.20] | [-0.03,0.05] | [-0.01,0.08] | [0.02,0.10] | [0.01,0.10] |
| $R^2$ | 0.174 | 0.246 | 0.218 | 0.303 | 0.321 |
| *Panel B* | | | | | |
| Mild preg. anemia | -0.20* | 0.03 | 0.01 | 0.06 | 0.05 |
| | [-0.41,0.00] | [-0.13,0.19] | [-0.13,0.15] | [-0.09,0.22] | [-0.08,0.17] |
| Moderate/severe preg. anemia | -0.57*** | 0.06 | 0.03 | 0.12* | 0.01 |
| | [-0.78,-0.36] | [-0.10,0.22] | [-0.12,0.19] | [-0.02,0.26] | [-0.12,0.15] |
| Hb (mother) | 0.15*** | 0.01 | 0.04* | 0.06*** | 0.05** |
| | [0.08,0.22] | [-0.03,0.05] | [-0.01,0.08] | [0.02,0.10] | [0.00,0.09] |
| $R^2$ | 0.172 | 0.246 | 0.218 | 0.303 | 0.321 |
| *Panel C* | | | | | |
| Hb (preg.) | 0.64*** | 0.16 | 0.03 | 0.21 | -0.01 |
| | [0.17,1.12] | [-0.12,0.45] | [-0.26,0.32] | [-0.05,0.47] | [-0.28,0.25] |
| Hb (preg.) $^2$ | -0.02** | -0.01 | -0.00 | -0.01* | -0.00 |
| | [-0.05,-0.00] | [-0.02,0.01] | [-0.02,0.01] | [-0.03,0.00] | [-0.01,0.01] |
| Hb (mother) | 0.13*** | 0.01 | 0.04 | 0.06*** | 0.05** |
| | [0.06,0.19] | [-0.03,0.05] | [-0.01,0.08] | [0.02,0.10] | [0.01,0.10] |
| $R^2$ | 0.178 | 0.247 | 0.218 | 0.305 | 0.321 |
| Controls | Yes | Yes | Yes | Yes | Yes |
| Tester fixed effects | Yes | No | No | No | No |
| FREDI fixed effects | No | Yes | Yes | Yes | Yes |
| N | 939 | 972 | 996 | 990 | 994 |

*Notes*: Outcome variables in column (2)-(5) are standardized test scores and the coefficients are shown in standard deviations. *Panel A* uses pregnancy Hb level of the mother as main explanatory variable. In *Panel B*, the two explanatory variables of interest are mild pregnancy anemia and moderate/severe pregnancy anemia, while the omitted category is no pregnancy anemia. In *Panel C*, the level of pregnancy Hb in quadratic from is included. 95% confidence intervals using standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding status, maternal age and literacy, first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in in column (2)-(5): test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

We did not find a statistically significant relationship of Hb or any type of pregnancy anemia with the child development in general, or any specific dimension of development. The coefficients were small in magnitude, indicating indeed zero-effects, rather than an imprecise estimation. For cognitive development, we found a small, negative and weakly statistically significant association with Hb level during pregnancy and moderate or severe pregnancy anemia. We found weak evidence for a U-shaped relationship between pregnancy Hb and cognitive skills, but not for motor, language or socio-emotional skills.

The heterogeneity analyses showed that there were only small differences in the estimates of pregnancy Hb on child Hb and skills on by gestational trimester, caste category, maternal literacy, sex and ANC take-up (**Supplemental Table S1**). In the case of language skills, we found a small, weakly statistically significant, negative interaction effect with ANC visits and a statistically significant, but positive interaction term for households belonging to a scheduled caste or tribe. Hence, for scheduled castes and tribes, we find evidence for a positive correlation of pregnancy anemia and early language skills.

Our findings were robust to the inclusion of additional, potentially confounding, covariates, namely birth spacing, body-mass-index and postnatal depression, panchayat fixed effects, and the exclusion of all covariates and fixed effects (**Supplemental Table S2 and S3**). Robustness checks including gestational month instead of gestational trimester, and the subgroup analysis by gestational trimester also confirmed our main results (**Supplemental Table S4 and S5**). We found no evidence for survival bias, i.e. that the surviving children were exposed to higher maternal Hb levels in-utero (**Supplemental Table S6).**

**Association of pregnancy anemia and child health indicators**

We found no indication that suboptimal Hb levels during pregnancy were associated with stunting, wasting or underweight of children, or increased the incidence of respiratory diseases or diarrhea (**Figure 2**).

*- Figure 2 here -*

### Association of childhood anemia with skill and child health

The lack of association between pregnancy anemia, early skills and infectious diseases, despite the strong correlation with early Hb levels, might be an indication that *childhood* anemia is not a risk factor for early childhood development or disease incidence in our study population. The cross-sectional analysis confirmed this hypothesis for diarrheal and respiratory diseases (**Table 3**). Yet, for the cumulative development scores, we found a small, but positive and statistically significant coefficient.

**Table 3. Association of child's hemoglobin (Hb) with their early skills and infectious disease incidence**

| | Cum. development z-score | Respiratory disease | Diarrhea |
|---|---|---|---|
| | Marginal effects | Odds ratios | Odd ratios |
| Hb (child) | 0.04*** | 1.02 | 0.99 |
| | [0.01,0.08] | [0.89,1.16] | [0.88,1.11] |
| Hb (mother) | 0.02 | 1.00 | 0.98 |
| | [-0.01,0.05] | [0.89,1.12] | [0.88,1.08] |
| (Adjusted) $R^2$ | 0.334 | 0.061 | 0.047 |
| Controls | Yes | Yes | Yes |
| Tester fixed effects | Yes | Yes | Yes |
| FREDI fixed effects | Yes | No | No |
| N | 915 | 938 | 933 |

*Notes*: The outcome variable in column (1) is the standardized total test score and the coefficients are shown in standard deviations. Outcomes in column (2) and (3) are binary variables and the coefficient are shown as odds ratios. 95% Confidence intervals using standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in column (1): test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

## DISCUSSION

### Interpretation

Our cohort study from rural Bihar, India, shows a strong association between maternal hemoglobin levels during pregnancy and the hemoglobin levels of the offspring between 22 and 32 months after birth. The association is strongly statistically significant and robust to the inclusion of several, potentially confounding, variables, such as current nutritional status of the mother or child age. The relationship does not differ by child sex, caste category, gestational trimester, ANC take-up or maternal literacy. Moderate and severe pregnancy anemia is

associated with lower Hb levels of children than mild pregnancy anemia. 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.

Our analysis extends the current literature that connects pregnancy anemia with direct birth outcomes in India, such as prematurity birth weight and size.[43, 44] The biological link between pregnancy anemia and child anemia more than two years after birth is unclear as the iron transferred from mother to child in-utero is typically consumed by the child's needs within the first year of life, before complementary feeding starts.[11] Our findings might be explained by a lower Hb trajectory since birth, initiated by the low hemoglobin environment in-utero as has been shown in Benin.[12] 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]

Overall, our findings on childhood anemia are in line with a recent study from the US, that showed an elevated risk ratio of infant anemia from anemia during pregnancy, in particular for moderate and severe anemic condition.[15] Similarly, an analysis with data from China showed reduced Hb levels of infants for maternal anemia during the 24 - 28 gestational week, the authors find however no association for the first gestational trimester.[16] 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]

We did not find any correlation between low Hb level during pregnancy and other child health outcomes, such disease incidence or growth indicators. The disadvantage of children born to anemic mothers, compared to their peers, hence seems to be limited to the low Hb levels. Our study does also not show any association between *childhood* Hb and infectious diseases, even though iron deficiency, a major cause of anemia, is known to weaken the immune functions.[46] In this regard, our findings contradict a study on Bedouin children in Israel, presenting a strong linkage between anemia, diarrhea and respiratory illnesses of children.[47]

Lastly, we find no evidence that pregnancy anemia is associated with lower early childhood development, even though children's Hb levels do correlate with early skills in our sample. Our results thereby go against the outcomes of several observational studies, most prominently

Chang et al.'s analysis in rural China, with children as a similar age. While they did not find an association of pregnancy anemia and psychomotor development, children of anemic mothers showed lower scores in language and cognitive development when 18 and 24 months old.[28]

Differences in the socio-economic characteristics of two study populations might explain this difference. Not only did the mothers in Chang et al.'s study have higher levels of education, but the prevalence of childhood undernutrition among them was about a fifth as large as in our study population. The poor nutritional status of children in Madhepura is likely not only a result of the insufficient intake of macro- and micronutrients but also of frequent gastrointestinal infections. The low coverage of improved toilet facilities and high prevalence of unsafe disposal of children's stool possibly led to greater exposure to fecal bacteria, thus facilitating the spread of diarrheal diseases and parasites. The constant exposure to fecal bacteria could also cause environmental enteropathy which hampers the absorption of nutrients and worsens malnutrition.[48–50]

Furthermore, it is important to note that the human brain development is largely driven by experience.[51] A lack of adequate learning opportunities and stimulation in the early years can have long lasting consequences for the functioning of the brain.[52, 53] The stimulation environment created by the caregivers for their children is limited in our study area. Only about half of the caregivers told stories, sung songs or read a book to the children during the three days prior to the survey. About a quarter of the mothers reported that no household member had played with the child in that time.

Taken together, all of these lead us to argue that the additional, possible adverse impact of pregnancy anemia on child development in our study setting was not large enough to be detectable in our estimations.

**Limitations**

Our analysis is based on the assumption that the single hemoglobin measure taken at baseline is informative of the hemoglobin status during the full course of pregnancy. Most Hb measurements of our participants were taken in the second trimester, allowing sufficient time for any improvement in the Hb levels during the remaining months. Such improvements after the baseline data collection would hence weaken the relationship of Hb that we measured with child outcomes. Given that half of the mothers in our sample reported not having consumed

any iron supplements during pregnancy or received any antenatal care, we believe that the extent of such attenuation bias is limited.

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.

Any systematic error in Hb measurement, depending on the Hb level, could be a bigger concern. This could have been induced by the differential recommendations our field team gave based on the blood test results. However, our data do not support this hypothesis. We did not observe a particular behavior change in more severely anemic women: even after delivery only 57% of the women with moderate or severe pregnancy anemia reported consuming iron supplements during pregnancy, compared to 60-61% in the mildly and non-anemic group. During pregnancy, the reported supplementation rates across the groups were as well similar (16% of the moderately or severely anemic women vs. 19-20% of the mildly or non-anemic women). This suggests that our advice did not alter the women's behavior. Two additional points should be kept in mind when interpreting our findings. First, we collected Hb levels but not ferritin levels, which leaves room for speculation on the origin of anemia in the study sample. Although iron deficiency is commonly believed to be the major reason for anemia, to our knowledge, no study has documented the actual share of iron deficiency anemia among the anemic pregnant women in rural Bihar. Second, it should be noted that the share of severely anemic pregnant women in our study is very small. Our estimated association of moderate or severe anemia and child development and Hb is hence mainly attributable to the group of women who were moderately anemic during pregnancy.

## 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. The relationship between pregnancy anemia and childhood Hb grows stronger with lower levels of Hb during pregnancy. Yet, diarrhea or respiratory disease incidence or child growth is not affected by either childhood Hb or pregnancy Hb. 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.

**Author contribution:** EH, MAS and SV designed research and the survey instruments, CM designed the child skill assessment. SV and MAS coordinated the first wave data collection, EH coordinated and supervised the second wave data collection, with the support by CM for the child testing. EH analyzed the data, wrote the paper, had primary responsibility for final content. All authors have read and approved the final manuscript.

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**Competing interests:** None declared.

**Patient consent for publication**: Not required.

**Ethics approval:** The study was approved by the ethics board of the IIT Gandhinagar (IEC/2014-15/2/MS/006, IEC/2016-17/2/MS/025) and the University of Goettingen (no IRB number available). We obtained written informed consent for the interviews, growth measurements, blood sample collections and the child development testing during each wave.

**Data availability statement:** No additional data available.

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## Figures

Figure 1 Attrition between 2015 (wave 1) and 2017 (wave 2) in childhood hemoglobin and skills sample

Figure 2 Weighted regression results on the association between maternal hemoglobin (Hb) levels during pregnancy and growth and disease incidence indicators (odds ratios)

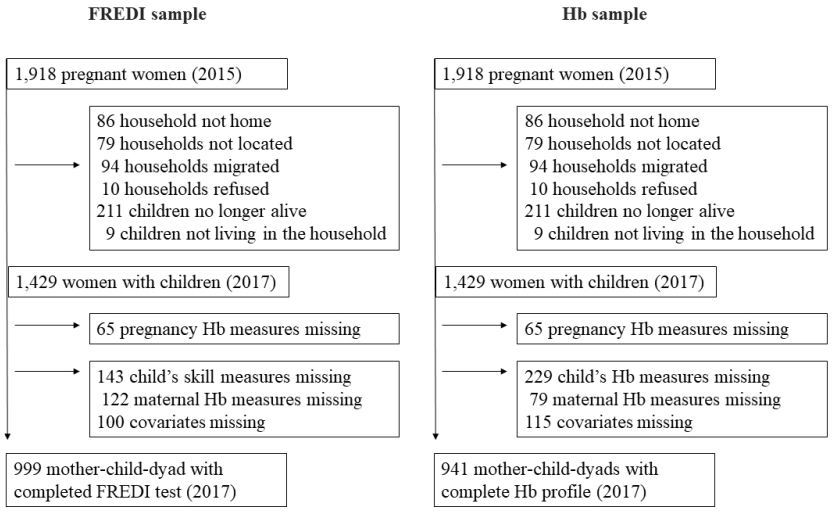



Figure 1
Attrition between 2015 (wave 1) and 2017 (wave 2) in childhood hemoglobin and skills sample

338x190mm (96 x 96 DPI)

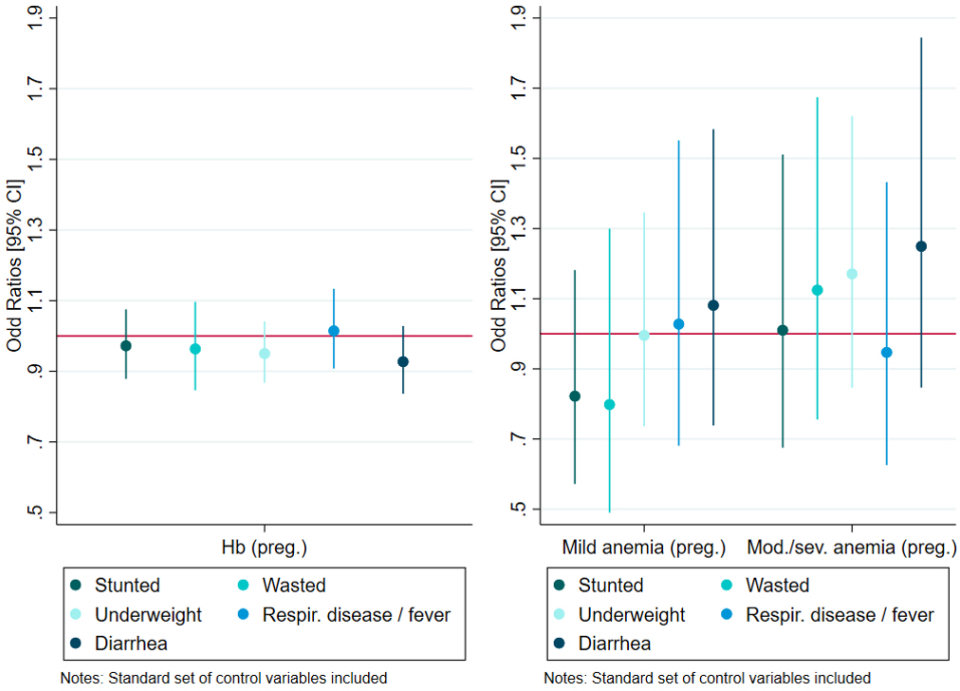
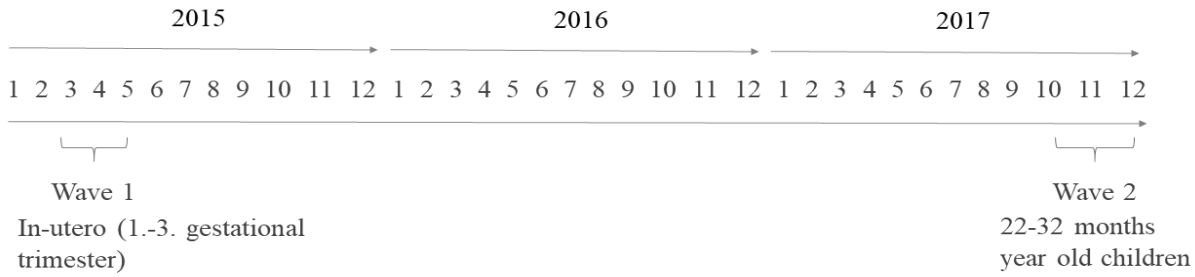


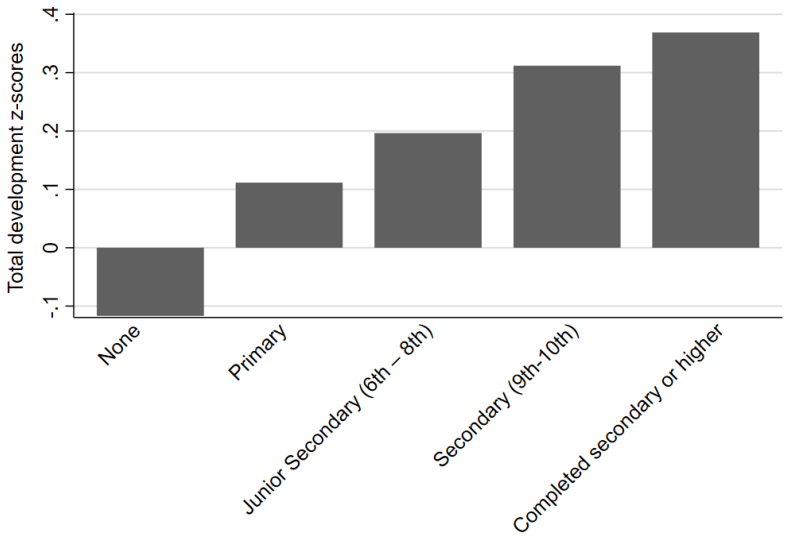

Figure 2
Weighted regression results on the association between maternal hemoglobin (Hb) levels during pregnancy and growth and disease incidence indicators (odds ratios)

207x151mm (129 x 129 DPI)

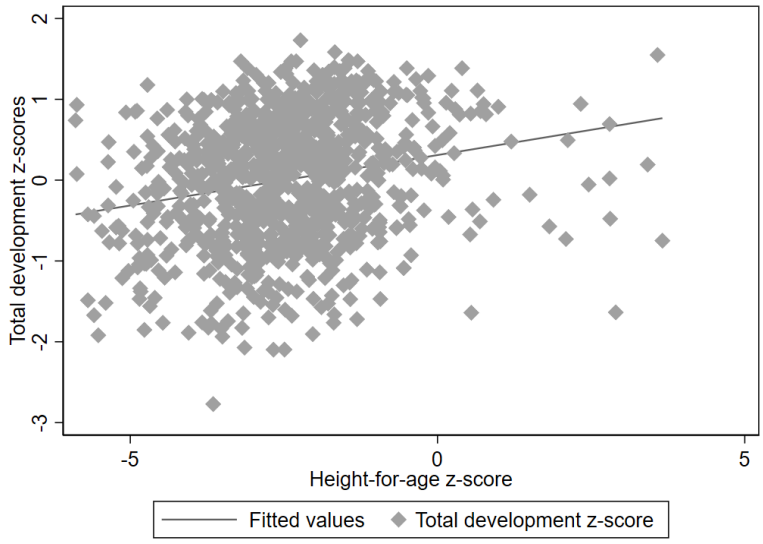
**Online Supporting Material**




**Supplemental Figure S1 Timeline of data collections and the respective age of the children**




**Supplemental Figure S2 Average cumulative development test scores by maternal education category**




**Supplemental Figure S3 Average cumulative development test scores by of FREDI by child growth**

**Supplemental Table S1 Heterogeneous treatment effects using a linear regression model on child hemoglobin levels and skills dimensions**

| | Hb (child) | Motor skills | Language skills | Cognition skills | Socio-emo. skill |
|---|---|---|---|---|---|
| **Panel A**: By antenatal care uptake | | | | | |
| Hb (preg.) | 0.162*** | -0.006 | 0.034 | -0.029 | 0.013 |
| | (0.042) | (0.029) | (0.030) | (0.025) | (0.025) |
| Any ANC # Hb (preg.) | 0.014 | -0.006 | -0.079* | -0.007 | -0.059* |
| | (0.058) | (0.035) | (0.041) | (0.034) | (0.035) |
| $R^2$ | 0.174 | 0.248 | 0.221 | 0.304 | 0.332 |
| N | 938 | 972 | 996 | 990 | 994 |
| **Panel B:** By child's sex | | | | | |
| Hb (preg.) | 0.208*** | 0.009 | -0.018 | -0.027 | -0.025 |
| | (0.041) | (0.028) | (0.025) | (0.027) | (0.024) |
| Male # Hb (preg.) | -0.077 | -0.037 | 0.024 | -0.009 | 0.015 |
| | (0.064) | (0.037) | (0.038) | (0.034) | (0.036) |
| $R^2$ | 0.176 | 0.247 | 0.218 | 0.303 | 0.321 |
| N | 939 | 972 | 996 | 990 | 994 |
| **Panel C:** By caste category | | | | | |
| Hb (preg.) | 0.164*** | -0.016 | -0.030 | -0.035* | -0.019 |
| | (0.033) | (0.023) | (0.026) | (0.019) | (0.022) |
| Scheduled caste or tribe # Hb (preg.) | 0.033 | 0.032 | 0.088** | 0.005 | -0.008 |
| | (0.079) | (0.039) | (0.044) | (0.041) | (0.039) |
| $R^2$ | 0.177 | 0.245 | 0.220 | 0.306 | 0.318 |
| N | 908 | 941 | 964 | 958 | 962 |
| **Panel D:** By maternal literacy | | | | | |
| Hb (preg.) | 0.166*** | -0.011 | -0.007 | -0.021 | -0.015 |
| | (0.035) | (0.022) | (0.023) | (0.020) | (0.021) |
| Maternal literacy # Hb (preg.) | 0.014 | 0.010 | 0.003 | -0.047 | -0.013 |
| | (0.072) | (0.050) | (0.047) | (0.047) | (0.038) |
| $R^2$ | 0.174 | 0.246 | 0.218 | 0.304 | 0.321 |
| N | 939 | 972 | 996 | 990 | 994 |
| **Panel E:** By gestational trimester | | | | | |
| Hb (preg.) | 0.173*** | -0.029 | -0.029 | -0.051 | -0.026 |
| | (0.054) | (0.038) | (0.040) | (0.037) | (0.034) |
| Second gestational trimester # Hb (preg.) | -0.031 | 0.039 | 0.061 | 0.029 | 0.041 |
| | (0.077) | (0.045) | (0.047) | (0.044) | (0.046) |
| Third gestational trimester # Hb (preg.) | 0.027 | 0.011 | -0.010 | 0.020 | -0.031 |
| | (0.073) | (0.052) | (0.053) | (0.050) | (0.040) |
| $R^2$ | 0.175 | 0.246 | 0.220 | 0.303 | 0.323 |
| N | 939 | 972 | 996 | 990 | 994 |
| Controls | Yes | Yes | Yes | Yes | Yes |
| Tester fixed effects | Yes | No | No | No | No |
| FREDI fixed effects | No | Yes | Yes | Yes | Yes |

Notes: Outcome variables in column (2)-(5) are standardized test scores and the coefficients are shown in standard deviations. Standard errors clustered on village level are in parentheses. Included control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, ANC visit (dummy), child's sex and age, and block dummies. Additional control variable in column (2)-(5): test version. Tester fixed effects are anthropometric test conductor fixed effects. FREDI fixed effects are child development test conductor fixed effects. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S2 Linear regression model results of haemoglobin level of children on haemoglobin levels during pregnancy with additional covariates**

| | Hb (child) | Hb (child) | Hb (child) | Hb (child) | Hb (child) |
|---|---|---|---|---|---|
| Hb (preg.) | 0.176*** | 0.163*** | 0.165*** | 0.154*** | 0.213*** |
| | (0.029) | (0.030) | (0.029) | (0.030) | (0.033) |
| Hb (mother) | 0.127*** | 0.133*** | 0.128*** | 0.134*** | |
| | (0.034) | (0.034) | (0.036) | (0.035) | |
| Mother gave birth in past 2 years | 0.013 | | | | |
| | (0.111) | | | | |
| BMI (preg.) | | 0.009 | | | |
| | | (0.016) | | | |
| Postnatal depression | | | 0.087 | | |
| | | | (0.118) | | |
| Controls | Yes | Yes | Yes | Yes | No |
| Tester fixed effects | Yes | Yes | Yes | Yes | No |
| Panchayat dummies | No | No | No | Yes | No |
| Block dummies | Yes | Yes | Yes | No | No |
| N | 896 | 933 | 807 | 939 | 939 |
| $R^2$ | 0.173 | 0.174 | 0.161 | 0.236 | 0.054 |

Notes: Each column presents the estimation results of the main estimation with the displayed covariates or spatial fixed effects. Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, ANC visit (dummy), child's sex and age.. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S3 Linear regression model results of cumulative test scores of children on haemoglobin levels during pregnancy with additional covariates**

| | Cum. development score | Cum. development score | Cum. development score | Cum. development score | Cum. development score |
|---|---|---|---|---|---|
| Hb (preg.) | -0.023 | -0.015 | -0.009 | -0.015 | -0.008 |
| | (0.015) | (0.014) | (0.016) | (0.016) | (0.017) |
| Hb (mother) | 0.042*** | 0.041** | 0.036** | 0.042** | |
| | (0.016) | (0.016) | (0.017) | (0.017) | |
| Mother gave birth in past 2 years | -0.017 | | | | |
| | (0.050) | | | | |
| BMI (preg.) | | 0.004 | | | |
| | | (0.008) | | | |
| Postnatal depression | | | 0.045 | | |
| | | | (0.051) | | |
| Controls | Yes | Yes | Yes | Yes | No |
| FREDI fixed effects | Yes | Yes | Yes | Yes | No |
| Panchayat dummies | No | No | No | Yes | No |
| Block dummies | Yes | Yes | Yes | No | No |
| N | 926 | 966 | 836 | 972 | 972 |
| $R^2$ | 0.335 | 0.325 | 0.325 | 0.375 | 0.000 |

Notes: Each column presents the estimation results of the main estimation with the displayed covariates or spatial fixed effects. Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, ANC visit (dummy), test version, child's sex and age. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S4 Linear regression model results of childhood Hb levels on Hb levels during pregnancy controlling for gestational month during Hb measurements and gestational trimester specific subgroup analyses**

| | Hb (child) | Hb (child) | Hb (child) | Hb (child) |
|---|---|---|---|---|
| Hb (preg.) | 0.17*** | 0.13** | 0.15*** | 0.20*** |
| | (0.03) | (0.06) | (0.05) | (0.05) |
| Hb (mother) | 0.13*** | 0.16** | 0.13** | 0.08 |
| | (0.03) | (0.06) | (0.06) | (0.06) |
| Gestational month during preg. Hb measure | 0.01 | | | |
| | (0.02) | | | |
| Controls | Yes | Yes | Yes | Yes |
| Tester fixed effects | Yes | Yes | Yes | Yes |
| Sample | Full sample | First trimester subsample | Second trimester subsample | Third trimester subsample |
| N | 972 | 245 | 467 | 260 |
| $R^2$ | 0.324 | 0.376 | 0.416 | 0.364 |

Notes: In column (1) the dummies for the gestational trimester during pregnancy Hb measurement are replaced by the gestational month. Column (2)-(4) present the sub-group analyses for each gestational trimester. Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), ANC visit (dummy), child's sex and age and block dummies. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S5 Linear regression model results of cumulative test scores of children on Hb levels during pregnancy controlling for gestational month during Hb measurements and gestational trimester specific subgroup analyses**

| | Cum. development score | Cum. development score | Cum. development score | Cum. development score |
|---|---|---|---|---|
| Hb (preg.) | -0.01 | 0.00 | 0.01 | -0.06* |
| | (0.01) | (0.03) | (0.02) | (0.03) |
| Hb (mother) | 0.04** | -0.04 | 0.04* | 0.07** |
| | (0.02) | (0.04) | (0.02) | (0.03) |
| Gestational month during preg. Hb measure | 0.03** | | | |
| | (0.01) | | | |
| Controls | Yes | Yes | Yes | Yes |
| FREDI fixed effects | Yes | Yes | Yes | Yes |
| Sample | Full sample | First trimester subsample | Second trimester subsample | Third trimester subsample |
| N | 972 | 245 | 467 | 260 |
| $R^2$ | 0.324 | 0.376 | 0.416 | 0.364 |

Notes: In column (1) the dummies for the gestational trimester during pregnancy Hb measurement are replaced by the gestational month. Column (2)-(4) present the sub-group analyses for each gestational trimester. Standard errors clustered on village level are in parentheses. Control variables: caste category, wealth quintile, food diversity in 2017, breast feeding, maternal age and literacy, first pregnancy (dummy), ANC visit (dummy), test version, child's sex and age. Hemoglobin is measured in g/dl. Inverse probability weight accounting for attrition applied. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

**Supplemental Table S6 Logit estimation results of child survival until wave 2 on Hb levels and anemia status during pregnancy**

| | Not alive | Not alive | Not alive | Not alive |
|---|---|---|---|---|
| Hb (preg.) | 1.017 | 0.991 | | |
| | (0.047) | (0.052) | | |
| Mild anemia (preg.) | | | 0.995 | 1.012 |
| | | | (0.183) | (0.205) |
| Moderate/severe anemia (preg.) | | | 1.080 | 1.170 |
| | | | (0.195) | (0.230) |
| Controls | No | Yes | No | Yes |
| N | 1821 | 1622 | 1821 | 1622 |

*Notes:* Standard errors clustered in village level are in in parentheses. The binary outcome variable equals 1 if a child had not survived from pregnancy to wave 2. The coefficients are reported in odds ratios. Column (1) and (2) uses pregnancy Hb level of the mother as main explanatory variable. In Column (3) and (4), the two explanatory variables of interest are mild pregnancy anemia and moderate/severe pregnancy anemia. The reference category in column (3) and (4) is "no pregnancy anemia" Control variables include maternal literacy, ANC visit (dummy), first pregnancy (dummy), gestational trimester during Hb (preg.) measurement, caste category and block dummies. Hemoglobin is measured in g/dl. Conventional significance level: * p<0.1, ** p<0.05, *** p<0.01.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation | Page No |
|---|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract | 2 |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported | 3 |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses | 3 |
| **Methods** | | | |
| Study design | 4 | Present key elements of study design early in the paper | 3,4,5 |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 4 |
| Participants | 6 | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up | 4 |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed | |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5,6 |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5,6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7,8 |
| Study size | 10 | Explain how the study size was arrived at | 10 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7 |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding | 6,7,8 |
| | | (*b*) Describe any methods used to examine subgroups and interactions | 7 |
| | | (*c*) Explain how missing data were addressed | 7 |
| | | (*d*) If applicable, explain how loss to follow-up was addressed | 8 |
| | | (*e*) Describe any sensitivity analyses | 7,8 |
| **Results** | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed | 10 |
| | | (b) Give reasons for non-participation at each stage | 10 |
| | | (c) Consider use of a flow diagram | 10 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders | 9 |
| | | (b) Indicate number of participants with missing data for each variable of interest | 9 |
| | | (c) Summarise follow-up time (eg, average and total amount) | 4 |
| Outcome data | 15* | Report numbers of outcome events or summary measures over time | 9 |

| | | | |
|---|---|---|---|
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included<br>(*b*) Report category boundaries when continuous variables were categorized<br>(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | 11<br>5 |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 11,12 |
| **Discussion** | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13,14 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 15,16 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 16 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 5,16 |
| **Other information** | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 16,17 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.