Risk factors for maternal anaemia and low birth weight in pregnant women living in rural India: A prospective cohort study

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Abstract

Objective: The aim of this prospective study was to estimate the prevalence and risk factors for maternal anaemia and low birth weight in pregnant women living in Maharashtra state, India.

Study design: Prospective study

Methods: Women between 3 to 5 months of pregnancy were recruited from 34 villages based in Maharashtra state. Baseline data collection, anthropometric measurements and blood investigations were performed. Participants were followed-up to record birth weight.

Results: In total, 303 women were eligible, and 287 (95%) provided data. 77% were anaemic defined as haemoglobin less than 11.0 g/dL at the time of recruitment, with a mean corpuscular volume (MCV) of 80.5 fl/cell, (standard deviation: 7.22, range: 53.4 to 93.8). Increased risk of anaemia was seen in women with consanguineous marriages (odds ratio (OR): 2.41, 95% Confidence Interval (CI): 1.16 to 5.01, p=0.01) after adjustment for potential confounding factors. Post-delivery data from full-term singleton live births demonstrated a 7% prevalence of low birth weight. Consanguineous marriage was a major risk for low birth weight (OR: 4.10, 95% CI: 1.25 to 13.41, p=0.02). The presence of maternal anaemia during 3 to 5 months of pregnancy was associated with lower risk of low birth weight (unadjusted OR: 0.34, 95% CI: 0.13 to 0.92, p= 0.03).

Conclusion: About 30% of our study participants were in a consanguineous marriage, which was identified as a potentially avoidable risk factor for both anaemia and low birth weight.

Abbreviations: ANC, Antenatal Care; BMI, Body Mass Index; BP, Blood Pressure; CBC, Complete Blood Count; g/dL, Grams Per Decilitre; Haemoglobin; Hb, HMF, Halo Medical Foundation; IFA, Iron Folic Acid; LBW, Low Birth Weight; MAS, Maharashtra Anaemia Study; MCV, Mean Corpuscular Volume; MUAC, Mid-upper arm circumference; NFHS, National Family Health Survey; RBS, Random Blood Sugar; VHW, Village Health Worker.

Background

Anaemia is a very common condition, which leads to a decrease in red blood cells and circulating haemoglobin in the blood, resulting in lower oxygen carrying capacity [1]. This is widely observed among pregnant women in developing countries such as India [1]. Anaemia leads to conditions such as general fatigue, weakness, shortness of breath [1].

The National Family Health Survey of India (NFHS 3) reported that 56% of women (between 15 to 49 years) were anaemic with a greater prevalence in rural areas [2]. The high prevalence of anaemia in a rural Indian setting is exacerbated by limited medical infrastructure to diagnose and treat anaemia [3,4]. Poor medical care during pregnancy may affect obstetric outcomes such as birth weight [2]. If the weight at birth is less than 2.5 kg then it is labelled as low birth weight (LBW), which may hamper neonatal health outcomes [2]. The NFHS 3 suggested that LBW prevalence is about 22% in the country with higher occurrence in rural areas compared to urban regions [2]. Higher prevalence was seen in younger women (< 20 years) at the time of delivery, and the prevalence decreased with an increase in education and wealth [2].

There is limited information on risk factors for either anaemia during pregnancy or low birth weight (LBW) from rural areas of India. A community based cohort study to identify determinants of LBW was conducted in rural areas of Maharashtra state in 1994 [5]. In this study, an increased risk of LBW was associated with i) low maternal haemoglobin (Hb) (less than 9.0 g/dL), ii) third trimestral bleeding, and iii) low maternal body mass index (BMI). However, the prevalence and risk factors associated with maternal anaemia were not assessed in this study. To our knowledge, no cohort studies on this important public health issue have been conducted in rural areas of the country.

The aim of this study of pregnant women from rural areas of Maharashtra state of India was to estimate the prevalence of anaemia, and investigate associated risk factors during the early stages of pregnancy. The study also examined exposures associated with low birth weight in the rural population. This research is a part of the project known as 'Maharashtra Anaemia Study' (MAS).

Material and Methods

Study population

The Maharashtra Anaemia Study (MAS) is a joint collaboration between Halo Medical Foundation India (HMF) and the University of Nottingham, United Kingdom (UK) [6]. The research area included 34 villages (approximately 65,000 population) from Tuljapur and Lohara blocks of Osmanabad district. Osmanabad district has a population of 1.6 million individuals; 85% reside in rural areas, with a low mean annual income (600 GBP per capita), and limited healthcare infrastructure [7]. The majority of pregnant women receive obstetric care in this community from government healthcare providers such as nurses.

A pilot process to test the study recruitment and data collection methods was conducted from January to March 2014. Study equipment was tested on the 1st working day of each month to generate an equipment performance report. The HMF's hospital in Andur provided the laboratory investigation support for this study [8].

The study was approved by the Institutional Ethics Committee of Government Medical College Aurangabad, Maharashtra, India (reference number: Pharma/IEC/GMA/196/2014), and the University of Nottingham (UK) Medical School Ethics Committee (reference number: E10102013).

Recruitment and data collection

Data were collected at three stages: (a) pre-delivery data collection from pregnant women participants, (b) village level data collection and (c) post-delivery data collection.

(a) Pre-delivery data collection: Pregnant women were identified through monthly household surveys of women in the reproductive age group (15 to 49 years), conducted by HMF's village based healthcare workers. All self-reported pregnancies identified between April 1, 2014 and December 31, 2014 were eligible. No formal sample size calculation was conducted, as the project was designed as a feasibility study to inform future research priorities. Eligible study participants were approached and provided with a summary of the proposed study. Those who decided to enter the study provided written consent before entry to the study.

The questionnaire was administered in the local language by a trained data assistant or the primary investigator (PI) himself across the project tenure. The individual questionnaire had sections on sociodemographic characteristics, medical/obstetric history, iron supplements, vaccinations, dietary preference, 7-day diet recall, family assets, ante-natal care (ANC) access and self-reported birth outcomes, in accordance with the standard operating procedures (SOP) [9]. The blood sample was collected by a trained member of the study team. Anthropometric measurements including height and mid-upper arm circumference (MUAC) of the dominant hand, were recorded using measuring tapes. Weight was ascertained using a digital weighing machine (OMRON Healthcare, India). Blood pressure (BP) was measured using the right arm in a sitting position with an automated digital BP monitor following manufacturer's recommendations (OMRON Healthcare, India). Venous blood withdrawal was performed in a supine position by a qualified and a trained laboratory technician. The complete blood count (CBC) was measured using Sysmex XP-100 automated analyser (Sysmex Corporation, Japan), and random blood sugar (RBS) was calculated using semi-automated device Erba Chem Touch (Erba Mannheim, Germany) at HMF hospital. All data collection and investigation were performed in the presence of the

PI.Additional information on data collection, blood withdrawal, transport, investigation and quality controls is published elsewhere [10].

Anaemia was defined based on a haemoglobin (Hb) level of less than 11.0 g/dL, and additionally further categorisation is performed using Hb levels as follows: severe anaemia (Hb < 7.0 g/dL), moderate anaemia Hb 7.0 to 9.9 g/dL, and mild anaemia (Hb 10.0 to 10.9 g/dL) [11]. LBW was defined as baby weight less than 2.5 kilograms (Kg) at birth [12]. Diabetes was defined as RBS ≥ 200 mg/dL [13]. Any pathological changes in white blood cells, platelets and red blood cells reported by the Sysmex XP-100 analyser were recorded. Hypertension was diagnosed if the mean of two consecutive measurements was more than 140/90 mm of Hg [14]. Consanguineous marriage was defined as an union between two individuals such as second cousins or closer who share blood relatives [15].

(b) Village level data collection: The village profile included information on village based healthcare facilities, infrastructure such as water supplies and transport resources, and data were obtained from HMF's village health workers (VHWs), government nurses and local government offices.

(c) Post-delivery data collection: All participants were followed up through HMF's village health workers network, and were also contacted twice during their pregnancy over the telephone to inquire about their wellbeing. Post-delivery data were collected by a telephone call to the participants within one week of delivery. Data were collected on the number of hospital visits during pregnancy, any disease/condition diagnosed following the baseline recruitment, blood transfusion, delivery location (hospital/home), delivery type (normal/assisted/caesarean), birth weight and information on any maternal/neonatal

complications. Birth weight data were obtained from post-natal health records for in-hospital deliveries, and for home deliveries, weight was recorded within the first two hours of birth at the nearest health centre. Post-delivery data collection was completed on 30th June 2015.

Data analysis

Analysis was performed using Stata statistical software (v13.1, Texas, USA).

Pre-delivery data: Logistic regression was performed to investigate the association between the risk factors and maternal anaemia (binary variable). Linear regression was used to investigate the association between potential risk factors and maternal Hb levels (continuous variable).

Village data: Village level risk factors were also investigated for the risk of anaemia using both linear and logistic regression.

Post –delivery data: Logistic regression was performed to investigate the association between the risk factors and low birth weight (binary variable). Linear regression was used to investigate the association between maternal covariates and birth weight (continuous variable).

Multivariable analysis: For the models, covariates were selected based on their theoretical relationship with maternal anaemia based on both our understanding of the study setting and the published literature. This resulted in three groups of covariates for the three main analyses conducted in this study (Figure 1). Initially unadjusted analysis to study the association of individual covariates with the outcome of interest was carried out for all three analyses outlined above. The final model was built using a parsimonious stepwise approach that combined all univariate associations with a *P* value of less than 0.05, discarding any where the *P* value increased to >0.05 in the multivariable model. A fully adjusted analysis used all factors in each of the three groups of covariates (Figure 1) to determine the robustness of the observations detected. Due to multicollinearity between height, weight, and calculated body mass index, MUAC was the only anthropocentric measurement included in the model.

Results

(a) Pre-delivery data findings: During the recruitment period (8 months), we identified 303 pregnant women from 34 villages, of whom 287 (95%) aged 15 to 36 years provided complete data (Figure 2). The overall anaemia prevalence was 77% (N=221, Hb < 11.0 g/dL); 3% had severe anaemia (Hb < 7.0 g/dL), 38% had moderate anaemia (Hb: 7.0 to 9.9 g/dL), and 36% had mild anaemia (Hb: 10 to 10.9 g/dL). About 26% (N=75), were teenage pregnancies (maternal age ≤19 years), and anaemia prevalence did not vary by maternal age (Table 1). The majority of women (67%) had a normal BMI (18.5 to 24.9 kg/m^2), while 28% were underweight (BMI < 18.5 kg/m^2). Of 287, 85 participants (29.6%) were in consanguineous marriage relationship.

The mean Hb was 10.5 g/dL (Standard Deviation (SD): 1.29, Range: 5.5 to 14), and Hb values followed a normal distribution (Supplementary Figure 1a). The average mean corpuscular volume (MCV) was 80.5 fl/cell, (SD: 7.22, Range 53.4 to 93.8) (Supplementary Figure 1b) [16]. Other Red Blood Cell indices are detailed in the Supplementary Table 1. No abnormalities relating to white blood cells and platelets were detected. None of the participants reported any history of a systemic disease/treatment (such as malaria, blood transfusion, diabetes, hypertension) in the previous 12 months at the time of recruitment. A consanguineous marriage was the only risk factor associated with anaemia in both unadjusted and adjusted analysis (adjusted Odds Ratio (OR): 2.41, 95% Confidence Interval (CI): 1.16 to 5.01, P = 0.01) (Tables 1 and 4).

(b) Village level data findings: All villages (N=34) were allocated government nurses. The majority (N=29) were visited by nurses once a month for ANC check-ups (providing vaccinations and IFA supplementation), while in the remaining 5 villages, pregnant women

had daily access to healthcare staff stationed in the same village. Normal obstetric delivery facilities were available in one village only. Of 34 villages, piped water supplies were available in 24 villages, and 11 villages had operational community toilets. Our study area had limited transport services to the nearest secondary care hospitals, and was mostly serviced by private vehicle operators. Daily access to a government nurse was the only factor associated with a lower risk of anaemia in the unadjusted analysis (OR: 0.48, 95% CI: 0.25 to 0.93, *P* =0.03) (Table 2), although this association did not persist in the multivariable analysis adjusting for all possible confounding factors (Table 4).

(c) Post-delivery data findings: There were 86% (N=248) full-term singleton live births (Figure 3). Of 35 complications identified in the post-delivery data (Figure 3), 11 participants were in consanguineous relationships, among whom 7 preterm deliveries, 2 stillbirths and 2 neonatal deaths were recorded. No maternal deaths were recorded. None of the participants were diagnosed with systemic conditions such as hypertension or diabetes during pregnancy.

Of 248 full-term singleton live births, 211 (85%) were uncomplicated vaginal deliveries and 37 (15%) were caesarean sections. In total, 236 participants (95%) delivered in a healthcare facility, and the remaining 12 (5%) were home deliveries (Table 3). There were 18 (7%) LBW babies, and the mean birth weight was 2.89 Kg (SD: 0.42 Kg). About 49.6% (123/248) were female births. No association was seen in the odds of LBW or mean birth weight by baby's gender. There was a higher likelihood of LBW among women who had a consanguineous marriage (adjusted OR: 4.10, 95% CI: 1.25 to 13.41, *P* =0.02) compared to those who did not. The presence of maternal anaemia in the third to fifth months of pregnancy was associated with lower risk of LBW (unadjusted OR: 0.34, 95% CI: 0.13 to

0.92, *P* = 0.03). The association of anaemia was consistent after adjusting for consanguineous marriage (OR: 0.25, 95% CI: 0.09 to 0.73, *P* =0.01). On conducting a linear regression of two continuous variables of interest, birth weight (outcome) and maternal haemoglobin (exposure), a borderline association was observed (β coefficient: -0.36 Kg, 95% CI: -0.74 to 0.02, *P* =0.06).

Discussion

A high prevalence of anaemia (77%) was observed in pregnant women living in rural Maharashtra during the third to fifth months of pregnancy. Consanguineous marriage was a potentially avoidable risk factor for both maternal anaemia and LBW. Maternal anaemia was associated with a lower risk of LBW. In addition, limited access to an antenatal nurse was associated with a higher likelihood of anaemia.

Strengths and limitations

This is the first prospective study involving Indian rural population, which investigated both individual and community level risk factors for maternal anaemia and birth outcomes. While operating in challenging remote areas, our ability to conduct recruitment, venous blood investigations, quality controls, and participant follow-up are major strengths of the study. However, we were unable to perform more detailed measurements on obstetric observations such as haematinic blood tests or *in utero* growth using ultrasound imaging due to limited resources. Another limitation is that the women were recruited over a range of pregnancy time points (i.e. between the third and fifth month). As anaemia is a normal physiological response to pregnancy over this period, defining anaemia is challenging, while comparing women at slightly different points during pregnancy may have introduced some non-differential bias to our analysis. Risk factors associated with maternal anaemia in rural population may differ from those in urban communities because of differences in health service access, socioeconomic status and environmental conditions, which may impact on the generalizability of these observations. Finally we did not have the ability to measure detailed haematinic micronutrients such as vitamin B_{12}, folate and iron to investigate the root causes of anaemia.

Synthesis

In the Indian national family health surveys (NFHS-2 and NFHS-3), pregnant women from all stages/trimesters were included in the anaemia assessment [17] . Results from the NFHS 2 (1998-99, N= 5619 pregnancies from 28 Indian states) reported an overall prevalence of anaemia of 47%, with mainly mild and moderate anaemic cases [18]. Findings from the NFHS 3 (2005-06) reported an increase in anaemia prevalence (59%, N= 6028 pregnant women)[19]. Our study reported a higher prevalence compared to both NFHSs. These surveys showed that the prevalence varied across and within Indian states suggesting the influence of local lifestyles and cultural norms, individual medical conditions, infrastructure, and healthcare services. For example, a study in a remote north-eastern region (Assam State) reported a prevalence of 90% with major difference in birth weight among anaemic and non-anaemic women [20]. Our study from Central India reported a high anaemia prevalence in pregnancy, where lower risk of LBW was observed in anaemic mothers compared to non-anaemic. The findings should be interpreted cautiously, as our study had a smaller sample size, and further research is essential to confirm such association. Published studies from Maharashtra state showed a 30% to 65% anaemia prevalence in pregnancy with higher prevalence in rural regions [2,5,21,22]. Studies from South India found 55% to 60% anaemia among pregnant women in a region where consanguineous marriage is a common practice [15].

A recent Indian Human Development Survey reported a national prevalence of 16% of consanguineous marriages, with a higher occurrence in Maharashtra state (28%) [15]. In such relationships, the maternal cousin (maternal uncle’s son) is the most preferred partner in our study area, and these marriages are favoured due to local cultural and economic reasons.

Currently there is no other published evidence on the association between consanguinity and maternal anaemia and this needs further independent investigation.

Findings from one of the oldest Indian studies showed that on an average babies born to consanguineous participants weighed 0.25 Kg less than babies from non-consanguineous relationships [23]. However, this observation was limited to poor socioeconomic groups when compared with participants from higher socioeconomic class. Findings from the prospective cohort of pregnant women (N=601) in rural regions of Karnataka state showed an increase in LBW incidence among consanguineous couples (P =0.04) [24]. The study also showed higher risks of stillbirth associated with consanguinity. A recent 2013 cross-sectional analysis from Karnataka state reported higher risk of LBW with consanguineous marriages [25]. Findings from these three Indian studies are in agreement with our results. Similarly, studies from Lebanon, Qatar, Pakistan and Jordan have reported higher risks of LBW in consanguineous relationship [26–29]. In consanguineous couples, a study from Lebanon reported a 1.8% decrease in birth weight [26], full term babies born in Qatar were nearly twice as likely to be LBW [27], the study from Pakistan showed 14% higher risk of small babies [28].

Analysis of the village level data showed lower likelihood of maternal anaemia associated with regular access to government nurses who conduct routine ANC checks-ups, provide vaccinations and free IFA supplies. In villages where nurses were permanently stationed, improved access to such services, compared to remote villages receiving monthly visits could explain this association. Alternatively, there are many environmental factors that may contribute to residual confounding.

Conclusion

The prevalence of anaemia during the third to fifth months of pregnancy in a rural population living in Maharashtra State was 77%. Consanguineous marriage was identified as a risk factor of maternal anaemia and low birth weight. Anaemia prevalence remained higher in the recent NFHS survey (2005-06) compared to the earlier survey data (1998-99) [2]. There is an urgent need for further research into clinical causes and optimal definitions of anaemia in Indian population, which can inform the design and implementation of suitable interventions. Lastly, community education on anaemia and impacts of consanguineous marriages may help in improving reproductive health outcomes.

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Table 1: Pre-delivery data: individual risk factors associated with maternal anaemia during pregnancy (linear and logistic regression, N= 287 pregnant women)

Characteristics	All study participants N (%)	Anaemic participants N (% with anaemia for each level of risk factor)	Unadjusted linear regression β (95% CI) (Hb in g/dL)	Unadjusted logistic regression OR (95% CI)
Maternal age				
Up to 19 years	75 (26.1)	61 (81.3)	0.01 (-0.04 to 0.06)	0.94 (0.86 to 1.03)
20 and above	212 (73.8)	160 (75.4)	-	-
Religion and category				
Hindu open category	137 (47.7)	106 (77.3)	Reference	Reference
Hindu reserved category	127 (44.2)	99 (77.9)	-0.06 (-0.38 to 0.25)	1.03 (0.57 to 1.84)
Muslim	23 (8.0)	16 (69.5)	0.15 (-0.42 to 0.73)	0.66 (0.25 to 1.77)
Annual income (in GBP)				
≤ 500	137 (47.7)	104 (75.9)	Reference	Reference
Between 501 to 1000	129 (44.9)	100 (77.5)	0.03 (-0.28 to 0.35)	1.09 (0.61 to 1.93)
≥ 1001	21 (7.3)	17 (80.9)	0.10 (-0.49 to 0.70)	1.34 (0.42 to 4.29)
Mid-upper arm circumference of dominant hand (MUAC)				
< 22 cm	25 (8.7)	19 (76.0)	Reference	Reference

≥ 22 cm	262 (91.2)	202 (77.0)	0.26 (-0.27 to 0.80)	1.06 (0.40 to 2.78)
Currently consuming Iron Folic Acid supplements				
No	74 (25.7)	55 (74.3)	Reference	Reference
Yes	213 (74.2)	166 (77.9)	-0.17 (-0.52 to 0.17)	1.22 (0.66 to 2.25)
Gravidity				
1	128 (44.6)	102 (79.6)	-0.04 (-0.21 to 0.12)	0.98 (0.73 to 1.34)
2	103 (35.8)	73 (70.8)	-	-
3 or more	56 (19.5)	46 (82.1)	-	-
Diet recall (recent 7 days history)				
Pure milk (not in the form of tea/coffee)				
≤ 2 times a week	159 (55.4)	122 (76.7)	Reference	Reference
≥ 3 times a week	128 (44.6)	99 (77.3)	0.10 (-0.19 to 0.41)	1.03 (0.59 to 1.80)
Green leafy vegetables				
≤ 2 times a week	165 (57.4)	130 (78.7)	Reference	Reference
≥ 3 times a week	122 (42.5)	91 (74.5)	-0.01 (-0.31 to 0.29)	0.79 (0.45 to 1.37)
Bean sprouts				

≤ 2 times a week	278 (96.8)	214 (76.9)	Reference	Reference
≥ 3 times a week	9 (3.1)	7 (77.7)	0.28 (-0.58 to 1.15)	1.04 (0.21 to 5.16)
Pulses-lentils				
≤ 2 times a week	74 (25.7)	59 (79.7)	Reference	Reference
≥ 3 times a week	213 (74.2)	162 (76.0)	0.06 (-0.28 to 0.40)	0.80 (0.42 to 1.54)
Fruits/Fruit juices				
≤ 2 times a week	154 (53.6)	118 (76.6)	Reference	Reference
≥ 3 times a week	133 (46.3)	103 (77.4)	-0.17 (-0.47 to 0.13)	1.04 (0.60 to 1.81)
Rice				
≤ 2 times a week	20 (6.9)	16 (80)	Reference	Reference
≥ 3 times a week	267 (93.0)	205 (76.7)	0.30 (-0.29 to 0.89)	0.82 (0.26 to 2.56)
Eggs				
≤ 2 times a week	271 (94.4)	209 (77.1)	Reference	Reference
≥ 3 times a week	16 (5.5)	12 (75)	0.16 (-0.50 to 0.82)	0.88 (0.27 to 2.85)
Chicken				
≤ 2 times a week	279 (97.2)	214 (76.7)	Reference	Reference
≥ 3 times a week	8 (2.7)	7 (87.5)	-0.20 (-1.12 to 0.72)	2.12 (0.25 to 17.60)
Goat/fish meat				

≤ 2 times a week	284 (98.9)	219 (77.1)	Reference	Reference
≥ 3 times a week	3 (1.0)	2 (66.6)	0.92 (-0.56 to 2.42)	0.59 (0.05 to 6.65)
Education completed				
Primary school	170 (59.2)	132 (77.6)	Reference	Reference
Secondary school	69 (24.0)	54 (78.2)	0.02 (-0.34 to 0.39)	1.03 (0.52 to 2.03)
≥ Higher secondary school	48 (16.7)	35 (72.9)	0.02 (-0.39 to 0.44)	0.77 (0.37 to 1.61)
Consanguineous marriage				
No	202 (70.3)	148 (73.2)	Reference	Reference
Yes	85 (29.6)	73 (85.8)	-0.00 (-0.34 to 0.32)	2.21 (1.11 to 4.40)*
Current pregnancy registration at health facility				
No	18 (6.2)	14 (77.7)	Reference	Reference
Yes	269 (93.7)	207 (76.9)	0.14 (-0.48 to 0.77)	0.95 (0.30 to 3.00)

Table 1 footnotes: * indicates *P* <0.05. For linear regression, haemoglobin values were used as a continuous measure (main outcome), and in the logistic regression anaemic or non-anaemic status was the main outcome. OR=odds ratio, β=linear regression correlation coefficient, CI= confidence interval Annual income is expressed in Great Britain Pound (GBP). 1 GBP was calculated as being equivalent to 100 INR. Maternal age and gravidity used as a continuous variable following linearity assessment.

Table 2: Village level data: Community risk factors associated with anaemia during pregnancy (linear and logistic regression, N= 287 pregnant women from 34 villages)

Village Characteristics	Village frequency (%)	All study participants N (%)	Anaemic participants N (% with anaemia for each level of risk factor)	Unadjusted linear regression β (95% CI) (Hb in g/dL)	Unadjusted logistic regression OR (95% CI)
Piped water supplied to houses					
No	10 (29.4)	68 (23.6)	50 (73.5)	Reference	Reference
Yes	24 (70.5)	219 (76.3)	171 (78.0)	-0.22 (-0.58 to 0.13)	1.28 (0.68 to 2.40)
Daily water supply (at least once a day)					
No	7 (20.5)	56 (19.5)	46 (82.1)	Reference	Reference
Yes	27 (79.4)	231 (80.4)	175 (75.7)	0.24 (-0.14 to 0.62)	0.67 (0.32 to 1.43)
Operational community toilets					
No	23 (67.6)	122 (42.5)	99 (81.1)	Reference	Reference
Yes	11 (32.3)	165 (57.4)	122 (73.9)	0.10 (-0.20 to 0.40)	0.65 (0.37 to 1.16)

Government health center availability					
No	24 (70.5)	177 (61.6)	141 (79.6)	Reference	Reference
Yes	10 (29.4)	110 (38.3)	80 (72.7)	0.22 (-0.08 to 0.54)	0.68 (0.39 to 1.18)
Accredited social health attendant (ASHA) appointed					
No	1 (2.9)	6 (2.0)	4 (66.6)	Reference	Reference
Yes	33 (97.0)	281 (97.9)	217 (77.2)	-0.95 (-2.01 to 0.10)	1.69 (0.30 to 9.46)
Daily access to government nurse					
No	29 (85.2)	235 (81.8)	187 (79.5)	Reference	Reference
Yes	5 (14.7)	52 (18.1)	34 (65.3)	0.24 (-0.14 to 0.63)	0.48 (0.25 to 0.93)*
Government nurse provides antenatal care services					
No	1 (2.9)	6 (2.0)	4 (66.6)	Reference	Reference
Yes	33 (97.0)	281 (97.9)	217 (77.22)	0.45 (-0.60 to 1.51)	1.69 (0.30 to 9.46)
Government nurse conducts					

haemoglobin investigation					
No	8 (23.5)	68 (23.6)	54 (79.4)	Reference	Reference
Yes	26 (76.4)	219 (76.3)	167 (76.2)	-0.13 (-0.49 to 0.22)	0.83 (0.42 to 1.61)
Private doctor availability					
No	31 (91.1)	236 (82.2)	184 (77.9)	Reference	Reference
Yes	3 (8.8)	51 (17.7)	37 (72.5)	0.34 (-0.04 to 0.74)	0.74 (0.37 to 1.48)
Travel time to nearest hospital	Continuous variable	-	-	-0.00 (-0.00 to 0.01)	1.00 (0.98 to 1.02)

Table 2 footnotes: * indicates P <0.05. For linear regression, haemoglobin values were used as a continuous measure (main outcome), and in the logistic regression anaemic or non-anaemic status was the main outcome. OR= Odds ratio, β=linear regression correlation coefficient, CI= confidence interval.

Table 3: Post-delivery data: individual risk factors associated with low birth weight (linear and logistic regression, N= 248 pregnant women)

Characteristics	All study participants N (%)	Low birth weight babies N (% with LBW for each level of risk factor)	Unadjusted linear regression β (95% CI) [birth weight in Kg]	Unadjusted logistic regression OR (95% CI)
Maternal age	248 (100)	18 (7.2)	0.00 (-0.01 to 0.01)	0.98 (0.83 to 1.16)
Religion and category				
Hindu open category	128 (51.6)	8 (6.2)	Reference	Reference
Hindu reserved category	105 (42.3)	9 (8.5)	0.01 (-0.09 to 0.12)	1.40 (0.52 to 3.78)
Muslim	15 (6.0)	1 (6.6)	0.10 (-0.12 to 0.33)	1.07 (0.12 to 9.20)
Annual income				
≤ 500 GBP	113 (45.5)	8 (7.0)	Reference	Reference
Between 501 and 1000 GBP	117 (47.1)	10 (8.5)	-0.05 (-0.16 to 0.05)	1.22 (0.45 to 3.22)
≥ 1001 GBP	18 (7.2)	0	0.06 (-0.15 to 0.27)	Omitted
Mid-upper arm circumference of dominant hand (MUAC)				
< 22	22 (8.8)	2 (9.0)	Reference	Reference

≥ 22 cm	226 (91.1)	16 (7.0)	0.11 (-0.06 to 0.30)	0.76 (0.16 to 3.55)
Currently consuming Iron Folic Acid supplements				
No	58 (23.3)	7 (12.0)	Reference	Reference
Yes	190 (76.6)	11 (5.7)	0.05 (-0.06 to 0.18)	0.44 (0.16 to 1.21)
Gravidity	248 (100)	18 (7.2)	0.02 (-0.03 to 0.07)	0.79 (0.44 to 1.41)
Diet recall (recent 7-days history)				
Pure milk (not in the form of tea/coffee)				
≤ 2 times a week	134 (54.0)	14 (10.4)	Reference	Reference
≥ 3 times a week	114 (45.9)	4 (3.5)	0.03 (-0.07 to 0.14)	0.31 (0.09 to 0.97)*
Green leafy vegetables				
≤ 2 times a week	140 (56.4)	14 (10)	Reference	Reference
≥ 3 times a week	108 (43.5)	4 (3.7)	0.04 (-0.06 to 0.14)	0.34 (0.11 to 1.08)
Bean sprouts				
≤ 2 times a week	241 (97.1)	18 (7.4)	Reference	Reference
≥ 3 times a week	7 (2.8)	0	0.04 (-0.28 to 0.36)	Omitted
Pulses-lentils				
≤ 2 times a week	69 (27.8)	6 (8.6)	Reference	Reference

≥ 3 times a week	179 (72.1)	12 (6.7)	0.07 (-0.04 to 0.19)	0.75 (0.27 to 2.09)
Fruits/Fruit juices				
≤ 2 times a week	134 (54.0)	13 (9.7)	Reference	Reference
≥ 3 times a week	114 (45.9)	5 (4.3)	0.08 (-0.01 to 0.19)	0.42 (0.14 to 1.23)
Rice				
≤ 2 times a week	18 (7.2)	3 (16.6)	Reference	Reference
≥ 3 times a week	230 (92.7)	15 (6.5)	0.03 (-0.16 to 0.24)	0.34 (0.09 to 1.33)
Eggs				
≤ 2 times a week	234 (94.3)	17 (7.2)	Reference	Reference
≥ 3 times a week	14 (5.6)	1 (7.1)	0.07 (-0.15 to 0.30)	0.98 (0.12 to 7.69)
Chicken				
≤ 2 times a week	242 (97.5)	18 (7.4)	Reference	Reference
≥ 3 times a week	6 (2.4)	0	0.31 (-0.02 to 0.66)	Omitted
Goat/fish meat				
≤ 2 times a week	247 (99.5)	18 (7.2)	Reference	Reference
≥ 3 times a week	1 (0.5)	0	-0.39 (-1.23 to 0.44)	Omitted
Education completed				
Primary school	138 (55.6)	13 (9.4)	Reference	Reference

Secondary school	66 (26.6)	5 (7.5)	0.00 (-0.12 to 0.12)	0.78 (0.26 to 2.31)
≥ Higher secondary school	44 (17.7)	0	0.07 (-0.07 to 0.21)	Omitted
Consanguineous marriage				
No	175 (70.5)	8 (4.5)	Reference	Reference
Yes	73 (29.4)	10 (13.6)	-0.11 (-0.23 to -0.00)*	3.13 (1.25 to 8.77)*
Current pregnancy registration at health facility				
No	15 (6.0)	0	Reference	Reference
Yes	233 (93.9)	18 (7.7)	0.03 (-0.19 to 0.25)	Omitted
Maternal anaemia status (Hb <11.0 g/dL)				
Not anaemic	58 (23.3)	8 (13.7)	Reference	Reference
Anaemic	190 (76.6)	10 (5.2)	0.21 (0.08 to 0.33)*	0.34 (0.13 to 0.92)*

Table 3 footnotes: * indicates *P* <0.05. Birth weight is used as a continuous measure in linear regression, and in the logistic regression it is classified as a binary categorical variable (LBW and non-LBW). Birth weight unit used: kilograms (Kg). OR= Odds ratio, β=linear regression correlation coefficient, CI= confidence interval. Income 1 Great Britain Pound (GBP) was considered equivalent to 100 Indian Rupees. Maternal age and gravidity used as a continuous variable following linearity assessment.

Table 4: Adjusted logistic regression analysis results for factors associated with maternal anaemia and low birth weight

Analysis	Risk factor	Unadjusted OR (95% CI)	Fully adjusted OR (95% CI)
a. Individual risk factors associated with anaemia during pregnancy	Consanguineous marriage	2.21 (1.11 to 4.40)*	2.41 (1.16 to 5.01)*[a]
b. Community risk factor associated with anaemia during pregnancy	Daily access to government nurse ^	0.48 (0.25 to 0.93)*	0.48 (0.19 to 1.19)* [b]
c. Individual risk factors associated with low birth weight	Consanguineous marriage	3.13 (1.25 to 8.77)*	4.10 (1.25 to 13.41)*[a]

Table 4 footnotes: * indicates *P* values <0.05. OR= Odds ratio, CI= confidence intervals. Details of adjustments made are shown below.

[a] Indicates adjusted results for maternal age, MUAC, IFA, gravidity, 7-day dietary recall, maternal education, religion, pregnancy registration with health facility and income.

[b] Indicates adjusted results for sanitation facilities (village water supplies, toilets), health facilities (government health centre, ASHA, private doctor, ANC care, Hb testing services, private doctor availability, travel time to hospital).

^ Access to government nurse was the associated factors in the unadjusted analysis (Table 2 community risk factor group), however on full adjustments no associations were observed.

Figure 1: Modelling strategy for linear and logistic regression analyses

Pre delivery data- Individual risk factors for maternal anaemia assessment

Individual factors included in the regression analyses for anaemia risk assessment (10 covariates, outcome- maternal anaemia): Maternal age, mid upper arm circumference (MUAC), currently consuming IFA supplementation, gravidity, 7-days diet recall, education completed, consanguineous marriage, current pregnancy registration at health facility, religion/category and annual income.

Village level data- Community risk factors for maternal anaemia assessment

Community risk factors included in the regression analyses (10 covariates, outcome- maternal anaemia): Water supplies, community toilets, government health centre, access to ASHA worker, availability of government nurse, services provided by the government nurse (Hb investigation, ANC), access to private doctor and travel time to nearby hospital.

Post-delivery data- Individual risk factors for low birth weight assessment

Individual factors included in the regression analyses for the birth weight assessment (10 covariates, outcome- low birth weight): Maternal age, mid upper arm circumference, currently consuming IFA supplementation, gravidity, 7-days diet recall, education completed, consanguineous marriage, current pregnancy registration at health facility, religion/category and annual income.

Figure 2: Pre-delivery analysis (final sample size N=287)

Total eligible population approached
N=303 pregnant women from 34 villages

Declined to participate (N=7).

Reason for declining- 6 were registered with private hospitals where blood investigations were performed, and 1 did not provide any reason

Agreed and participated in data collection
N=296

Declined for blood withdrawal (N=9).

Reason for decline – recently had same blood investigation in preceding 7 days

Data and blood investigation reports obtained from 287 participants (N= 287, response rate 95%)

Figure 3: Post-delivery analysis (final sample size N=248)

Data and haemoglobin measurement obtained from 287 participants (N= 287)

Pre-delivery complications (N=4)

Miscarriages = 3, Induced abortion due to fetal anomaly= 1

Number of women delivered= 283

Post-delivery complications (N=35)

Pre-term deliveries= 21, Stillbirth=7 and Neonatal deaths=6.

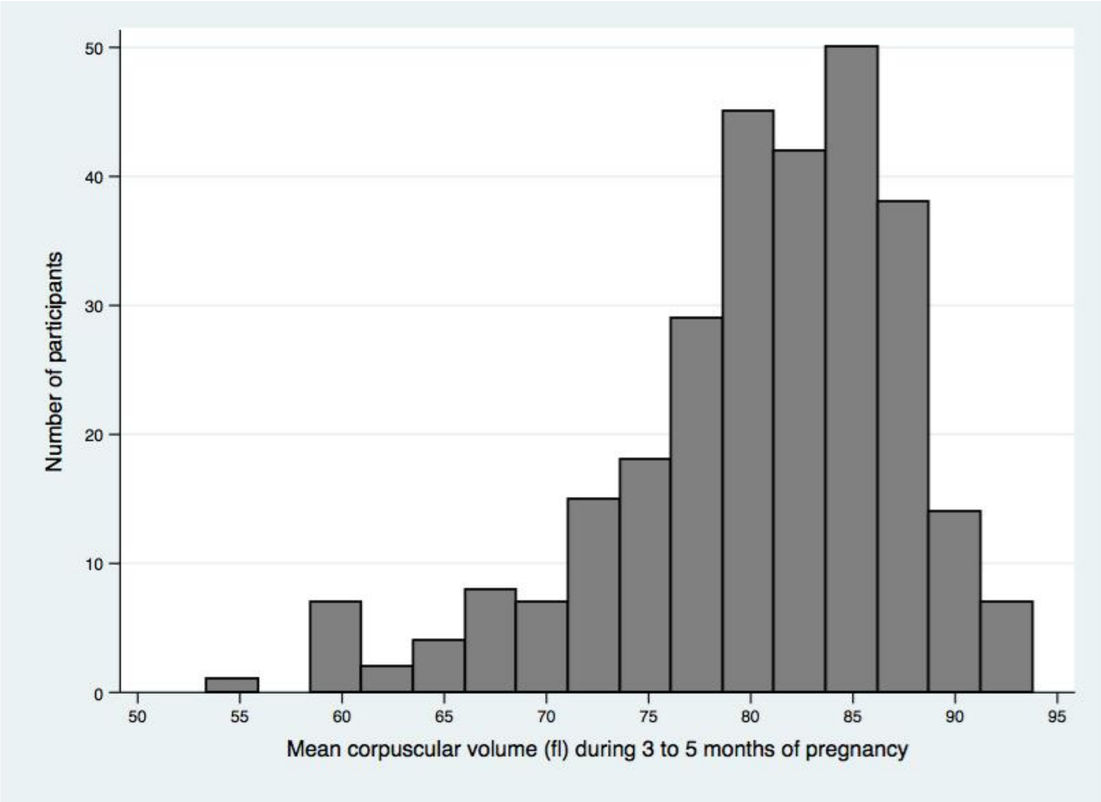
Loss to follow-up=1

Post-delivery data available for birth weight analysis from 248 women (N=248)

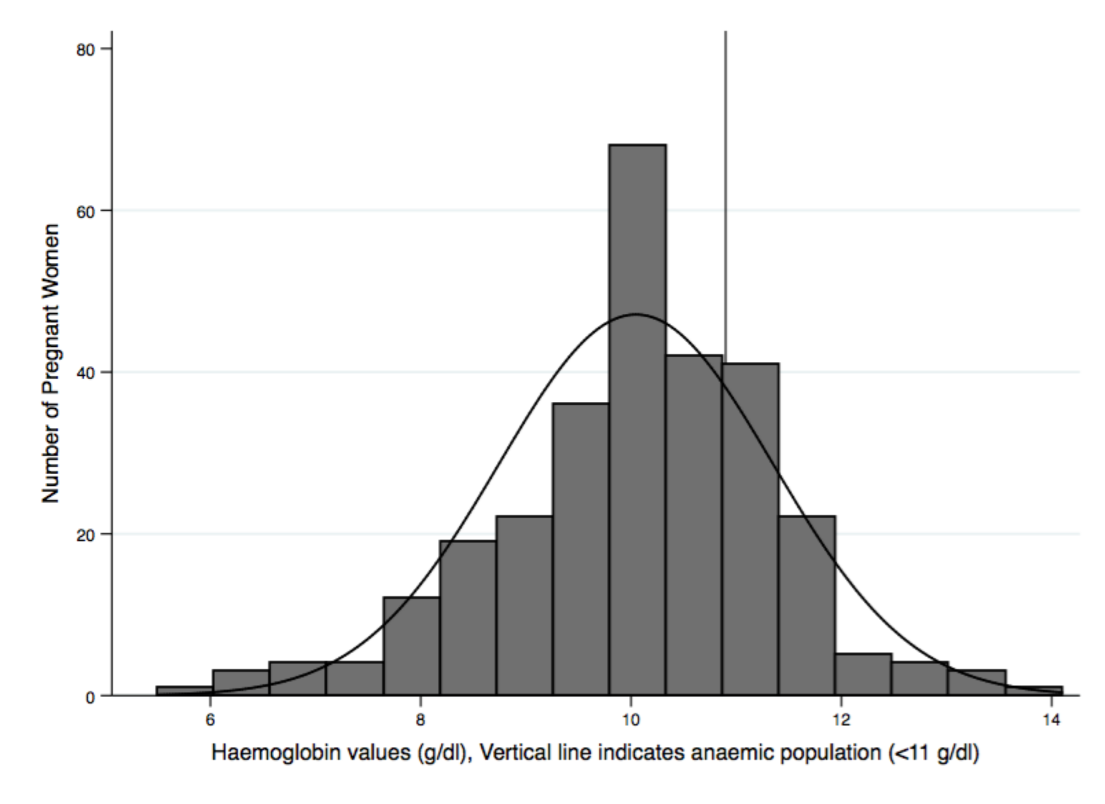
Supplementary Table 1: Red Blood Cell (RBC) indices (N= 287, pre-delivery data)

Parameter	Mean	Standard Deviation	Range
Red Blood Cell Count	3.5×10^6/mm	0.36	2.7 to 4.9
Haemoglobin (Hb)	10.04 g/dL	1.29	5.5 to 14.0
Haematocrit	28.7%	2.85	20.2 to 38.5
Mean Corpuscular Haemoglobin (MCH)	28.2 pg/cell	3.63	14 to 35.1
Mean Corpuscular Volume (MCV)	80.5 fl/cell	7.22	53.4 to 93.8
Mean Corpuscular Haemoglobin Concentration (MCHC)	34.9 g/dL	1.82	26.2 to 38.8
RBC Distribution Width	42.5 fl	4.26	12.8 to 60.7
Reticulocytes %	14.3%	2.41	9.4 to 33.2

Supplementary table 1 footnotes: Measurement units used: RBC count was measured in million cells per microliter. Hb and MCHC were measured in grams per decilitre. Haemotocrit and Reticulocytes were measured in percentages (%). MCH was measured in pictograms per cell (pg/cell), while the MCV and RBC width were measured in femtoliteres (fl) per cell. RBS was measured in milligram per decilitre.



Supplementary Figure 1b Histogram of MCV among pregnant women



Supplementary Figure 1a Histogram of Hb distribution in pregnant women

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

Paper title: Risk factor for maternal anaemia and low birth weight in pregnant women living in rural India: A prospective cohort study

	Item No	Recommendation (*indicated with page numbers of the manuscript*)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (Page 1 and 2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (Page 1 and 2)
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported (page 3 and 4)
Objectives	3	State specific objectives, including any prespecified hypotheses (page 3 and 4)
Methods		
Study design	4	Present key elements of study design early in the paper (page 5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (page 5 and 6)
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (page 5 and 6)
		(*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
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 (page 6 and 7)
Bias	9	Describe any efforts to address potential sources of bias (page 8)
Study size	10	Explain how the study size was arrived at (page 5, 10)

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (page 8 and 9)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (page 8 and 9)
		(*b*) Describe any methods used to examine subgroups and interactions (page 8 and 9)
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how loss to follow-up was addressed
		(*e*) Describe any sensitivity analyses (page 8 and 9)
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 (page 10 and 11, and figures 2, 3)
		(b) Give reasons for non-participation at each stage (page 10 and 11, and figures 2, 3)
		(c) Consider use of a flow diagram (figure 2, 3)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (page 10)
		(b) Indicate number of participants with missing data for each variable of interest (page 11, 12, figure 3)
		(c) Summarise follow-up time (eg, average and total amount) (page 11, 12, figure 3)
Outcome data	15*	Report numbers of outcome events or summary measures over time (page 11, 12, figure 3)
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 (page 10, 11, 12, and Tables 1-3)
		(*b*) Report category boundaries when continuous variables were categorized (page 10, 11, 12, and Tables 1-3)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (page 10, 11, 12, and Table 4)

Discussion		
Key results	18	Summarise key results with reference to study objectives (Page 13)
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 (Page 13)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (Page 14, 15)
Generalisability	21	Discuss the generalisability (external validity) of the study results (Page 14, 15, 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 (additional information will appear at the end of the manuscript, submitted as an additional file according to the journal requirement)

*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.