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Article title: The association between maternal anaemia and pregnancy outcomes: a cohort study in Assam, India

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On behalf of the IndOSS-Assam steering committee

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List of abbreviations

AMCH	Assam Medical College and Hospital
aOR	adjusted odds ratio
BMI	Body mass index
BPL	Belongs to below poverty line
CI	Confidence intervals
e-MDR	Electronic maternal death reporting system
FAAMCH	Fakhruddin Ali Ahmed Medical College and Hospital
GMCH	Guahati Medical College and Hospital
IMR	Infant Mortality Rate
IndOSS-Assam	Indian Obstetric Surveillance System - Assam
LMICs	Low and middle income countries
LR-test	Likelihood ratio tests
MMR	Maternal Mortality Ratio
OR	Odds ratio
PPH	Postpartum haemorrhage
RR	Risk ratio
UK	United Kingdom
UKOSS	UK Obstetric Surveillance System
UTI	Urinary tract infection
WHO	World Health Organisation

ABSTRACT

Objectives: To examine the association between maternal anaemia and adverse maternal and infant outcomes, and to assess the feasibility of conducting epidemiological studies through the Indian Obstetric Surveillance System–Assam (IndOSS-Assam)

Design: Retrospective cohort study using anonymised hospital records. Exposure: maternal iron deficiency anaemia; outcomes: postpartum haemorrhage (PPH), low birthweight, small-for-gestational age, perinatal death.

Setting: Five government medical colleges in Assam.

Study population: 1007 pregnant women who delivered in the five medical colleges from January to June 2015.

Main outcome measures: Odds ratios with 95% confidence intervals (CI) to estimate the association between maternal iron deficiency anaemia and the adverse maternal and infant outcomes. Potential interactive roles of infections and induction of labour on the adverse outcomes were explored.

Results: 35% (n=351) pregnant women had moderate-severe anaemia. Women with severe anaemia had a higher odds of PPH (adjusted odds ratio (aOR)=9·45; 95%CI=2·62-34·05), giving birth to low birthweight (aOR=6·19; 95%CI=1·44-26·71) and small-for-gestational age babies (aOR=8·72; 95%CI=1·66-45·67), and perinatal death (aOR=16·42; 95%CI=4·38-61·55). Odds of PPH increased 17-fold among women with moderate-severe anaemia who underwent induction of labour and increased 19-fold among women who had infection and moderate-severe anaemia.

Conclusion: Maternal iron deficiency anaemia is a major public health problem in Assam. Maternal anaemia was associated with increased risks of PPH, low birthweight, small-for-gestational age babies and perinatal death. While the best approach is prevention a large number of women present with severe anaemia late in pregnancy and there is no clear guidance on how these women should be managed during labour and delivery.

SUMMARY BOX

What is already known on this subject?

- Assam, a north-eastern state in India, is reported to have high maternal and infant mortality. It has been hypothesised that iron deficiency anaemia is a major causal factor. However, there is limited research evidence of a causal relationship with maternal and neonatal mortality in Assam.

What are the new findings?

- This study was part of a pilot study conducted to assess the feasibility of establishing an Indian obstetric surveillance and research system in the state of Assam (IndOSS-Assam) and demonstrated the feasibility of using routine medical records for future prospective epidemiological studies linked to the surveillance system.
- This study showed a high prevalence of iron deficiency anaemia among pregnant women in Assam which highlights that this is an important public health problem. Maternal anaemia was associated with increased risks of postpartum haemorrhage, low birthweight, small-for-gestational age babies and perinatal death.
- Notably the odds of postpartum haemorrhage increased 17-fold among women with moderate-severe anaemia who underwent induction of labour.

What are the recommendations for policy and practice?

- Clearly the best approach is to prevent anaemia or treat it during the antenatal period, but a large number of pregnant women in India present with severe anaemia either at the point of delivery or in the third trimester. At present, there is no clear guidance with regard to how women with severe anaemia should be managed during labour and delivery and further studies are required to generate evidence in order to inform policy and practice.

INTRODUCTION

Assam, a north-eastern state in India, is reported to have a high maternal mortality ratio (MMR) as well as a high infant mortality rate (IMR). Report published by the Office of the Registrar General, India, estimated the MMR to be 328/100,000 live births for the years 2010-12[1] and IMR to be 55/1000 live births for the year 2011.[2] It has been hypothesised that iron deficiency anaemia is a major cause of the high maternal and infant mortality in the state. Assam has the highest prevalence of iron deficiency anaemia among pregnant women (73%, Indian National Family Health Survey 2005-06).[3] A study in one tertiary hospital in the state showed that maternal haemoglobin levels <8 g/dl was associated with increased risk of low birthweight and small-for-gestational age babies,[4] but we did not find any study that examined the effects on maternal and perinatal outcomes.

Iron deficiency anaemia during pregnancy is a known risk factor for pre-term birth, low birthweight and small-for-gestational age babies,[5] and increases the risk of postpartum haemorrhage (PPH).[6] PPH is the leading cause of maternal mortality in India.[7] The incidence of PPH is much higher in India compared with the rest of the world and the Registrar General of India attributes this to high prevalence of anaemia among pregnant women.[8] A study investigating the causes of neonatal deaths among the tea-garden labourers in Assam reported a high prevalence of low birthweight (43%) and pre-term deliveries (34%) in the study population, contributing to more than 90% of the neonatal deaths.[9]

While untreated iron deficiency anaemia can itself lead to adverse outcomes, its negative effects may be aggravated by the presence of other risk factors such as infections during pregnancy (malaria,[10] urinary tract infection (UTI),[11] helminthic infections[12]). Additionally, studies have suggested that induction of labour is associated with increased risk of PPH.[13 14] These factors could augment the adverse effects of anaemia on pregnancy outcomes. The objectives of this study were to examine the association between maternal anaemia and PPH, low birthweight, small-for-gestational age, and perinatal death. In addition, we explored the potential interactive role of infections and induction of labour on the adverse effects of iron deficiency anaemia.

METHODS

We conducted a cohort study using anonymised hospital records of pregnant women (>20 weeks of gestation) who underwent delivery in five government medical colleges and hospitals in Assam: Gauhati Medical College and Hospital (GMCH), Guwahati; Assam Medical College and Hospital (AMCH), Dibrugarh; Silchar Medical College and Hospital, Silchar; Fakhruddin Ali Ahmed Medical College and Hospital (FAAMCH), Barpeta; and Jorhat Medical College and Hospital, Jorhat. This study was part of a pilot study conducted to assess the feasibility of establishing an Indian obstetric surveillance and research system in the state (IndOSS-Assam). Details of the feasibility study are described separately.[15] This retrospective study was undertaken to establish the feasibility of using routine medical records for future prospective epidemiological studies linked to the surveillance system.

A data collection form adapted from the Indian Demographic and Health Survey questionnaire and UK Obstetric Surveillance System[16] was used to collect anonymous information from the hospital and antenatal records of pregnant women. Data was collected sequentially from the five study sites, for one month per site, over a period of six months (January to June 2015) to include an average of 200 women per site for an even distribution of the required sample. All women admitted to the postnatal ward of the hospital during the study month were included (until the sample size of 200 was reached) and data was collected from their hospital records by field staff from the Regional Resource Centre, Guwahati, Assam.

The primary exposure for this cohort study was maternal iron deficiency anaemia defined using the WHO criteria for moderate anaemia (pregnant women with a haemoglobin level of 7-9·9 g/dl) and severe anaemia (haemoglobin level <7 g/dl).[17] The other exposures, malaria, UTI, helminthic infections, and induction of labour were recorded based on reported

diagnoses by clinicians from the women's hospital and antenatal records. The primary outcomes of interest were: (i) primary PPH defined as pregnant women of 20 weeks gestation or more identified as having a blood loss of 500 ml or more from the genital tract within 24 hours of birth of a baby[18] and (ii) low birthweight defined as weight at birth of less than 2500 grams. In addition we examined two other secondary infant outcomes: small-for-gestational age and perinatal death which included stillbirths and neonatal deaths within the first week of life.

The hospital notes did not have information on haemoglobin levels recorded at different gestational period for each woman. Therefore, we included most recently recorded antenatal haemoglobin concentration and noted the gestational age at which it was recorded. We tested the continuous variable 'haemoglobin level' for deviations from linearity. Fitting fractional polynomials showed the presence of a non-linear relationship of haemoglobin level with the outcomes PPH, low birthweight and small-for-gestational age. We therefore categorised pregnant women into three groups: mild anaemia/ normal haemoglobin, moderate anaemia and severe anaemia.

Gender specific z-scores and centiles for birthweight-for-gestational age were generated using the INTERGROWTH-21st tool[19] for all singleton pregnancies. Infants below the 10th centile or z-score <-1·28 were grouped as small-for-gestational age.

We also collected information on known confounders and risk factors for maternal and infant morbidity and mortality including socio-demographic factors maternal age, body mass index (BMI), caste (social class), religion, residence, below poverty line status, women's employment status, husband's occupational status and whether he was a tea garden worker; pregnancy related factors such as parity, previous caesarean section, inter-pregnancy interval, previous pregnancy problems, pre-existing medical problems, multiple pregnancies, number of antenatal visits, whether folic acid tablets were received and mode of delivery. BMI was calculated from the earliest available information on weight and height of pregnant women measured during the antenatal period, but we do not know the exact time point at which it was measured.

Study power and sample

India is reported to have a high incidence of PPH,[8] therefore a risk of 16% was used for estimating the sample size. For the outcome low birthweight, the reported overall incidence of 37·3% in the state[20] was used for the purpose of sample size calculation. Assuming an effect size for the risk ratio (RR) to be 1·5, power as 80%, alpha 5%, and ratio of unexposed to exposed 2, for the above proportions of risk in the unexposed we estimated a sample of 230 in the exposed group for the outcome PPH and 70 in the exposed group for low birthweight. These estimates were inflated to account for cluster sampling and possible missing information to give a final estimated sample size of 980.

Statistical analyses

We conducted an initial descriptive analysis to compare the distribution of the confounders and other risk factors across the three exposure groups and univariable analyses to examine the crude odds ratios for the association between exposure and outcomes. We conducted explanatory multivariable logistic regression analyses using separate models to investigate the associations of maternal anaemia with PPH, low birthweight, small-for-gestational age, and perinatal death.

We conducted tests for interaction by fitting interaction terms for maternal anaemia and infections during pregnancy for the outcomes PPH, low birthweight and small-for-gestational age, and maternal anaemia and induction of labour for PPH followed by likelihood ratio tests (LR-test). We found significant interaction between these variables, therefore dummy variables were generated to ascertain the effects of these interactions on the outcomes in separate multivariable logistic regression analyses.

Tests for correlation between the independent variables showed a high correlation between parity and inter-pregnancy interval; this variable was therefore not included in the multivariable model. We also found a significant correlation between husband's employment status and below poverty line household, as well as between husband's employment status and tea-garden worker, thus husband's employment status was excluded to improve model parsimony. The final multivariable models therefore adjusted for the following risk factors: caste, religion, residence, below poverty line status, women's employment status, tea garden worker, parity, BMI, maternal age, previous caesarean section, previous pregnancy problems, placental problems in index pregnancy, pre-existing medical problems, multiple pregnancies, number of antenatal visits, whether folic acid tablets were received, and mode of delivery. Mode of delivery was not included in the infant outcomes model as this is not likely to be causally associated.

A substantial proportion of the data was missing for BMI, parity, previous pregnancy problems and below poverty line status. We did not assume the data to be missing at random and generated proxy variables with missing as a separate group for all variables. However, we also conducted complete case analysis for all outcomes and sensitivity analyses by redistributing the missing observations into the different categories of the variables. The results of complete case analyses and sensitivity analyses did not materially differ from that of the main models other than widen the 95% CI, except for small-for-gestational age for which results could not be obtained as the dependant variable did not vary within the severe anaemia group in complete case analysis (Table-S1).

Based on the sampling frame, clustering of data was at the level of the tertiary hospital which caters to population within a definite geographical boundary. We did not consider the intraclass correlation coefficient to be high because the geographical areas that the five tertiary hospitals serve are large with heterogeneous population. Further, the sampling used a single stage cluster design with five large clusters, thus we employed the Hubert-White robust standard errors method to account for any clustering effect. All associations were considered to be significant at a p-value of <0·05 (two-tailed) and all analyses were performed using Stata version 13·1, SE (StataCorp, College Station, TX).

RESULTS

Information was collected for a total of 1,011 pregnant women. After excluding one woman who delivered at <20 weeks gestation and three women for whom information on haemoglobin level was not available, we had a sample of 1,007 women (Figure-1).

Based on the information available from the hospital records, 33 out of 1,007 women (3.3%) had a PPH. Eleven women were reported to have a blood loss of 500–999 ml, 6 women between 1000–1499 ml, another 6 women had >1500 ml of blood loss in the first 24 hours after delivery and for the remaining 10, estimates of blood loss were not available. In 73% of the PPH cases, the reported cause was uterine atony (n=24), other causes included placenta praevia (n=2), placental abruption (n=1), uterine infection (n=2), tears/injuries (n=2), and retained placenta (n=2).

We found a high prevalence (n=263 out of 969, 27%) of low birthweight among live born singleton pregnancies in the study population and a higher prevalence of small-for-gestational age (n=409 out of 969, 42%). There were 33 perinatal deaths out of 989 singleton pregnancies (3·3%), 20 stillbirths, and 13 neonatal deaths which occurred either on the day of delivery or within 2 days of birth.

Among the 1,007 pregnant women, 28% (n=282) had the most recent recorded haemoglobin in the first trimester (1-12 weeks of gestation), 37% (n=372) in the second trimester (13-27 weeks), 30% (n=302) in the third trimester (28-40 weeks) and for the remaining 5% (n=51) the date of haemoglobin record was not available. Overall 65·1% had mild anaemia/normal haemoglobin levels (356 women with normal haemoglobin ≥11 g/dl, and 300 with mild anaemia 10-10·9 g/dl), 32·8% had moderate anaemia (n=330) and 2·1% (n=21) had severe anaemia during the antenatal period. Just over a quarter of women (n=141) received

treatment for anaemia including iron supplementation, blood transfusion or other treatments (such as herbal remedies).

Table-1: Comparison of maternal and infant characteristics across the exposure groups

Characteristics (N=1007, except where specified)	Maternal anaemia		
	Normal haemoglobin/mild n=656	Moderate n=330	Severe n=21
	Frequency (%)	Frequency (%)	Frequency (%)
Maternal age			
<20 years	75 (11·4)	29 (8·8)	0 (0)
20-25 years	394 (60·1)	204 (61·8)	13 (61·9)
26-30 years	149 (22·7)	78 (23·6)	6 (28·6)
≥30 years	37 (5·6)	19 (5·8)	2 (9·5)
Not known	1 (0·2)	0 (0)	0 (0)
Body mass index (BMI) in Kg/m^2			
Underweight	120 (18·3)	61 (18·5)	2 (9·5)
Normal	338 (51·5)	172 (52·1)	7 (33·3)
Overweight	42 (6·4)	21 (6·4)	3 (14·3)
Obese	11 (1·7)	8 (2·4)	0 (0)
Not known	145 (22·1)	68 (20·6)	9 (42·9)
Pregnancy related factors			
Parity*			
Nulliparous	98 (14·9)	36 (10·9)	0 (0)
Multiparous	274 (41·8)	184 (55·8)	19 (90·5)
Not known	284 (43·3)	110 (33·3)	2 (9·5)
Previous caesarean sections (multiparous women n=477)			
No	180 (65·7)	132 (71·7)	15 (78·9)
Yes	52 (19·0)	31 (16·9)	3 (15·8)
Not known	42 (15·3)	21 (11·4)	1 (5·3)
Previous pregnancy problems*			
No	267 (40·7)	168 (50·9)	11 (52·4)
Yes	44 (6·7)	20 (6·1)	3 (14·3)
Not known	345 (52·6)	142 (43·0)	7 (33·3)
Anaemia during previous pregnancy*			
No	203 (30·9)	108 (32·7)	3 (14·3)
Yes	7 (1·1)	22 (6·7)	10 (47·6)
Not known	446 (68·0)	200 (60·6)	8 (38·1)
Pre-existing medical problems			
No	637 (97·1)	313 (94·8)	19 (90·5)
Yes	18 (2·7)	17 (5·2)	2 (9·5)
Not known	1 (0·2)	0 (0)	0 (0)
Index pregnancy			
Multiple pregnancy			
No	647 (98·6)	321 (97·3)	21 (100)
Yes	9 (1·4)	9 (2·7)	0 (0)
Not known	0	0	0
Number of antenatal check-ups*			
≥ four	335 (51·1)	183 (55·5)	9 (42·9)
At least one but < four	232 (35·4)	96 (29·1)	7 (33·3)
None	46 (7·0)	38 (11·5)	5 (23·8)
Not known	43 (6·5)	13 (3·9)	0 (0)
Received folic acid tablets			
No	160 (24·4)	88 (26·7)	9 (42·9)
Yes	495 (75·4)	242 (73·3)	12 (57·1)
Not known	1 (0·2)	0 (0)	0 (0)
Received any treatment (women diagnosed with anaemia, n=499)*			
No	155 (23·6)	107 (32·4)	3 (14·3)
Yes	44 (6·7)	81 (24·6)	16 (76·2)
Not known	457 (69·7)	142 (43·0)	2 (9·5)
Infection during pregnancy (Malaria/ UTI / Helminthic infection)*			
No	602 (91·8)	282 (85·5)	10 (47·6)
Yes	17 (2·6)	13 (3·9)	3 (14·3)
Not known	37 (5·6)	35 (10·6)	8 (38·1)
Placental problems during index pregnancy			
No	646 (98·5)	323 (97·9)	21 (100)

Yes	9 (1·4)	7 (2·1)	0 (0)
Not known	1 (0·1)	0 (0)	0 (0)
Mode of delivery*			
Spontaneous vaginal	289 (44·1)	202 (61·2)	14 (66·7)
Ventouse	6 (0·9)	1 (0·3)	0 (0)
Forceps	12 (1·8)	3 (0·9)	1 (4·7)
Breech	3 (0·5)	1 (0·3)	0 (0)
Elective caesarean section	72 (11·0)	31 (9·4)	0 (0)
Emergency caesarean section	273 (41·6)	91 (27·6)	6 (28·6)
Not known	1 (0·1)	1 (0·3)	0 (0)
Delivery induced			
No	567 (86·4)	287 (87·0)	17 (81·0)
Yes	60 (9·2)	22 (6·7)	3 (14·3)
Not known	29 (4·4)	21 (6·3)	1 (4·7)
Socio-demographic factors			
Caste/ social class*			
Schedule caste	68 (10·4)	26 (7·9)	2 (9·5)
Schedule tribe	81 (12·3)	61 (18·5)	6 (28·6)
Other backward classes	75 (11·4)	57 (17·3)	6 (28·6)
General	431 (65·7)	186 (56·4)	7 (33·3)
Not known	1 (0·2)	0 (0)	0 (0)
Religion*			
Hindu	456 (69·5)	236 (71·5)	16 (76·2)
Muslim	190 (28·9)	66 (20·0)	2 (9·5)
Others	5 (0·8)	8 (2·4)	2 (9·5)
Not known	5 (0·8)	20 (6·1)	1 (4·8)
Residence			
Rural	560 (85·3)	282 (85·5)	19 (90·5)
Urban	95 (14·5)	48 (14·5)	2 (9·5)
Not known	1 (0·2)	0 (0)	0 (0)
Belongs to below poverty line (BPL) household*			
No	309 (47·1)	132 (40·0)	6 (28·6)
Yes	286 (43·6)	143 (43·3)	12 (57·1)
Not known	61 (9·3)	55 (16·7)	3 (14·3)
Husband in paid employment			
Employed	594 (90·6)	300 (90·9)	18 (85·7)
Unemployed	14 (2·1)	6 (1·8)	0 (0)
Not known	48 (7·3)	24 (7·3)	3 (14·3)
Women in paid employment*			
No	571 (87·0)	263 (79·7)	14 (66·7)
Yes	33 (5·0)	31 (9·4)	6 (28·6)
Not known	52 (7·9)	36 (10·9)	1 (4·7)
Husband tea-garden worker*			
No	562 (85·7)	261 (79·1)	12 (57·1)
Yes	46 (7·0)	45 (13·6)	6 (28·6)
Not known	48 (7·3)	24 (7·3)	3 (14·3)

*$p<0·05$ for χ^2 test for difference in proportion

The socio-demographic and pregnancy related characteristics of the women are shown in Table 1. After adjusting for the socio-demographic and pregnancy related characteristics, we found that compared to women with mild anaemia/normal haemoglobin level, women with severe anaemia had more than nine-fold higher odds of PPH (adjusted odds ratio (aOR) 9·45; 95% CI 2·62 to 34·05), six-fold higher odds of giving birth to a low birthweight baby (aOR 6·19; 95% CI 1·44 to 26·71), and almost nine-fold higher odds of having a small-for-gestational age baby (aOR 8·72; 95% CI 1·66 to 45·67) (Table-2). Women with moderate anaemia also had higher odds of PPH, low birthweight and small-for-gestational age babies compared to women with mild anaemia/normal haemoglobin level, but the association was statistically significant only for the low birthweight outcome (Table 2).

The odds of PPH were 17-fold higher among women who had moderate-severe anaemia and underwent induction of labour compared to women who had neither of these exposures. Similarly, the odds of PPH were almost 19-fold higher among women who had moderate-severe anaemia and infection during pregnancy compared to women who had none of these risk factors (Table-2). The associations between moderate-severe anaemia and infant outcomes, low birthweight and small-for-gestational age, were not observed to vary

according to the presence of maternal infection during the antenatal period. Women with severe anaemia were observed to have 16-fold higher odds of perinatal mortality compared to women with mild anaemia/normal haemoglobin levels (Table-2).

Table-2: Association between maternal anaemia and maternal and infant outcomes

	Outcome present n (%)	Outcome absent n (%)	Unadjusted OR (95% Robust CI)	Adjusted OR (95% Robust CI)
Postpartum haemorrhage (n=1006)				
Anaemia				
Normal/mild (≥10 grams/dl)	15 (2·3)	641 (97·7)	1 (ref)	1 (ref)
Moderate (7-9·9 grams/dl)	13 (3·9)	316 (96·1)	1·76 (0·79 to 3·90)	1·50 (0·80 to 2·80)
Severe (<7 grams/dl)	5 (23·8)	16 (76·2)	13·35 (4·88 to 36·54)	9·45 (2·62 to 34·05)
Anaemia and/ or induction of labour				
None	12 (2·1)	555 (97·9)	1 (ref)	1 (ref)
Either	14 (3·9)	350 (96·1)	1·85 (0·91 to 3·75)	1·76 (0·96 to 3·22)
Both	5 (20·0)	20 (80·0)	11·56 (2·38 to 56·13)	17·39 (3·73 to 80·97)
Missing	2 (4·0)	48 (96·0)	1·93 (0·95 to 3·89)	2·14 (1·15 to 3·99)
Anaemia and/ or infection				
None	14 (2·3)	588 (97·7)	1 (ref)	1 (ref)
Either	12 (3·9)	296 (96·1)	1·70 (1·06 to 2·75)	1·39 (0·89 to 2·15)
Both	4 (25·0)	12 (75·0)	14·00 (1·66 to 118·07)	18·80 (3·57 to 98·93)
Missing	3 (3·8)	77 (96·2)	1·64 (0·31 to 8·56)	2·64 (0·21 to 33·45)
Low birth weight (singleton live born only n=966)				
Anaemia				
Normal/mild (≥10 grams/dl)	160 (25·2)	474 (74·8)	1 (ref)	1 (ref)
Moderate (7-9·9 grams/dl)	91 (29·2)	221 (70·8)	1·22 (0·94 to 1·58)	1·26 (1·04 to 1·53)
Severe (<7 grams/dl)	12 (60·0)	8 (40·0)	4·44 (1·75 to 11·28)	6·19 (1·44 to 26·71)
Anaemia and/ or infection				
None	142 (24·4)	440 (75·6)	1 (ref)	1 (ref)
Either	86 (29·3)	208 (70·7)	1·28 (0·99 to 1·65)	1·27 (0·99 to 1·65)
Both	3 (21·4)	11 (78·6)	0·85 (0·37 to 1·95)	1·21 (0·40 to 3·59)
Missing	32 (42·1)	44 (57·9)	2·25 (1·18 to 4·29)	1·32 (0·70 to 2·49)
Small-for-gestational age (singleton live born n=881)				
Anaemia				
Normal/mild (≥10 grams/dl)	251 (43·7)	323 (56·3)	1 (ref)	1 (ref)
Moderate (7-9·9 grams/dl)	143 (49·5)	146 (50·5)	1·26 (0·82 to 1·94)	1·25 (0·91 to 1·73)
Severe (<7 grams/dl)	15 (83·3)	3 (16·7)	6·43 (0·87 to 47·39)	8·72 (1·66 to 45·67)
Anaemia and/ or infection				
None	228 (43·1)	301 (56·9)	1 (ref)	1 (ref)
Either	134 (49·8)	135 (50·2)	1·31 (0·83 to 2·07)	1·20 (0·80 to 1·80)
Both	6 (42·9)	8 (57·1)	0·99 (0·50 to 1·98)	1·40 (0·67 to 2·93)
Missing	41 (59·4)	28 (40·6)	1·93 (1·26 to 2·97)	1·35 (0·87 to 2·11)
Perinatal deaths (singleton pregnancies, n=987)				
Anaemia				
Normal/mild (≥10 grams/dl)	18 (2·8)	628 (97·2)	1 (ref)	1 (ref)
Moderate (7-9·9 grams/dl)	10 (3·1)	310 (96·9)	1·13 (0·65 to 1·96)	1·18 (0·64 to 2·19)
Severe (<7 grams/dl)	4 (19·1)	17 (80·9)	8·21 (3·20 to 21·09)	16·42 (4·38 to 61·55)

OR – odds ratio; CI – confidence intervals

The final multivariable models were adjusted for the following risk factors: caste, religion, residence, below poverty line status, women's employment status, tea garden worker, parity, BMI, maternal age, previous caesarean section, previous pregnancy problems, pre-existing medical problems, multiple pregnancies, number of antenatal visits, whether folic acid tablets were received, and mode of delivery. Mode of delivery was not included in the infant outcomes model as this is not likely to be causally associated. . Gestational age was included in the low birthweight model.

Calculated study power based on estimated prevalence of the outcomes in the study population

The prevalence of the outcomes PPH and low birthweight were found to be lower than the figures available from the literature therefore we calculated the study power for each outcome based on the estimated prevalence of the outcomes in the study population and the final sample. For a prevalence of 2.3% PPH in the unexposed population, with 351 participants in the exposed group (ratio of unexposed (mild anaemia/ normal Hb) to exposed

(moderate-severe anaemia) =1.9) our study had 80% power to detect significantly an odds ratio of 2.28 or greater associated with maternal anaemia at p<0.05 (two-tailed). For the prevalence of 25% low birthweight babies in the unexposed population, with 351 participants in the exposed group (ratio of unexposed to exposed=1.9) our study had 80% power to detect significantly an odds ratio of 1.29 or greater. For a prevalence of 44% small-for-gestational age babies in the unexposed group (351 participants in the exposed group, ratio unexposed to exposed=1.9) our study had 80% power to detect significantly an odds ratio of 1.19 or greater, and for a prevalence of 2.8% perinatal deaths in the unexposed group (351 participants in the exposed group, ratio unexposed to exposed=1.9) our study had 80% power to detect significantly an odds ratio of 1.16 or greater.

DISCUSSION

We were able to use the IndOSS-Assam surveillance and research system successfully to conduct this cohort study. The study showed that after adjusting for other risk factors, women with severe anaemia were at a significantly higher risk of PPH, giving birth to low birthweight and small-for-gestational age babies, and having a baby who died in the perinatal period. The odds of these adverse outcomes were also higher among women with moderate anaemia, but only the association with low birthweight was statistically significant. Importantly the risk of PPH increased 17-fold among women with moderate-severe anaemia who underwent induction of labour.

Limitations of this study

This was a retrospective cohort study based on information collected from the hospital records of pregnant women. There are known issues related to estimation of blood loss in the peripartum period and after delivery through observation alone, therefore we cannot ignore the possibility of misclassification. We were also not able to grade the severity of PPH. As discussed above, the hospital notes did not have records of haemoglobin level at different gestational period, thus effects of anaemia in relation to the different gestational periods could not be ascertained. Another inherent limitation of collecting data from hospital records is a high proportion of missing data which was evident in our study. However, the estimated odds ratios using complete case analysis and sensitivity analyses were not substantively different from the outputs of the main models.

Findings in relation to other studies

A number of studies in India and in other LMICs have shown that anaemia during pregnancy is associated with increased risk of PPH[6 21], low birthweight[4 5 21] and small-for-gestational age babies[4 21]. Our results, in relation to the adverse neonatal outcomes, are comparable to the other study in Assam conducted in one hospital (n=421).[4] Studies from other countries show varying results when examining the association between maternal anaemia on the risk of perinatal death. One study in Pakistan found maternal anaemia to be associated with increased risk of stillbirth,[22] but another in China showed that a haemoglobin level <9 g/dl in the third trimester was associated with a reduced risk of stillbirth and maternal anaemia was not associated with neonatal death.[23] A study that used National Maternal and Infant Health Survey data in the USA found moderate anaemia to be associated with an increased risk of stillbirth among non-black women, but found no statistically significant association among black women.[24]

Individual associations of infections during pregnancy and maternal anaemia with adverse maternal and fetal outcomes have been shown by several studies.[5 6 10 12] It is known that there is a causal relationship between infections and anaemia during pregnancy.[11] We found a significant additive effect of infection and moderate-severe anaemia on increased risk of PPH, but this was not observed for the infant outcomes.

We observed a 17-fold increased risk of PPH among pregnant women with moderate-severe anaemia who underwent induction of labour. A few small studies have suggested that women with severe anaemia are more likely to have uterine atony due to impaired transport of oxygen and haemoglobin to the uterus,[6 21] but there is no strong evidence. This could be a possible explanation for the observed increased risk of PPH among pregnant women with

moderate-severe anaemia who underwent induction of labour in this study, but it is also possible that other mechanisms exist.

Implications and future research

We found a high prevalence of iron deficiency anaemia among pregnant women in Assam which highlights that this is an important public health problem. Maternal anaemia was associated with increased risks of PPH, low birthweight, small-for-gestational age babies and perinatal death. Clearly the best approach would be to prevent anaemia or treat it during the antenatal period, but the Annual Health Survey showed that only 23% of the pregnant women in Assam consumed the complete course of iron-folic acid tablets in 2012-13 (a slight improvement from 15.3% in 2010-11).[25] A large number of pregnant women in India present with severe anaemia either at the point of delivery or in the third trimester.[26 27] In this study, 62% of the women with severe anaemia had low haemoglobin levels during the 3rd trimester of pregnancy when there is little scope for prevention or iron treatment.

At present, there is no clear guidance with regard to how women with severe anaemia should be managed during labour and delivery. WHO guidelines on induction of labour do not provide any recommendation with regards to whether induction of labour should be carried out in women with severe anaemia[28], nor do guidelines on management of anaemia make recommendations for management of women with moderate-severe anaemia during labour and delivery.[17] Considering the high prevalence (35%) of moderate-severe anaemia among pregnant women in the study population and in several LMICs (an estimated 42% of pregnant women suffer from anaemia, globally[29]), and its association with PPH and high risk of maternal death, further consideration needs to be given to generating evidence on the appropriate management of pregnant women with moderate-severe anaemia during the peripartum period to prevent maternal morbidity and mortality.

By successfully conducting this pilot study, we were able to demonstrate that the IndOSS-Assam platform can be used to conduct epidemiological research. A number of studies will be undertaken through this platform in the near future to test the hypotheses generated in this study, and to examine the risk factors, management and outcomes of other pregnancy complications in order to improve care.

Competing interests: We have read and understood the BMJ policy on declaration of interests and declare that we have no competing interests.

Ethics approvals: The IndOSS-Assam feasibility study, of which this retrospective study was a part, was approved by the institutional ethics committee of Srimanta Sankaradeva University of Health Sciences (No SSUHS/Ethics/2014/1, dated 27 June 2014); the Health Ministry's Screening Committee, Indian Council of Medical Research (No 5/7/12 12/14-RCH, dated 25.09.2014) and the University of Oxford's Oxford Tropical Research Ethics Committee (OxTRTEC Reference 57-14).

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Authorship and contributions: MN designed the study, trained the field staff, conducted literature searches, planned and carried out the data analysis, interpreted the data, and wrote the first draft of the article. MKC designed the study, trained the field staff, supervised data collection and data entry, and edited the manuscript. SSC designed the study, trained the field staff, supervised data collection and data entry, and edited the manuscript. SDK

designed the study, trained the field staff, and edited the manuscript. UCS designed the study, trained the field staff, supervised data collection, and edited the manuscript. PW designed the study, and edited the manuscript. MK designed the study, contributed to the data analysis plan, data interpretation, and to writing the article.

Data sharing statement: This was a pilot project and there is no additional data from the study.

Transparency declaration: MN is the guarantor and affirms that the manuscript is an honest, accurate, and transparent account of the study being reported and no important aspects of the study have been omitted.

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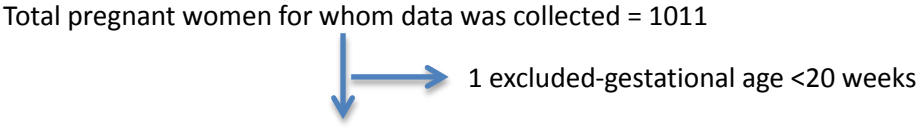
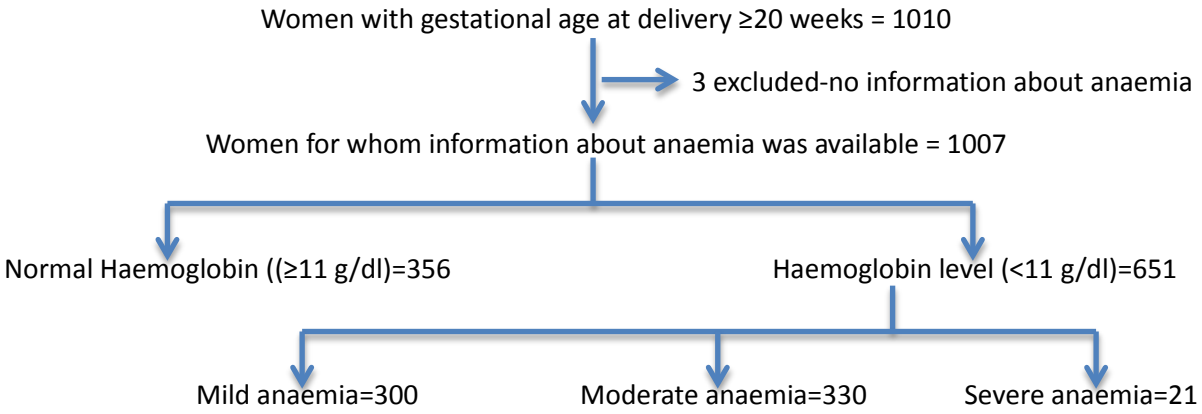
Figure-1: Flow chart to show the study population




Women with gestational age at delivery ≥20 weeks = 1010
3 excluded-no information about anaemia
Women for whom information about anaemia was available = 1007
Normal Haemoglobin ((≥11 g/dl)=356
Haemoglobin level (<11 g/dl)=651
Mild anaemia=300
Moderate anaemia=330
Severe anaemia=21