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Spectrum of Anemia and Hemoglobinopathies in Antenatal Women Referred for Hematological Evaluation at a Tertiary Care Hospital: A 12-Month Cross-Sectional Study

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Abstract | **Original Research Article**

Background: Anemia in pregnancy is one of the leading public health problems in India, highly contributing to maternal and perinatal morbidity and mortality. Madhya Pradesh is a high-prevalence zone for hemoglobinopathies [HBPs] such as sickle cell disease and beta-thalassemia with several areas showing more than 50% prevalence of HBP, including the East Nimar region. This study aimed to determine the spectrum of anemia and hemoglobinopathies in antenatal women referred for hematological evaluation at a major tertiary care center in this region of India. ***Methods:*** A hospital-based cross-sectional study was conducted over a period of one year at the laboratory facilities of Nandkumar Singh Chouhan Government Medical College, Khandwa. The study included 400 antenatal women who gave consent to participate. Blood samples received in the Department of Pathology were analyzed using complete blood count [CBC] and High-Performance Liquid Chromatography [HPLC] for detection of hemoglobinopathies. Statistical analysis of the collected data was carried out using SPSS version 25.0. ***Results:*** Among the 400 antenatal women included in the study, anemia [hemoglobin <11 g/dL] was observed in 246 women [61.5%]. Mild anemia was the most common type, seen in 140 women [35.0%], followed by moderate anemia in 91 women [22.75%] and severe anemia in 15 women [3.75%]. Hemoglobinopathies were detected in 45 participants [11.25%]. Sickle cell trait [HbAS] was the most frequently identified abnormality and was present in 24 women [6.0%]. Beta-thalassemia trait [BTT] was detected in 18 women [4.5%], while sickle cell disease [HbSS] was identified in 3 women [0.75%]. Women with beta-thalassemia trait showed lower mean MCV values along with elevated HbA2 levels when compared to participants with normal hemoglobin patterns. ***Conclusion:*** The present study showed that both nutritional anemia and inherited hemoglobinopathies are common among antenatal women in this region of Madhya Pradesh. These findings show the significance of early screening during pregnancy for early diagnosis and appropriate treatment. Although routine CBC still is useful as an initial screening tool, HPLC is useful in confirming the diagnosis of hemoglobinopathies and identifying pregnant women who will benefit from further investigations and genetic counselling. Early detection will help improving maternal care and reduce the risk of severe hemoglobin disorders in future progeny.

Keywords: Anemia, Pregnancy, Hemoglobinopathy, Sickle Cell, Beta-Thalassemia, Antenatal Screening, Madhya Pradesh.

INTRODUCTION

Anemia during pregnancy is a major public health problem, especially in low- and middle-income countries where nutritional deficiencies and limited access to healthcare is very common. According to the World Health Organization, around 35.5% of pregnant women worldwide were suffering from anemia in 2023, and India contributes majorly to this burden [1]. Anemia in pregnancy is defined as hemoglobin levels less than 11 g/dL and is considered one of the most common medical conditions complicating pregnancies [2]. Generally, iron and folate deficiency are the major causes, anemia may also occur due to chronic infections, parasitic infestations, and inherited disorders which lead to hemoglobin synthesis disorders.

Maternal anemia can have negative impacts on both the mother and the developing fetus. Women who

are pregnant and anemic are at a higher risk of experiencing fatigue, frequent infections, early labor, bleeding after childbirth, and in severe instances, heart-related issues. For the fetus, complications such as restricted growth within the womb, low birth weight, premature birth, and higher rates of perinatal mortality are often linked to maternal anemia. The severity of anemia often affects how serious the complications are, so finding and treating it early is important. Nutritional anemia is common in pregnancy, but inherited blood disorders, called hemoglobinopathies, are also a major cause of anemia in India. These include sickle cell disease and beta-thalassemia, which are inherited through families. These disorders may lead to long-term health complications, and severe cases may require repeated blood transfusions. Since these conditions are inherited, there is also a risk of transmission to the offspring [3].

India continues to face a significant burden of hemoglobinopathies because of its large and genetically diverse population. The cumulative gene frequency is estimated to be around 4.2%, while beta-thalassemia trait is present in nearly 3–4% of the population [4]. Sickle cell disorders are more commonly seen in tribal and central Indian populations, especially in Madhya Pradesh, which forms part of the "sickle cell belt" of India [5]. In the East Nimar region of Madhya Pradesh, sickle cell trait and sickle cell disease are frequently encountered among tribal communities, making antenatal screening particularly important in this region [6].

Identification of hemoglobinopathies during pregnancy is important not only for maternal health but also for assessment of fetal risk. Women carrying these traits may remain asymptomatic or may present with only mild anemia. However, if both parents are carriers, there is a possibility that the child may inherit a severe hemoglobin disorder. Early diagnosis allows partner screening, genetic counselling, prenatal diagnosis, and informed reproductive planning, which may help reduce the burden of severe inherited disorders in future generations [7].

Routine antenatal investigations such as hemoglobin estimation and complete blood count [CBC] are commonly used for screening and classification of anemia. However, CBC findings alone may not always differentiate nutritional anemia from inherited hemoglobin disorders. Red cell indices like mean corpuscular volume [MCV] and mean corpuscular hemoglobin [MCH] can provide useful clues, particularly in beta-thalassemia trait, but confirmatory testing remains necessary. High Performance Liquid Chromatography [HPLC] is widely used for identification of hemoglobin variants and is considered a reliable method for screening common hemoglobinopathies [8].

In many parts of central India, antenatal screening programs continue to focus mainly on nutritional anemia, while screening for hemoglobinopathies often receives less attention. In regions such as East Nimar, where both anemia and inherited hemoglobin disorders are frequently seen, a more comprehensive screening approach is needed for better antenatal care [9].

Keeping this in view, the present study was undertaken to evaluate the spectrum of anemia and common hemoglobinopathies among antenatal women undergoing hematological evaluation at a tertiary care hospital in Khandwa, Madhya Pradesh.

MATERIALS AND METHODS

Study Design and Setting:

The present study was a hospital-based cross-sectional observational study conducted in the Department of Pathology at Nandkumar Singh Chouhan Government Medical College, Khandwa, Madhya Pradesh, which serves as a tertiary care centre. The study was carried out over a duration of 12 months from January 2025 to December 2025.

Study Population and Sampling:

The study included antenatal women whose blood samples were referred to the Department of Pathology for Complete Blood Count [CBC] and High-Performance Liquid Chromatography [HPLC] during the study period. Consecutive sampling was used for participant enrolment.

Sample Size – A total of 400 antenatal women were included in the study.

Inclusion Criteria:

The study included all consenting antenatal women attending the antenatal clinic or admitted during the study period whose blood samples were received for Complete Blood Count [CBC] and High-Performance Liquid Chromatography [HPLC] analysis. Pregnant women from all gestational age groups were included in the study.

Exclusion Criteria -

Women with a previously diagnosed hemoglobinopathy who were already on treatment were excluded from the study. Patients with a history of blood transfusion within the preceding three months, known chronic hematological malignancy, inadequate or hemolyzed blood samples, and those who did not consent to participate were also excluded.

Ethical Consideration -

The study protocol was reviewed and approved by the Institutional Ethics Committee prior to the commencement of the study.

Data Collection Procedure –

Socio-demographic information including age, parity, residence, and educational status was recorded for all participants. Following this, 3 mL of venous blood was collected in an EDTA vacutainer under aseptic precautions and transported to the central laboratory for further analysis.

Laboratory Analysis -

- **Complete Blood Count [CBC]:** All blood samples were analyzed using an automated hematology analyzer [Sysmex XN-1000] for estimation of hemoglobin [Hb] concentration, red blood cell [RBC] count, Mean Corpuscular Volume [MCV], and Mean Corpuscular Hemoglobin [MCH]. Anemia was defined and classified according to the WHO 2024 criteria for pregnancy [1] as follows:
  - Mild anemia: 10.0–10.9 g/dL
  - Moderate anemia: 7.0–9.9 g/dL
  - Severe anemia: <7.0 g/dL

- **Hemoglobinopathy Screening:** All 400 samples were further analyzed using High Performance Liquid Chromatography [HPLC] on the Bio-Rad D-10 Hemoglobin Testing System for detection of hemoglobinopathies. Beta-thalassemia trait [BTT] was diagnosed when HbA2 levels were >3.5%. Sickle cell trait [SCT] was identified by the presence of HbS ranging between 30–45% along with HbA, whereas sickle cell disease [SCD] was diagnosed when HbS levels were >80% with absent HbA [10].

- **Statistical Analysis –**

The collected data were analyzed using SPSS version 25.0. Quantitative variables were expressed as mean ± standard deviation [SD], while qualitative variables were presented as frequency and percentage. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 400 pregnant women were enrolled. The socio-demographic characteristics are presented in Table 1.

Table 1: Socio-Demographic Characteristics of Study Participants [N=400]

Characteristic	Category	Frequency [n]	Percentage [%]
Age Group [Years]	≤ 20	60	15.0
	21-30	288	72.0
	> 30	52	13.0
Residence	Urban	262	65.5
	Rural	138	34.5
Education	Illiterate	48	12.0
	Primary/Middle School	154	38.5
	High School/Intermediate	136	34.0
	Graduate & above	62	15.5
Parity	Primigravida	168	42.0
	Multigravida	232	58.0

A total of 400 antenatal women were included in the study during the 12-month study period. The mean age of the participants was 25.4 ± 4.2 years, with most women belonging to the reproductive age group.

The majority of participants [72.0%] were in the 21–30 years age group, followed by women aged ≤20 years [15.0%] and those above 30 years [13.0%]. The age range in this study was typical for pregnant women groups. Most women, 65.5%, lived in cities, while 34.5% were from rural areas. More urban participants might be because they have better access to healthcare. For education, 38.5% had primary or middle school education, and 34.0% had high school education. About 12.0% could not read or write, and 15.5% had finished college or higher education. Most women in the study, 58.0%, had been pregnant before, while 42.0% were pregnant for the first time.

Prevalence and Severity of Anemia:

Anemia Category	Hemoglobin Level [g/dL]	Frequency [n]	Percentage [%]
No Anemia	≥ 11.0	154	38.5
Mild Anemia	10.0 – 10.9	140	35.0
Moderate Anemia	7.0 – 9.9	91	22.75
Severe Anemia	< 7.0	15	3.75
Total	—	**400**	**100.0**

The distribution of anemia severity is shown in Figure 1.

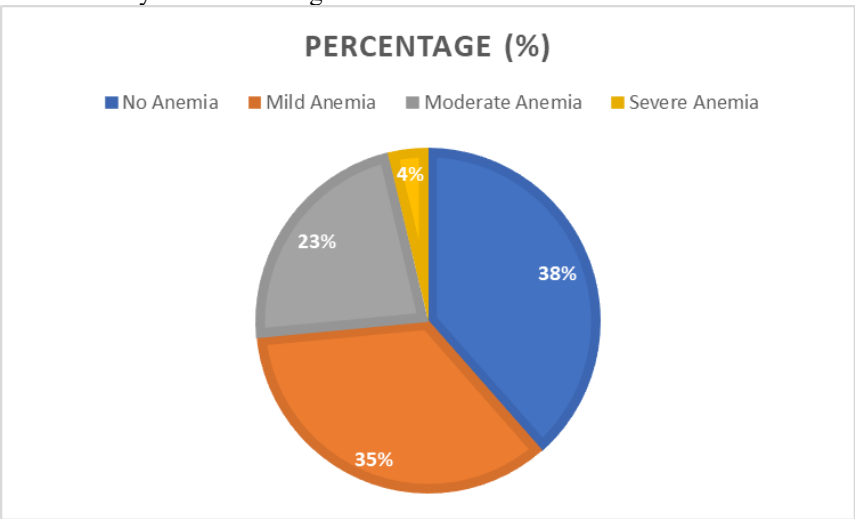

Figure 1: Distribution of Anemia Severity among Participants [N=400]

Among the 400 antenatal women included in the study, anemia [Hb <11 g/dL] was detected in 246 women, giving an overall prevalence of 61.5%.

Mild anemia was the most common category and was seen in 140 women [35.0%]. Moderate anemia was observed in 91 women [22.75%], while severe anemia was present in 15 women [3.75%].

A total of 154 participants [38.5%] had hemoglobin levels within the normal range. Overall, mild and moderate anemia together formed the major proportion of anemia cases in the study population.

Prevalence and Spectrum of Hemoglobinopathies:
The findings are presented in Table 2 and Figure 2.

Table 2: Spectrum of Hemoglobinopathies Detected by HPLC [N=400]

HPLC Finding	Hemoglobin Pattern	Frequency [n]	Percentage [%]
Normal	HbAA	355	88.75
Sickle Cell Trait	HbAS	24	6.0
Beta-Thalassemia Trait	High HbA2	18	4.5
Sickle Cell Disease	HbSS	3	0.75
Total Hemoglobinopathies		**45**	**11.25**

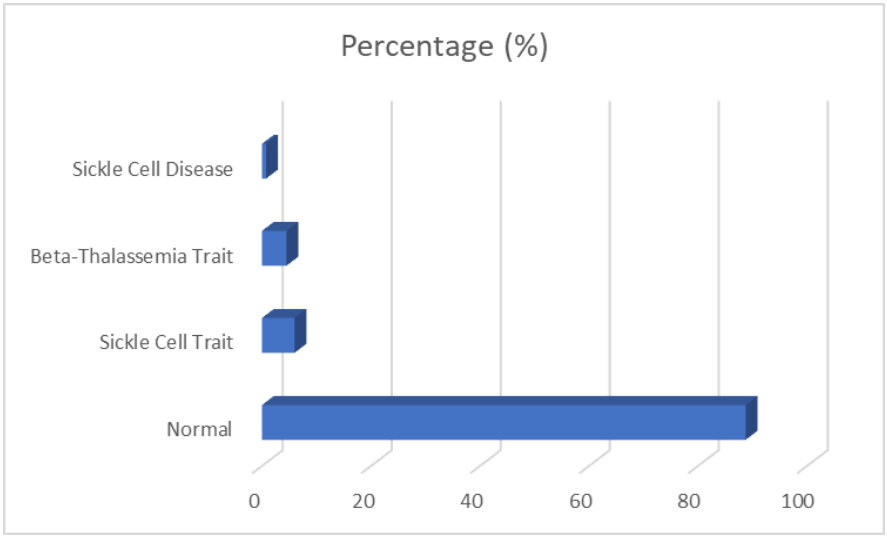

Figure 2: Spectrum of Hemoglobinopathies Detected [N=400]

HPLC analysis showed a normal hemoglobin pattern [HbAA] in 355 participants [88.75%].

Hemoglobinopathies were identified in 45 women, giving an overall frequency of 11.25% in the study population.

Sickle cell trait [HbAS] was the most commonly detected hemoglobinopathy and was observed in 24 participants [6.0%]. Beta-thalassemia trait was identified in 18 women [4.5%], making it the second most common abnormality detected on HPLC analysis.

Sickle cell disease [HbSS] was diagnosed in 3 participants [0.75%]. These cases are clinically important because such patients may require closer monitoring during pregnancy due to increased maternal and fetal risks.

The higher frequency of sickle cell trait observed in the study is in keeping with the known prevalence of hemoglobinopathies in the central Indian sickle cell belt.

Comparison of Hematological Parameters:
The findings are shown in Table 3.

Table 3: Comparison of Mean Hematological Parameters Across Different Groups

Parameter	Normal [n=355]	Beta-Thalassemia Trait [n=18]	Sickle Cell Trait [n=24]	Sickle Cell Disease [n=3]	p-value*
Hemoglobin [g/dL]	11.2 ± 1.5	9.8 ± 0.9	10.1 ± 1.1	7.8 ± 1.3	<0.001
MCV [fL]	83.5 ± 6.8	64.2 ± 5.1	81.7 ± 7.2	86.5 ± 7.5	<0.001
MCH [pg]	28.1 ± 2.4	21.5 ± 1.9	27.5 ± 2.6	28.5 ± 3.0	<0.001
HbA2 [%]	2.6 ± 0.4	5.1 ± 0.6	2.8 ± 0.5	2.3 ± 0.4	<0.001
HbS [%]	0	0	36.8 ± 4.2	91.0 ± 4.0	NA

As shown in Table 3, women with beta-thalassemia trait [BTT] had lower mean hemoglobin, MCV, and MCH values compared to participants with normal hemoglobin patterns, and the difference was statistically significant [p <0.001]. The mean HbA2 level in the BTT group was elevated [5.1 ± 0.6%], which supported the diagnosis of beta-thalassemia trait.

Participants with sickle cell disease [SCD] showed the lowest mean hemoglobin level [7.8 ± 1.3 g/dL], indicating comparatively more severe anemia. The mean HbS percentage was 36.8 ± 4.2% in sickle cell trait [SCT] cases and was markedly higher in sickle cell disease cases [91.0 ± 4.0%].

Overall, significant differences were observed in hematological parameters among the study groups [p <0.001]. The findings show that CBC parameters can help with initial screening, while HPLC is important for confirming and understanding hemoglobin disorders.

DISCUSSION

The present study showed that both nutritional anemia and inherited hemoglobinopathies were commonly seen among antenatal women attending our tertiary care centre in the East Nimar region of Madhya Pradesh. The coexistence of these conditions creates an additional challenge during antenatal management, particularly in regions where hemoglobin disorders are already known to be prevalent.

Anemia was observed in 61.5% of the study population. This was slightly higher than the prevalence reported in the NFHS-5 survey for Madhya Pradesh, where anemia was reported in 52.2% of pregnant women [11]. Since our study was conducted at a tertiary care hospital, the higher prevalence may be related to referral of comparatively high-risk patients. Nutritional deficiencies, repeated pregnancies, and socioeconomic factors may also have contributed to the increased burden.

The prevalence observed in our study was close to the findings of Yadav *et al.,*[12], who also reported anemia in more than half of antenatal women. In comparison, Unadkat *et al.,*[13] documented a much higher prevalence of 91.5%. Differences between studies may be due to variation in study population, nutritional status, healthcare accessibility, and regional dietary habits.

Mild anemia was the most frequently observed category in our study, accounting for 35.0% of cases, followed by moderate anemia [22.75%] and severe anemia [3.75%]. Similar findings have been described in other Indian antenatal studies [14,15]. Identification of mild anemia remains important because many patients can be managed effectively with dietary advice, iron supplementation, and regular follow-up during pregnancy.

A notable feature of the present study was that all 400 antenatal women underwent High Performance Liquid Chromatography [HPLC] testing. This allowed detailed evaluation of inherited hemoglobin disorders in the study population. Hemoglobinopathies were detected in 11.25% of participants, suggesting that inherited disorders contribute substantially to the overall anemia burden in this region.

Sickle cell trait was the most commonly detected hemoglobinopathy and was identified in 6.0% of cases. This finding is expected because Madhya Pradesh lies within the "sickle cell belt" of India [16]. The prevalence observed in our study was higher than that reported by Agarwal *et al.,*[17], who found sickle cell trait in 3.5% of antenatal women. The higher frequency in our study may be related to the greater proportion of tribal populations in the East Nimar region, where the sickle cell gene is more prevalent.

Sickle cell disease was identified in three women [0.75%]. Although the number of cases was small, these patients are clinically important because pregnancy in sickle cell disease is associated with increased maternal and fetal complications. Similar observations have been reported in studies from other parts of central India [18,19], supporting the importance of early diagnosis and close antenatal monitoring in such patients.

Beta-thalassemia trait was detected in 4.5% of antenatal women in the present study. This finding was comparable to the prevalence reported by Bhukhanvala *et al.,* [20], supporting the continued presence of beta-thalassemia carriers in Indian antenatal populations. However, Unadkat *et al.,*[13] reported a higher prevalence of 9%, which may be due to regional and ethnic variation in carrier frequency.

Women with beta-thalassemia trait in our study showed significantly lower hemoglobin levels along with marked microcytosis and hypochromia, as reflected by low MCV and MCH values. Similar hematological findings were described by Unadkat *et al.,*[13], who also reported low MCV and MCH values in beta-thalassemia trait cases. Such findings continue to be useful indicators for suspecting beta-thalassemia trait during routine CBC analysis.

The mean HbA2 level in beta-thalassemia trait cases was significantly elevated in our study and remained consistent with standard diagnostic criteria and previously published literature [21], where HbA2 levels above 3.5% are considered diagnostic of beta-thalassemia trait. This supports the usefulness of HPLC in confirming beta-thalassemia carriers.

Although CBC parameters such as low MCV and low MCH may provide important clues for hemoglobinopathy screening, overlap with iron deficiency anemia can make interpretation difficult, especially during pregnancy where nutritional anemia is common. Therefore, CBC findings alone may not always reliably differentiate inherited hemoglobin disorders from nutritional causes of anemia.

The present study also demonstrated the usefulness of HPLC in accurately identifying and characterizing hemoglobin variants. Detection of hemoglobinopathies during pregnancy has important implications because it allows early counselling, partner screening, prenatal diagnosis, and assessment of fetal risk for severe inherited disorders [10].

Overall, our findings suggest that antenatal screening strategies in regions with high prevalence of anemia and hemoglobinopathies should not rely only on routine hemoglobin estimation. A combined approach using CBC screening along with HPLC in high-risk or suspected cases may help in early diagnosis, better antenatal care, and prevention of severe hemoglobin disorders in future generations.

Limitations

The present study was conducted at a single tertiary care centre, which may limit the generalizability of the findings to the wider population, particularly rural and tribal communities. Since this was a hospital-based study, referral bias may have contributed to overestimation of the burden of anemia and hemoglobinopathies. In addition, rare co-inheritance patterns and uncommon hemoglobin variants may not have been completely identified by routine HPLC interpretation alone.

CONCLUSION

The present study showed a high prevalence of both anemia and hemoglobinopathies among antenatal women in the East Nimar region of Madhya Pradesh. Mild and moderate anemia formed the major proportion of cases, while sickle cell trait and beta-thalassemia trait were the most commonly detected hemoglobinopathies. Early pregnancy screenings are important for quick diagnosis and proper care. CBC is useful for initial checks, and HPLC is key for confirming blood disorders. In places where inherited blood disorders are common, targeted screening and advice can improve health for mothers and babies and reduce severe genetic issues in future generations.

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