Original Article

Role of colour Doppler in predicting foetal outcome in maternal anaemia

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Abstract

Context: Anaemia is the most common nutritional deficiency in Indian pregnant females. It ensues foetal hypoxia resulting in different compensatory mechanisms in the foetus which may result in adverse perinatal outcomes. Colour Doppler can be used to measure these hemodynamic changes of the foetus ahead of the clinical manifestations, guiding the obstetrician for the appropriate management and circumventing any dire complication. **Aims:** The aim of this study is to detect foetal haemodynamic changes associated with maternal anaemia and assess the parameters which predict these changes accurately. **Settings and Design:** Prospective cohort study. **Materials and Methods:** Two hundred and forty pregnant females in the third trimester, divided into four groups based on their haemoglobin levels in the non-anaemic, mild, moderate and severe anaemic groups, were included in the study. These patients were followed up for foetal outcome in terms of effective foetal weight, APGAR score and neonatal intensive care unit admission. **Statistical Analysis Used:** Analysis of Variance Appearance, Pulse, Grimace, Activity and Respiration (ANOVA) test was used to compare the quantitative variables. SPSS software was used. **Results:** The middle cerebral artery (MCA) Doppler indices and cerebroplacental ratio (CPR) values were increasing while umbilical Doppler indices were decreasing with the increasing severity of anaemia. CPR was found to be the most sensitive predictor for foetal outcome. **Conclusions:** Maternal anaemia results in foetal hypoxia which can be measured in terms of foetal Doppler indices. CPR was found to be more sensitive than the umbilical or MCA in predicting foetal hypoxia and in turn the perinatal outcome of foetuses of anaemic pregnant females. The foetuses with low CPR values will require urgent intervention to improve the outcomes.

Keywords: Anaemia, Doppler ultrasound, pregnant

Introduction

Anaemia is the most common disorder during pregnancy which encounter serious global health concerns, especially among the poorer segments of the population and those with higher natality. Iron deficiency is found to be the most common cause of anaemia as the demand for iron increases during late pregnancy by six to seven times.[1] As per World Health Organisation (WHO) anaemia in pregnant females is defined as haemoglobin levels less than 13 g/dL in adult men, less than 12 g/dL in non-pregnant adult women and less than 11 g/dL in pregnant females.[2] The prevalence of iron deficiency anaemia is approximately 53.9% of pregnant females in Madhya Pradesh, India.[3]

Maternal anaemia results in foetal hypoxia and is a risk factor for prematurity, preterm delivery, small for gestational age and even perinatal mortality in some. In order to compensate for foetal hypoxia, foetal cerebral vasodilatation along with placental circulation variations ensues.

Ultrasound colour Doppler being a safe, non-invasive and reliable modality can easily assess these variations in pregnant females since the hemodynamic changes are demonstrated well before the clinical manifestation. Early recognition of these parameters

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will help in preventing complications and initiating respective preventive and therapeutic measures by the obstetricians. This in turn will result in a significant reduction in maternal and foetal morbidity and mortality.

The aim of this study is to detect these haemodynamic changes associated with maternal anaemia and to assess the parameters with the highest sensitivity which predict these changes accurately and help improve the perinatal outcome.

Materials and Methods

The study was conducted in the Department of Radio Diagnosis on a total of 240 pregnant females who attended the obstetrics outpatient and inpatient department in the third trimester from April 2021 to October 2022. They were randomised into four subgroups according to their haemoglobin values into non-anaemic, mild, moderate and severe anaemic groups. The pregnant females in active labour, with any hemoglobinopathies, any diagnosed maternal morbidity or with congenital foetal anomaly were excluded from this study.

The study was performed using real-time greyscale and colour Doppler ultrasound using convex probes of frequency ranging from 3 to 5 MHZ. The scan was performed with the patients in a supine position on an exam table. For umbilical artery (UmA) Doppler, free loop along the longitudinal axis of the vessels was focussed and then with adequate sample gate and scaling Doppler pattern was measured. UmA Doppler values were considered abnormal when more than >95th percentile. For the middle cerebral artery (MCA) Doppler, a circle of Willis and then proximal MCA was identified. The pulsed-wave Doppler gate was placed at the proximal third of the MCA and <5th percentile of MCA Doppler values were considered abnormal. The cerebroplacental ratio (CPR) was calculated by dividing the umbilical artery Pulsatility Index (PI) and the MCA PI. Adverse pregnancy outcomes included effective foetal weight (EFW) <2500 g, 5 min APGAR score of 7 and the neonatal intensive care unit (NICU) admission. Abbreviations are listed below in the Appendix-A.

Statistical analysis

Summarisation of continuous variables like gestational age and ratios was done using mean and standard deviation. ANOVA test was calculated to find the correlation between quantitative variables. Sensitivity and specificity were calculated for Doppler indices. Statistical significance is checked at a 5% level of significance (*P*-value <0.05). The statistical software SPSS 15.0 was used for analysis of the data and Microsoft Word and Excel were used to generate graphs and tables.

Results

Each group in the present study had 60 patients. The maximum number of patients, 91 (37.9%) belonged to 20–25 years of age group. All the studied groups had a maximum of 84 (35%) patients as primiparous. The detailed distribution with respect to age and parity is as shown in Figures 1 and 2.

All the patients were studied in the third trimester group with most patients 113 (47.08%) in 28–32 weeks of gestation age range. The mean values of the demographic data compared between the studied groups are shown in Table 1.

Doppler assessments of foetal circulation were compared among each studied group as depicted in Table 2. MCA PI and RI showed a decreasing trend with the increasing severity of anaemia with the mean value of 1.64 ± 0.15 and 0.79 ± 0.05 in non-anaemic group, 1.59 ± 0.17 and 0.76 ± 0.05 in mild anaemia group, 1.50 ± 0.27 and 0.74 ± 0.07 in moderate anaemia group, and 1.34 ± 0.21 and 0.67 ± 0.08 in severe anaemia group, while UmA PI and RI are found to be increasing with the increasing severity of anaemia with the mean value of 0.81 ± 0.08 and 0.58 ± 0.04 in non-anaemic group, 0.89 ± 0.12 and 0.59 ± 0.05 in mild anaemia group, 0.96 ± 0.13 and 0.64 ± 0.06 in moderate anaemia group, and 1.15 ± 0.21 and 0.69 ± 0.09 in severe anaemia group. MCA peak systolic velocity (PSV) was found to be increasing with anaemia severity though the difference was insignificant (*P* > 0.05). UmA PSV did not show any association with the degree of anaemia. CPR reflects the hemodynamic status and is found to be decreasing with the severity of anaemia with the mean value of 1.99 ± 0.24 in the non-anaemic group, 1.77 ± 0.26 in the mild anaemia group, 1.66 ± 0.36 in the moderate anaemia group and 1.16 ± 0.31 in the severe anaemia group.

Table 1: Comparison between the different studied groups regarding demographic data

	Non-anaemic	Mild	Moderate	Severe	*P**
Age	25.05±3.73	25.30±4.10	24.01±3.52	24.50±3.83	0.147
Parity	1.65±0.89	1.99±0.90	1.80±0.81	1.86±0.76	0.720
Gestation age	36.6±2.17	36.05±2.44	35.4±3.28	34.9±3.33	0.947
Haemoglobin	13.73±0.56	10.58±0.27	9.12±0.56	6.47±0.93	0.001

**P* considered significant at <0.005

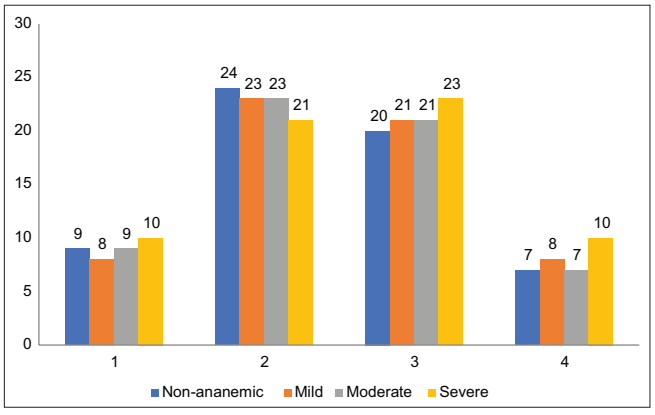

Figure 1: Figure showing distribution of patients in the studied groups as per their age groups

Table 2: Comparison between the different studied groups regarding MCA and UmA Doppler indices

Doppler indices	Non-anaemic	Mild	Moderate	Severe	*P**
UmA PSV**					
Range	22-76	22-74	21-84	18-68	0.2521
Mean±SD	42.4±14.2	39.4±15.8	41.6±9.2	38.8±9.9	
UmA PI					
Range	0.6-0.9	0.6-1.0	0.7-1.2	0.8-1.7	0.003
Mean±SD	0.81±0.08	0.89±0.12	0.96±0.13	1.15±0.21	
UmA RI					
Range	0.5-0.7	0.51-0.72	0.54-0.79	0.62-0.97	0.008
Mean±SD	0.58±0.04	0.59±0.05	0.64±0.06	0.69±0.09	
MCA PSV**					
Range	24-70	25-75	26-94	24-68	0.421
Mean±SD	54±10.2	50.31±9.3	48.43±10.3	46.8±11.1	
MCA PI					
Range	1.35-1.70	1.21-1.64	1.12-1.56	0.98-1.51	0.001
Mean±SD	1.64±0.15	1.59±0.17	1.50±0.27	1.34±0.21	
MCA RI					
Range	0.73-0.98	0.62-0.95	0.60-0.90	0.48-0.81	0.001
Mean±SD	0.79±0.05	0.76±0.05	0.74±0.07	0.67±0.08	
CPR					
Range	0.97-3.12	0.94-3.10	0.90-2.54	0.64-2.0	0.001
Mean±SD	1.99±0.24	1.77±0.26	1.66±0.36	1.16 0.31	

*P considered significant at <0.005. **PSV as cm/s

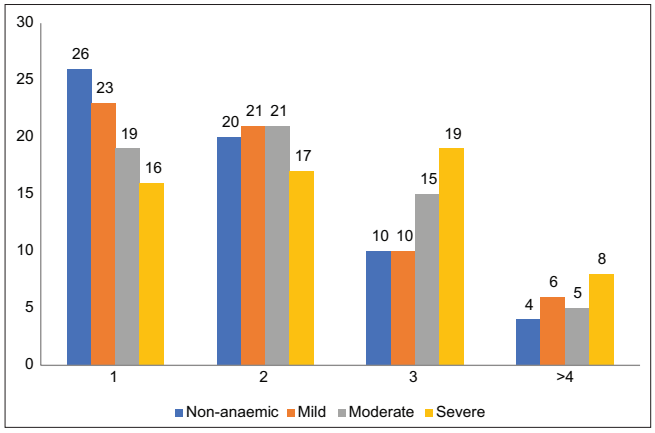

Figure 2: Figure showing distribution of patients in the studied groups as per their parity

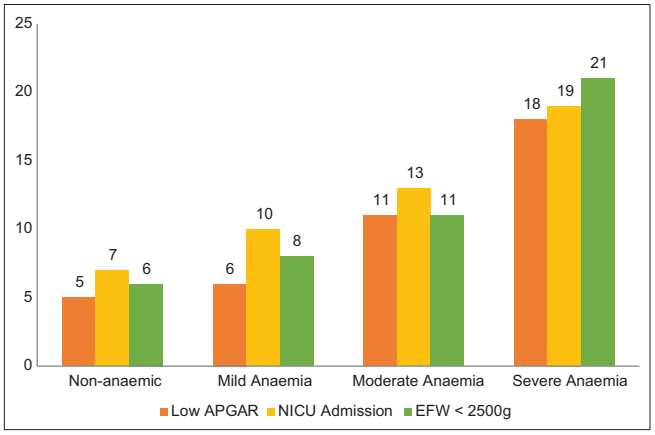

Figure 3: Distribution of patients as per adverse perinatal outcome

In our study, 18.7% of the newborn had EFW <2500 g, 17.5% had low APGAR scores at 5 min and 20.4% were admitted to the NICU as shown in Figure 3. Abnormal values of the Doppler indices in each group were calculated and shown in Table 3 where MCA RI was found to be the most (23.7%) abnormal. On evaluation, CPR was found to be the most sensitive and specific for foetal hypoxia as a result of maternal anaemia with sensitivity at 71.4% and specificity at 98.4% [Table 4].

Discussion

As per the study done in our department, most of the pregnant females 91 (37.9%) belonged to group 20-25 years group with 40%, 38.4%, 38.4% and 35% in non-anaemic, mild, moderate and severe anaemia group, respectively. All the groups were matched and no statistically significant difference was found. In terms of parity, the maximum number of patients, 84 (35%), were primiparous. However, no significant association was found between parity and degree of anaemia in pregnant females. Gestation age in all groups at the time of Doppler scan showed no significant difference. The maximum number of pregnant females, 113 (47%), presented in the 28-32 weeks of gestation age.

In our study, Umbilical Artery PSV (in cm/s) in the non-anaemic anaemia group ranged from 22 to 76, in the mild anaemia group ranged from 22 to 74, in the moderate anaemia group ranged from 21 to 84 and in the severe anaemia group ranged from 18 to 68. The mean values did not show any significant difference ($P > 0.05$). Kessous *et al.*[4] did a similar study where he found no association of UmA PSV with the outcome.

Table 3: Abnormal values of Doppler indices in each studied group

	Non-anaemic	Mild anaemia	Moderate anaemia	Severe anaemia	Total
UmA PI	2 (3.3%)	5 (8.3%)	16 (26.6%)	22 (36.6%)	45 (18.7%)
UmA RI	1 (0.4%)	4 (6.6%)	15 (25%)	21 (35%)	41 (17%)
MCA PI	0	6 (10%)	16 (26.6%)	28 (46.6%)	50 (20.8%)
MCA RI	1 (0.4%)	3 (5%)	20 (33.3%)	33 (55%)	57 (23.7%)
CPR	0	3 (5%)	9 (15%)	21 (35%)	33 (13.75%)
Total	60 (100%)	60 (100%)	60 (100%)	60 (100%)	240 (100%)

Table 4: Prediction of foetal hypoxia as per abnormal Doppler velocimetry

	Sensitivity	Specificity	PPV*	NPV*
UmA PI	40.4%	85.8%	37.7%	87.2%
UmA RI	38%	87.3%	39%	86.9%
MCA PI	57%	86.8%	48%	90.5%
MCA RI	69%	85.8%	50.8%	92.8%
CPR	71.4%	98.4%	90.9%	94.2%

*PPVs=Positive predictive value, NPVs=Negative predictive value

Umbilical artery pulsatility index in the non-anaemic group ranged from 0.60 to 0.90, in the mild anaemia group ranged from 0.60 to 1.0, in the moderate anaemia group ranged from 0.70 to 1.2 and in the severe anaemia group ranged from 0.80 to 1.7. Mean UmA PI values in all groups showed a statistically significant increase ($P < 0.05$) with the increasing severity of anaemia. Similar results were seen in a study done by Abdel Samie AS *et al.*[5] with mean UmA PI higher in the severe anaemia group at 1.11 ± 0.27 than the mild or moderate anaemia groups ($P < 0.000$). Studies done by Mohamad Ihab Md *et al.*,[6] Ghada A *et al.*[7] and Rafiq S *et al.*[8] showed similar results.

The umbilical artery resistance index showed a rising trend with the severity of anaemia in our study. The UmA RI in the non-anaemic anaemia group ranged from 0.50 to 0.7, in the mild anaemia group ranged from 0.51 to 0.72, in the moderate anaemia group ranged from 0.54 to 0.79 and in the severe anaemia group ranged from 0.62 to 0.97. The increasing trend of the UmA RI showed statistical significance between the non-anaemic group and different anaemia groups along with the increasing severity of anaemia. Normally with the progression of the pregnancy, there is a decrease in the resistance index of UmA thus allowing continuous flow throughout the cardiac cycle. In pregnancies complicated by anaemia, the end-diastolic flow is reduced causing a rise in resistance of placental flow. Studies done by Rafiq S *et al.*,[8] Abdel-Megeed AM *et al.*[9] and Abdel Samie AS *et al.*[5] reported similar results.

On evaluation, the MCA peak systolic velocity (PSV, in cm/s) in the non-anaemic group ranged from 24 to 70, in the mild anaemia group ranged from 25 to 75, in the moderate anaemia group ranged from 26 to 94, in the severe anaemia group ranged from 24 to 68. There was no statistically significant difference in anaemia groups ($P > 0.05$) with respect to MCA PSV. These findings are similar to the findings described by Ali A *et al.*[10] showing no association of MCA PSV with anaemia.

The MCA pulsatility index in the non-anaemic group ranged from 1.35 to 1.70, in the mild anaemia group ranged from 1.21 to 1.64, in the moderate anaemia group ranged from 1.12 to 1.56 and in the severe anaemia group ranged from 0.98 to 1.51. The decrease in MCA PI in our study was significant ($P < 0.05$) and corroborated well with the findings of Abdel Megeed AM *et al.*[9], Ali *et al.*[10] and Abdel Samie[5] *et al.* The results also showed a decreasing trend of MCA PI with the severity of anaemia.

In the present study, the MCA resistance index had a decreasing trend with the severity of anaemia. The MCA RI in the non-anaemic group ranged from 0.73 to 0.98, in the mild anaemia group ranged from 0.62 to 0.95, in the moderate anaemia group ranged from 0.60 to 0.90 and in the severe anaemia group ranged from 0.48 to 0.81. The results of our study correlated well with the findings of Abdel Megeed AM *et al.*[9] where a progressive significant decline ($P < 0.05$) was seen with anaemia severity and in the non-anaemic group. Ali A *et al.*[10] also found that the MCA RI was significantly lower in the anaemia group than in the non-anaemic group. Foetal cerebral circulation flow is a high resistance flow which gradually rises with the progression of pregnancy. But in cases with maternal anaemia, the foetal cerebral circulation undergoes vasodilatation in order to maintain the foetal oxygenation at satisfactory levels. The most profound effect is seen in severe maternal anaemia causing a decrease in resistance of the cerebral artery.

In our study, the CPR was calculated using the ratio of MCA PI with UA PI. In the non-anaemic group, the CPR value ranged from 0.97 to 3.12, in the mild anaemia group from 0.94 to 3.10, in the moderate anaemia group ranged from 0.90 to 2.54 and in the severe anaemia group it ranged from 0.64 to 2.0. There was a statistically significant decline in the mean value of CPR between different study groups. This ratio was found to be more reliable and accurate for the prediction of foetal hypoxia, foetal growth retardation than the UA or MCA PI in the evaluation of various antenatal and perinatal complications.[11,12] The CP ratio reflects the status of redistribution of the cardiac output to the cerebral circulation, which improves accuracy in predicting adverse outcomes compared to MCA and UA Doppler alone. It should be >1 in normal foetuses but foetuses with hypoxia as in severe anaemia will have this ratio at <1 value. Thus, it is supposed to be more physiological in the measurement of centralisation of foetal blood flow. The CP ratio is proven to be an important adjunct parameter to help in monitoring perinatal and antenatal complications in pregnancies complicated with anaemia.[13]

This is concerned with the results of Abdel Megeed AM *et al.*,[9] Stefanović M *et al.*[14] where CPR values were less than 1 in severely anaemic pregnant females. Ali A *et al.*[10] also detected decreased CPR values on admission of anaemic pregnant females which improved after treatment.

We assessed the foetal status after delivery in terms of EFW, APGAR score at 5 min and the number of NICU admissions. About 18.7% of the newborn had EFW <2500 g, 17.5% had low APGAR scores and 20.4% were admitted NICU admission. Based on the clinical setting, we calculated the sensitivity and specificity of Doppler indices and found CPR to be the most sensitive and specific parameter to detect any hypoxic changes. Vollgraff Heidweiller Schreurs CA *et al.*[15] did a meta-analysis and evaluated the prognostic accuracy of CPR where they found that CPR outperformed UmA and MCA Doppler in the prediction of adverse outcomes, and Hwang HS *et al.*[16] assessed the umbilical artery sensitivity in maternal anaemia and found it to be 30% sensitive, similar to our study.

The need for Doppler Ultrasonography (USG) in pregnant females is unmatchable. Thus, Doppler enables a better understanding of the hemodynamic changes in the foetus in response to the various alterations in maternal physiology. The precise quantification of the vascular response in terms of Doppler indices helps the obstetrician in initiating any necessary intervention if needed. Therefore, it has become one of the most important clinical tools for feto-maternal surveillance in high-risk pregnancies and thus, can be credited with causing a significant decrease in perinatal mortality and morbidity. The CPR was found to be more sensitive than the umbilical or MCA in predicting foetal hypoxia and in turn the perinatal outcome of foetuses of anaemic pregnant females. The foetuses with low CPR values will require urgent intervention to improve the outcomes.

Ethical policy and institutional review board statement

Ethics and Scientific review Committee, M.G.M. Medical College, Indore, Madhya Pradesh (India), EC/MGM/April – 21/16.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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Appendix-A

List of abbreviations

Abbreviation	Definition
APGAR	Appearance, Pulse, Grimace, Activity and Respiration
ANOVA	Analysis of Variance
MCA	Middle Cerebral Artery
CPR	Cerebroplacental Ratio
PI	Pulsatility Index
WHO	World Health Organisation
UmA	Umbilical artery
EFW	Effective foetal weight
NICU	Neonatal intensive care unit
RI	Resistive Index

Reporting guidelines: STROBE (2007).

	Item No	Recommendation	Yes/No
Title and abstract	1	*(a)* Indicate the study's design with a commonly used term in the title or the abstract	Yes
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found. Structured abstract: Aims and Objectives, Materials and Methods, Results, Conclusion Format to be consistent	Yes
		Introduction	
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes
Objectives	3a	State specific objectives, including any prespecified hypotheses. The research objective should not be biased	Yes
	3b	Statements to be appropriately cited	Yes
		Methods: Structured methods section (with subheadings) is preferred	
Study design	4a	Present key elements of study design early in the paper (cross-sectional/cohort/case-non-anaemic)	Yes
	4b	Is the study design robust and well-justified?	Yes
Setting	5a	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Yes
	5b #	Mention the details of the Supplier/manufacturer of the equipment/materials (e.g., Chemicals) used in the study	No
	5c #	Mention the details of the drugs (manufacturer, dosage, dilution, frequency and route of administration, monitoring equipment) used in the study	No
	5d #	Mention the details about the cell lines (names and where it was obtained from)	No
	5e #	Mention the details of plant sample collection (Location, time period, validation of the specimen, Institution where the specimen is submitted and the voucher specimen number)	No
Participants	6	*(a) Cohort study*—Give the eligibility criteria (inclusion/exclusion), and the sources and methods of selection of participants. Describe methods of follow-up	Yes
		Case-Non-anaemic study—Give the eligibility criteria (Inclusion/exclusion), and the sources and methods of case ascertainment and non-anaemic selection. Give the rationale for the choice of cases and non-anaemics	No
		Cross-sectional study—Give the eligibility criteria (Inclusion/exclusion), and the sources and methods of selection of participants	No
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed	No
		Case-Non-anaemic study—For matched studies, give matching criteria and the number of non-anaemics per case	No
Variables	7a	Clearly define all outcomes (primary and secondary), exposures, predictors, potential confounders and effect modifiers	No
	7b	Give diagnostic criteria, if applicable	No
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	Yes
Bias	9	Describe any efforts to address potential sources of bias	No
Study size	10	Explain how the study size (sample size) was arrived at	Yes
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Yes
Statistical methods (a separate heading needed)	12	*(a)* Describe all statistical methods, including those used to non-anaemic for confounding	Yes
		(b) Describe any methods used to examine subgroups and interactions	Yes
		(c) Explain how missing data were addressed	No

Contd...

Methods: Structured methods section (with subheadings) is preferred			
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed *Case-Non-anaemic study*—If applicable, explain how matching of cases and Non-anaemics was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	No
		(e) Describe any sensitivity analyses	Yes
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Yes
		(b) Give reasons for non-participation at each stage	No
		(c) Consider use of a flow diagram	No
Descriptive data	14*	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	Yes
		(b) Indicate number of participants with missing data for each variable of interest	Yes
		(c) *Cohort study*—Summarise follow-up time (e.g., average and total amount)	Yes
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Yes
		Case-Non-anaemic study—Report numbers in each exposure category, or summary measures of exposure	No
		Cross-sectional study—Report numbers of outcome events or summary measures	No
Main results	16	*(a)* Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Yes
		(b) Report category boundaries when continuous variables were categorised	Yes
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	Yes
Presentation	18a	Tables and graphs properly depicted with no repetition of the data in the text	Yes
	18b	Annotation/footnotes to be mentioned appropriately	Yes
	18c	Abbreviations to be defined in the footnotes	Yes
Discussion			
Key results	19	Summarise key results with reference to study objectives	Yes
Limitations	20	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	No
Interpretation	21	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Yes
Generalisability	22	Discuss the generalisability (external validity) of the study results	
Citations	23a	The statements should be adequately cited	Yes
	23b	Recent citations (last 5 years) to be cited in a greater proportion	Yes
Other information			
Funding	24a	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	No
	24b	Mention the Grant Number	No
Ethical approval and Patient Consent	25a	Mention the IRB approval and the approval number (For animal and human subjects)	Yes
	25b	Mention if the study has been conducted in accordance with the ethical principles mentioned in the Declaration of Helsinski (2013)	Yes
	25c	Mention if the patients have consented to participate in the study To mention if consent has been waived/exempted by IRB	Yes
Conflict of Interest	26	Mention the financial, commercial, legal, or professional relationship of the author (or the author's employer) with sponsors/organisations that could potentially influence the research	No
Language	27	The language should be understandable without grammatical errors that hinders the readability	Yes

*Give information separately for cases and non-anaemics in case-non-anaemic studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. # Give information depending on the study sample