Section: Biochemistry

Original Research Article

ANTENATAL DETECTION OF HEMOGLOBINOPATHIES USING RED BLOOD CELL INDICES AS A SCREENING TOOL

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ABSTRACT

Background: Hemoglobinopathies are common inherited disorders in India and contribute significantly to anemia burden. Carrier states such as β-thalassemia trait and sickle cell trait are often clinically silent and may be missed during pregnancy unless appropriate screening and confirmatory testing are performed. Red blood cell indices can serve as an initial low-cost screening tool, while hemoglobin electrophoresis provides biochemical confirmation through hemoglobin fraction profiling. The aim is to detect hemoglobinopathy carrier states in antenatal women using red blood cell indices as a screening tool followed by biochemical confirmation through hemoglobin electrophoresis.

Materials and Methods: This hospital-based observational study was conducted over two months (1st August to 30th September 2016) at a tertiary care center. A total of 80 antenatal women aged 20–40 years with microcytic hypochromic anemia (MCV ≤ 80 fL and MCH ≤ 27 pg) were included. Complete blood count was performed using a Sysmex KX-21 automated hematology analyzer. Hemoglobin electrophoresis was performed using Helena SAS-MX. HbA2 > 3.5% was considered diagnostic of β-thalassemia trait, and the presence of both HbA and HbS bands was considered indicative of sickle cell trait.

Results: The mean age of participants was 24 ± 4 years and the mean hemoglobin was 9.08 ± 1.7 g/dL. Severe anemia (Hb < 7 g/dL) was observed in 7.5% of women, moderate anemia (Hb 7–9.9 g/dL) in 53.75%, and mild anemia (Hb 10–10.9 g/dL) in 22.5%. Hemoglobin electrophoresis detected β-thalassemia trait in 6.25% (5/80) women based on elevated HbA2 (>3.5%). Sickle cell trait was detected in 3.75% (3/80) women by the presence of HbA and HbS bands. Overall, hemoglobinopathy carrier states were confirmed in 10% (8/80) of screened antenatal women.

Conclusion: Antenatal screening using red blood cell indices followed by hemoglobin electrophoresis is a practical and cost-effective strategy for detecting hemoglobinopathy carriers. Biochemical identification of Hb variants and HbA2 elevation enables early counseling, spouse screening, and preventive planning to reduce the burden of severe hemoglobinopathy births.

Keywords: Hemoglobinopathy; β-thalassemia trait; Sickle cell trait; HbA2; Hemoglobin electrophoresis; Antenatal screening; Microcytic hypochromic anemia; Red blood cell indices.

INTRODUCTION

Hemoglobinopathies are among the most common inherited genetic disorders of hemoglobin worldwide and are responsible for a significant proportion of severe anemia cases in many regions. These disorders are inherited in an autosomal recessive pattern and include quantitative defects in globin chain synthesis

(thalassemias) as well as qualitative structural hemoglobin variants such as sickle hemoglobin (HbS). The clinical manifestations range from asymptomatic carrier states to severe transfusion-dependent conditions, with significant morbidity and mortality, especially in low- and middle-income countries.

Globally, hemoglobinopathies and other hereditary hemolytic anemias affect an estimated billions of people, with the World Health Organization classifying sickle cell disease (SCD) and thalassemia among the most serious public health concerns due to their prevalence, long-term complications, and burden on healthcare systems. According to the Global Burden of Disease study, the number of individuals living with SCD worldwide increased to approximately 7.7 million in 2021, and global births affected by SCD have risen significantly, underscoring the impact of population growth and migration on hemoglobinopathy distribution (Global Burden of Disease Collaborative Network, 2025).[1] Systematic analyses suggest that the prevalence of sickle cell trait (SCT) globally is around 5.9%, with sickle cell disease affecting approximately 1.17% of the population in varied study settings (Rao et al., 2024).[2]

India lies within the so-called "thalassemia belt" and carries a disproportionately high burden of hemoglobinopathy carriers due to its large population and diverse ethnic groups. The average frequency of β-thalassemia carriers in India has been reported at 3–4%, while sickle cell allele prevalence ranges widely from 0% to over 40% in specific tribal and regional populations (Prenatal hemoglobinopathy screening & prevention in India study, 2025).[3] Population-based surveys have documented SCT prevalence of approximately 9.87% in tribal communities in Rajasthan (Purohit et al., 2025),[4] and large screening initiatives, such as a state-wide campaign in Maharashtra, identified over 82,000 carriers and nearly 9,700 new sickle cell disease cases, reinforcing the high local burden and clustering of these conditions (The Times of India, Pune report, 2026).[5] Such regional heterogeneity reflects the complex interplay of genetic, socio-cultural, and historical factors that influence gene distribution across Indian populations.

Pregnancy presents a crucial opportunity for hemoglobinopathy screening, as antenatal women are routinely evaluated with hematological investigations and are often motivated to engage with preventive health services. Early identification of carrier status enables spouse screening, genetic counseling, and referral for prenatal diagnosis in at-risk couples, thereby reducing the likelihood of births affected by severe forms such as thalassemia major and sickle cell disease. Simple red cell indices like mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) serve as cost-effective preliminary screening tools for microcytic hypochromic anemia, which, when coupled with biochemical confirmatory tests such as hemoglobin electrophoresis or high-performance liquid chromatography (HPLC), facilitates accurate hemoglobinopathy detection in resource-limited settings.

AIM

To detect hemoglobinopathy carrier states in antenatal women using red blood cell indices as a screening tool followed by biochemical confirmation through hemoglobin electrophoresis.

Objectives

1. To screen antenatal women with microcytic hypochromic indices (MCV ≤ 80 fL and MCH ≤ 27 pg) for suspected hemoglobinopathies.
2. To identify and classify hemoglobin variants using hemoglobin electrophoresis, with emphasis on HbA2 elevation for β-thalassemia trait and HbS detection for sickle cell trait.

MATERIALS AND METHODS

Study Design and Setting: This hospital-based observational study was conducted at a tertiary care hospital in Telangana over a period of one year, from December 2024 to December 2025.

Selection of Subjects: Pregnant women attending the obstetric outpatient department (OPD) for antenatal care were evaluated for eligibility, particularly those with:

- History of unexplained anemia, and/or
- Family history suggestive of anemia or hemoglobinopathy.

Inclusion Criteria

Antenatal women aged 20–40 years, irrespective of trimester, with no known medical disorder were included. A total of 80 antenatal women with:

- Microcytic hypochromic picture on peripheral smear, and
- CBC showing MCV ≤ 80 fL and MCH ≤ 27 pg, were enrolled for further evaluation.

Exclusion Criteria

- Subjects already diagnosed with hemoglobinopathy or screened previously
- Subjects with confirmed iron deficiency anemia.

Ethical Clearance and Consent

Institutional Ethical Committee approval was obtained. Written informed consent was taken from all participants prior to sample collection.

Laboratory Investigations

Complete Blood Count

CBC was performed using a Sysmex automated hematology analyzer (KX-21). RBC indices including MCV and MCH were recorded.

Hemoglobin Variant Analysis

Hemoglobin electrophoresis was performed using a semi-automated Helena SAS-MX system. Hemolysate was prepared from EDTA blood samples and used for electrophoretic separation.

Normal adult hemoglobin fractions include:

- HbA: 95–98%
- HbA2: 2–3%
- HbF: up to 2%

HbA2 > 3.5% was taken as the cut-off for diagnosing β-thalassemia trait. Presence of HbA and HbS bands was considered indicative of sickle cell trait.

Biochemical Screening Strategy

Although red blood cell indices were used as the initial selection step, the primary screening endpoint of the study was the biochemical detection of hemoglobin variants using hemoglobin fraction analysis.

Laboratory Investigations

1. Complete Blood Count (CBC)

CBC was performed using an automated hematology analyzer (Sysmex KX-21). Hemoglobin, RBC count, and red cell indices (MCV, MCH) were recorded.

2. Hemoglobin Variant Analysis

Hemoglobin electrophoresis was performed using a semi-automated electrophoresis system (Helena SAS-MX).

Sample Collection

- Venous blood samples were collected in EDTA vacutainers.

Preparation of Hemolysate

Hemolysate was prepared from EDTA blood samples according to standard laboratory protocol for hemoglobin fraction separation.

Principle

Hemoglobin electrophoresis separates hemoglobin fractions based on:

- Net electrical charge,
- Structural amino acid substitutions, and
- Molecular mobility under an electric field.

This enables biochemical identification of common hemoglobin variants and carrier states.

Hemoglobin Fractions and Diagnostic Interpretation

Normal adult hemoglobin fractions include:

- HbA ($\alpha_2\beta_2$): 95–98%
- HbA2 ($\alpha_2\delta_2$): 2–3%
- HbF ($\alpha_2\gamma_2$): up to 2%

Diagnostic Cut-off Used

- HbA2 > 3.5% was considered diagnostic of β-thalassemia trait.

Identification of Sickle Cell Trait

Presence of both:

- HbA band, and
- HbS band was considered indicative of sickle cell trait.

RESULTS

A total of 80 antenatal women fulfilling the inclusion criteria of microcytic hypochromic anemia were enrolled in the study. All participants demonstrated microcytosis and hypochromia on hematological screening, defined by MCV ≤ 80 fL and MCH ≤ 27 pg, along with supportive peripheral smear findings.

Table 1: Baseline Hematological Characteristics of Study Population (N = 80)

Parameter	Mean ± SD
Age (years)	24 ± 4
Hemoglobin (g/dL)	9.08 ± 1.7
MCV (fL)	69.23 ± 7.55
MCH (pg)	20.90 ± 3.49
RBC Count (million/mm³)	4.3 ± 1.6

Table 2: Distribution of Anemia Severity Based on Hemoglobin Levels

Anemia Category	Hemoglobin Range (g/dL)	Number (n)	Percentage (%)
Severe Anemia	< 7	6	7.5%
Moderate Anemia	7 – 9.9	43	53.75%
Mild Anemia	10 – 10.9	18	22.5%
Hb ≥ 11 g/dL (Microcytic indices)	≥ 11	13	16.25%
Total		80	100%

Table 3: Hemoglobin Variant Detection by Electrophoresis

Electrophoresis Finding	Number (n)	Percentage (%)
β-thalassemia trait (HbA2 > 3.5%)	5	6.25%
Sickle Cell Trait (HbA + HbS)	3	3.75%
Normal Hemoglobin Pattern	72	90%
Total	80	100%

Table 4: Comparative Hematological Profile of Hemoglobin Variant Groups

Parameter	β-Thalassemia Trait (n=5) Mean ± SD	Sickle Cell Trait (n=3) Mean ± SD
Hemoglobin (g/dL)	8.8 ± 3.6	7.8 ± 4.4
MCV (fL)	60.2 ± 9.9	64.7 ± 21
MCH (pg)	17.0 ± 1.5	19.6 ± 8.8
RBC Count (million/mm³)	5.0 ± 0.9	3.9 ± 1.0

The mean age of the study population was 24 ± 4 years. The overall mean hemoglobin concentration was 9.08 ± 1.7 g/dL, indicating that the majority of the study population belonged to the anemic range. The mean MCV and MCH were 69.23 ± 7.55 fL and 20.90 ± 3.49 pg, respectively, confirming significant

microcytosis and reduced hemoglobinization of red blood cells. The mean RBC count was 4.3 ± 1.6 million/mm³ [Table 1].

Distribution of Anemia Severity: On categorizing anemia severity based on hemoglobin levels, 6 (7.5%) women were found to have severe anemia (Hb < 7 g/dL). A majority of participants, 43 (53.75%), had moderate anemia (Hb 7–9.9 g/dL). Mild anemia (Hb 10–10.9 g/dL) was observed in 18 (22.5%) women. Additionally, 13 (16.25%) women had hemoglobin levels ≥ 11 g/dL despite microcytic indices, suggesting the presence of early or compensated microcytosis in this subgroup [Table 2, Figure 2]. Overall, moderate anemia represented the most common clinical category in the study population.

Hemoglobin Variant Detection by Electrophoresis

Hemoglobin electrophoresis was performed as the confirmatory biochemical test to identify hemoglobin variants and carrier states. Among the 80 women evaluated, 5 (6.25%) showed elevated HbA2 fractions (> 3.5%), which is diagnostic of β-thalassemia trait [Table 3, Figure 1]. This biochemical finding reflects reduced β-globin chain synthesis with compensatory increase in δ-globin production, resulting in an increased HbA2 fraction.

Further, 3 (3.75%) women demonstrated the presence of both HbA and HbS bands on electrophoresis, consistent with sickle cell trait [Table 3, Figure 1]. This electrophoretic pattern reflects heterozygosity for the structurally abnormal β-globin chain (HbS). The remaining 72 women (90%) showed normal adult hemoglobin fractions without detectable abnormal hemoglobin variants.

Thus, hemoglobin electrophoresis confirmed hemoglobinopathy carrier status in 10% (8/80) of the screened antenatal women, emphasizing the diagnostic value of hemoglobin fraction profiling as a biochemical confirmatory tool in antenatal screening.

Comparative Hematological Pattern in Variant Groups

The hematological parameters of women with β-thalassemia trait and sickle cell trait showed characteristic differences (Table 4). Women with β-thalassemia trait demonstrated a pattern of more pronounced microcytosis and hypochromia with relatively higher RBC counts, consistent with the typical carrier phenotype. In contrast, women with sickle cell trait showed comparatively higher MCV and MCH values than the β-thalassemia trait group, while their hemoglobin and RBC counts tended to be lower.

The mean hemoglobin level in women with β-thalassemia trait was 8.8 ± 3.6 g/dL, while in those with sickle cell trait it was 7.8 ± 4.4 g/dL. The mean MCV values were 60.2 ± 9.9 fL in β-thalassemia trait and 64.7 ± 21 fL in sickle cell trait. The mean MCH values were 17.0 ± 1.5 pg and 19.6 ± 8.8 pg, respectively. The mean RBC count was 5.0 ± 0.9 million/mm³ in β-thalassemia trait and 3.9 ± 1.0 million/mm³ in sickle cell trait.

Summary of Screening Outcome

Overall, the study demonstrates that among antenatal women presenting with microcytic hypochromic indices, a significant subset harbors clinically silent hemoglobinopathy carrier states. The screening strategy used in this study—initial hematological selection followed by biochemical hemoglobin fraction analysis—enabled detection of β-thalassemia trait and sickle cell trait, thereby facilitating early identification of carrier couples and enabling appropriate genetic counseling and spouse screening.

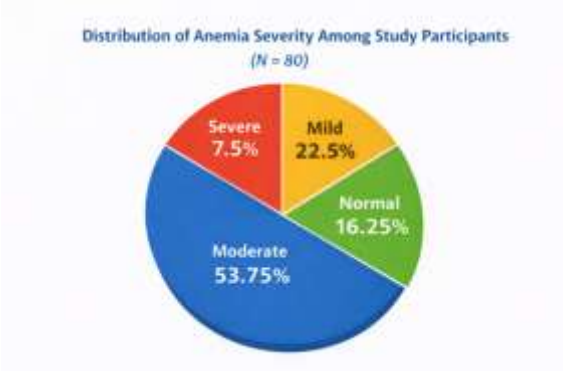

Figure 1: Distribution of Anemia Severity among study participants (N=80)

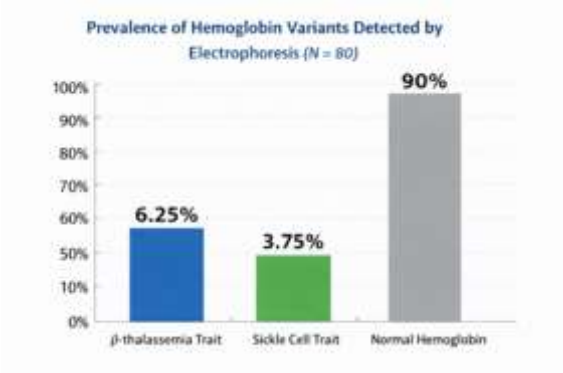

Figure. 2: Prevalence of Hemoglobin Variants Detected by Electrophoresis (N=80)

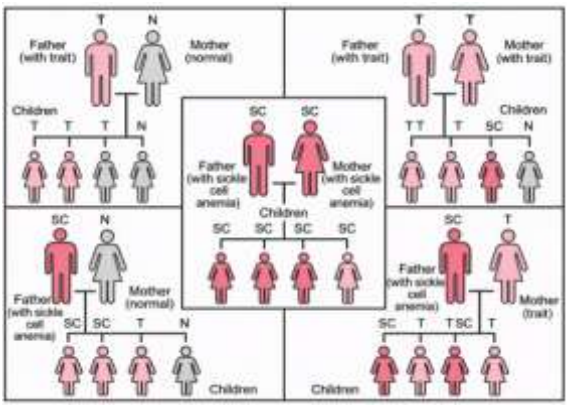

Figure 3: Pattern of inheritance for sickle cell anemia

DISCUSSION

Hemoglobinopathies are autosomal recessive inherited disorders and represent a major public health burden in India. These disorders include quantitative defects in globin chain synthesis (thalassemias) and qualitative structural hemoglobin variants such as HbS. In β-thalassemia trait, mutations in the β-globin gene reduce β-globin chain synthesis, leading to decreased HbA (α2β2). This biochemical imbalance is compensated by increased synthesis of δ-globin and γ-globin chains, resulting in an increase in HbA2 (α2δ2) and, in some cases, a mild rise in HbF. Therefore, HbA2 quantification is the most important biochemical marker for identifying heterozygous β-thalassemia trait, which may remain clinically silent in most carriers (National Health Mission, 2016).[12]

In the present study, antenatal screening using hematological triage followed by hemoglobin electrophoresis confirmed hemoglobinopathy carrier states in a significant proportion of cases. Among 80 antenatal women with microcytosis and hypochromia, β-thalassemia trait was detected in 6.25% (5/80) based on HbA2 > 3.5%, while sickle cell trait was detected in 3.75% (3/80) based on the presence of HbA and HbS bands on electrophoresis. Thus, hemoglobin fraction profiling confirmed carrier status in 10% (8/80) of the screened antenatal women. These findings highlight that a clinically silent carrier state can be detected during routine antenatal care when biochemical hemoglobin fraction analysis is incorporated.

The prevalence of β-thalassemia trait observed in our study (6.25%) is comparable with several Indian antenatal screening studies reporting clinically relevant carrier frequency. A tertiary care antenatal screening study reported β-thalassemia trait prevalence of approximately 2.78%, and emphasized the importance of partner testing and prenatal diagnostic referral in at-risk couples (Baxi et al., 2012).[13] Another antenatal screening study using NESTROFT-based triage reported a higher prevalence of β-thalassemia trait around 8.5%, demonstrating that prevalence varies with the screening tool, population structure, and geographic clustering (Kulkarni et al., 2013).[14] These differences are supported by broader evidence, where Indian prevalence studies show marked heterogeneity due to regional and community-based variation and differences in diagnostic methodology (Sumedha et al., 2023).[18] Therefore, the carrier prevalence observed in our cohort falls well within the nationally documented range and supports the continued relevance of antenatal screening.

The sickle cell trait prevalence in our study was 3.75% (3/80). This finding is clinically relevant because sickle cell trait is often undetected unless hemoglobin variant testing is performed. The biochemical basis of sickle cell disease involves a structural mutation in the β-globin gene (β6 Glu→Val), producing HbS, which can be detected as a distinct electrophoretic band. Detection of HbS carriers during pregnancy is therefore an important public health opportunity for genetic counseling and spouse screening, particularly in populations where hemoglobinopathy traits are prevalent (National Health Mission, 2016).[12]

A major strength of the approach used in this study is the combination of low-cost hematological triage with confirmatory biochemical hemoglobin fraction analysis. RBC indices such as MCV and MCH are useful screening tools because β-thalassemia trait typically produces microcytosis and hypochromia. However, indices alone cannot provide definitive diagnosis, as microcytosis is also common in iron deficiency anemia and other microcytic disorders. Several studies have shown that RBC indices and derived discrimination formulas can help enrich a suspected carrier pool, but confirmatory testing remains mandatory (Parthasarathy et al., 2012).[15] In this context, hemoglobin electrophoresis and HPLC are critical diagnostic tools. HPLC is widely regarded as superior for HbA2 quantification and for identifying additional hemoglobin variants with greater analytical precision (Khera et al., 2014).[16] Recent comparative evidence also supports that capillary electrophoresis and HPLC provide robust diagnostic accuracy, particularly in borderline HbA2 ranges (Kaur et al., 2023).[19]

The genetic counseling implications of carrier detection are significant. If one parent is a carrier for β-thalassemia and the other is normal, there is a 50% chance of the child inheriting the carrier state in each pregnancy. If both parents are carriers, there is a 25% chance of a child being affected with thalassemia major and a 50% chance of being a carrier. Similar Mendelian inheritance applies to sickle cell disease. Therefore, detection of hemoglobinopathy traits during antenatal screening must be followed by spouse screening, as recommended in national guidelines (National Health Mission, 2016).[12] The inheritance pattern diagram (Figure 4) provides a practical counseling tool to explain risk to families and encourage partner testing.

From a public health perspective, antenatal screening is a highly feasible and impactful strategy in India. While premarital screening may be ideal, it is not universally acceptable or implementable across all communities. The antenatal period is a time when families are receptive to health education, and preventive counseling is more likely to be accepted. National guidance emphasizes that early detection, preferably during the first trimester, enables timely spouse testing and referral for prenatal diagnosis in at-risk couples (National Health Mission, 2016).[12] Several states have initiated hemoglobinopathy control programs, and the integration of antenatal screening into routine maternal health services remains essential for long-term disease burden reduction.

Limitations of the study

The study had certain limitations. First, the sample size was relatively small (N=80), which limits generalizability. Second, hemoglobin electrophoresis was used as the confirmatory method; however,

HPLC provides superior quantification of HbA2 and improved detection of additional variants, and would strengthen diagnostic certainty, especially in borderline HbA2 ranges . Third, molecular confirmation by DNA analysis was not performed. DNA testing would be ideal for confirming the exact mutation in β-thalassemia trait, detecting α-thalassemia, and resolving complex cases . Finally, in routine antenatal practice, iron deficiency and hemoglobinopathy traits may coexist, and ferritin-based biochemical stratification would improve interpretation.

CONCLUSION

Hemoglobinopathies are preventable inherited disorders with significant national health impact. In this study, hemoglobin fraction analysis identified β-thalassemia trait in 6.25% (5/80) and sickle cell trait in 3.75% (3/80) of antenatal women selected by microcytic hypochromic indices, confirming carrier status in 10% (8/80). A practical and cost-effective screening strategy is to use CBC and RBC indices for initial triage, followed by hemoglobin electrophoresis or preferably HPLC for biochemical confirmation. Early antenatal detection enables spouse screening, genetic counseling, and preventive planning, thereby contributing to control and reduction of severe hemoglobinopathy births.

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