ORIGINAL ARTICLE

Assessment of Hemoglobinopathies in Antenatal Females

Charusheela Gore[1], Mallika Agarwal[2*], Parag Ratnakar[3], Hemant Deshpande[4], Archana Buch[5], Maharshi Dixit[6]

Received: 24 March 2025; *Accepted:* 09 June 2025

Abstract

Introduction: Hemoglobinopathies, a group of inherited disorders characterized by abnormal hemoglobin (Hb) production or structure, pose significant health risks during pregnancy. This study aims to assess the prevalence of hemoglobinopathies among antenatal females and establish guidelines for effective screening and management.

Materials and methods: An observational cross-sectional study was conducted over 18 months at Dr DY Patil Medical College, Hospital and Research Centre, involving 800 antenatal women. Demographic data, medical history, and blood samples were collected for complete blood count (CBC) and Hb electrophoresis. Statistical analysis was performed using SPSS software.

Results: A study evaluated 800 antenatal females aged 20–39 years, with an almost equal distribution between age-groups 20–29 (49.23%) and 30–39 (50.77%) years. Peripheral blood smear analysis revealed 97.69% had microcytic hypochromic anemia, indicating a high prevalence of iron deficiency, while 2.31% exhibited normocytic normochromic anemia. Hb electrophoresis identified hemoglobinopathies in 1.72% of cases, with 1.53% cases identified as beta-thalassemia carriers, and 0.19% with sickle cell trait (SCT). Among the abnormal cases, beta-thalassemia (55.56%) was found to be the most common, followed by HbE heterozygous (11.11%), HbE homozygous (11.11%), and double heterozygous (11.11%), with a single case (11.11%) of sickle cell disease (SCD). Beta-thalassemia was the most prevalent hemoglobinopathy. CBC parameters showed significant variations among hemoglobinopathy types, with analysis of variance (ANOVA) *p*-values of 0.0001 for Hb, mean corpuscular volume (MCV), and mean corpuscular Hb. These findings underscore the significance of microcytic hypochromic anemia and the relatively low prevalence of hemoglobinopathies in the antenatal population.

Discussion: The low prevalence of hemoglobinopathies in this region contrasts with higher rates reported elsewhere in India, indicating potential regional genetic factors. The predominant finding of microcytic hypochromic anemia underscores the urgent need for targeted interventions addressing iron deficiency in antenatal care.

Conclusion: This study emphasizes the importance of routine screening for hemoglobinopathies in pregnant women, particularly in regions with known genetic predispositions. Increased awareness and follow-up molecular analysis are recommended for accurate diagnosis and management, ultimately improving maternal and fetal health outcomes. Future research should expand to larger, multicentric studies to further validate these findings.

Journal of The Association of Physicians of India (2025): 10.59556/japi.73.1193

Introduction

Hemoglobinopathies constitute a heterogeneous group of single-gene disorders that are inherited. They are characterized by either abnormal production or structure of the hemoglobin (Hb) molecule. The abnormality may be quantitative or qualitative.

Qualitative defects can occur due to genetic mutations that involve globin protein chains. These defects include either amino acid deletions or substitutions, which cause structural variations of the globin chain that manifest in the form of HbS, HbD, HbE, etc.[1]

Quantitative defects can lead to a decrease in the synthesis of structurally normal globin chains.

These include disorders such as:

Thalassemia, which is a genetically heterogeneous autosomal recessive disorder of Hb synthesis caused by germline mutations, is characterized by the absence or decreased synthesis of alpha or beta globin chains of Hb. Thus, it leads to anemia, tissue hypoxia, and red cell hemolysis connected to an imbalance in globin chain synthesis.[2]

Alpha-thalassemia is characterized by deficient synthesis of alpha globin chains. Beta-thalassemia is caused by a deficient synthesis of beta globin chains. Two alpha chains in HbA are encoded by an identical pair of alpha globin genes present on chromosome 16. Beta chains are encoded by a single globin gene on chromosome 11.

World literature states that heterozygous carriers of hereditary disorders of Hb are >270 million. Among them, at least 3,00,000 affected homozygotes or compound heterozygotes are born each year.[3] The purpose of this document is to review the most common hemoglobinopathies and to formulate policies and guidelines for screening and clinical management of hemoglobinopathies during pregnancy. Alpha-thalassemia is distributed in Southeast Asia, the Eastern Mediterranean region, and the Middle East.[4] Beta-thalassemia is distributed in the Mediterranean region, Africa, the Middle East, India, Pakistan, and Southeast Asia.[5]

Sickle cell disease (SCD) and thalassemia are the common hemoglobinopathies caused by mutations in genes for Hb, resulting in significant morbidity and mortality. There is usually a great disparity in the outcome of these diseases between resource-rich and resource-poor nations.

The aim of this study was to assess the frequency and prevalence of hemoglobinopathies in antenatal mothers in Western India.

The objective was to evaluate the hematological parameters and Hb electrophoresis in different hemoglobinopathies and to study the demographic profile of antenatal females with hemoglobinopathies.

Materials and Methods

An observational cross-sectional analytical study was performed in the Department of Pathology in collaboration with the Department of Obstetrics and Gynecology, Dr DY Patil Medical College, Hospital and Research Centre, Pimpri, Pune, for a period of 18 months. An Institutional Ethics Committee Clearance (IECC) was obtained before the start of the study.

Informed and written consent was taken from the antenatal females who were included in the study. The study included 800 antenatal females as cases. Antenatal females during their first visit to the antenatal OPD, irrespective of their gestational age, in the Department of

[1]Professor and Head; [2]Resident; [3]Lab Director, Department of Pathology; [4]Professor and Head of Department, Department of Obstetrics and Gynecology; [5]Professor; [6]Consultant, Department of Pathology, Dr DY Patil Medical College, Hospital and Research Centre, Dr DY Patil Vidyapeeth, Pune, Maharashtra, India; *Corresponding Author

How to cite this article: Gore C, Agarwal M, Ratnakar P, *et al.* Assessment of Hemoglobinopathies in Antenatal Females. J Assoc Physicians India 2025;73(10):72–75.

Obstetrics and Gynecology, were included as cases. Patients with a previous history of blood transfusion were excluded from the study for a year. Relevant history was taken regarding their demographic profile, past, and family history. A 3 mL blood sample of antenatal females was collected in vacuum collection tubes containing ethylenediaminetetraacetic acid (EDTA), and 2 mL of blood sample was collected in a plain vial.

Complete blood cell count was done by a five-part cell counter. Peripheral blood smears were prepared and analyzed. EDTA samples were stored at 2–8°C for 4 days. They were allowed to reach room temperature prior to analysis.

Automated cation-exchange high-performance liquid chromatography (HPLC) was used for the detection of hemoglobinopathies. About 2 mL of blood sample, which was collected in plain vials, was used for the HPLC machine: D-10 Dual HbA2/F/A1c Program 220-0201, manufacturer: Bio-Rad Laboratories, model: D-10 Hemoglobin Testing System. The principle of the procedure is based on chromatographic separation of the analytes by ion-exchange HPLC. The data obtained is displayed as a chromatogram and converted into peaks as per the retention time. The separated Hb with the percentage is displayed.

Data were collected and entered into Microsoft Excel and were analyzed using SPSS software version 26. Quantitative data were expressed in mean and standard deviation. Categorical variables were expressed in numbers and percentages. An independent *t*-test was applied to compare means. A *p*-value < 0.05 was considered statistically significant. Antenatal females were called and counselled for further screening of their spouse for prevention and emphasizing the need for early detection and screening of their offspring for hemoglobinopathies.

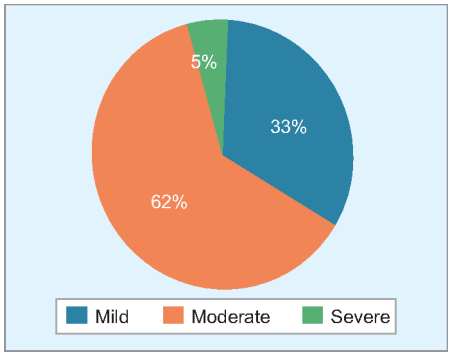

Fig. 1: Distribution of cases according to anemia grading

Results

A total of 800 antenatal females were screened. The females with an age range between 20 and 29 years were 394 (49.21 %), whereas those between 30 and 39 years were 406 (50.77%). Thus, showing a nearly equal distribution among the two age-groups. Among the total 800 cases, 520 (65%) were diagnosed with anemia, while 280 (35%) showed normal Hb levels.

In terms of anemia grading, the majority of cases fell into the moderate anemia category (Hb levels between 7.1 and 9.9 gm/dL), with 323 cases making up 62% of the total. Mild anemia (Hb levels between 10 and 10.9 gm/dL) was observed in 172 (33%) cases. Severe anemia (Hb levels of 7 gm/dL or lower) was noted in 25 cases, representing 4.80% of the total, as shown in Figure 1.

Table 1: Distribution of cases according to findings on Hb electrophoresis

Hb electrophoresis	*Number*	*Percentage (%)*
SCT	01	0.19
Normal	511	98.26
Thalassemia	08	1.53
Total	520	12.50

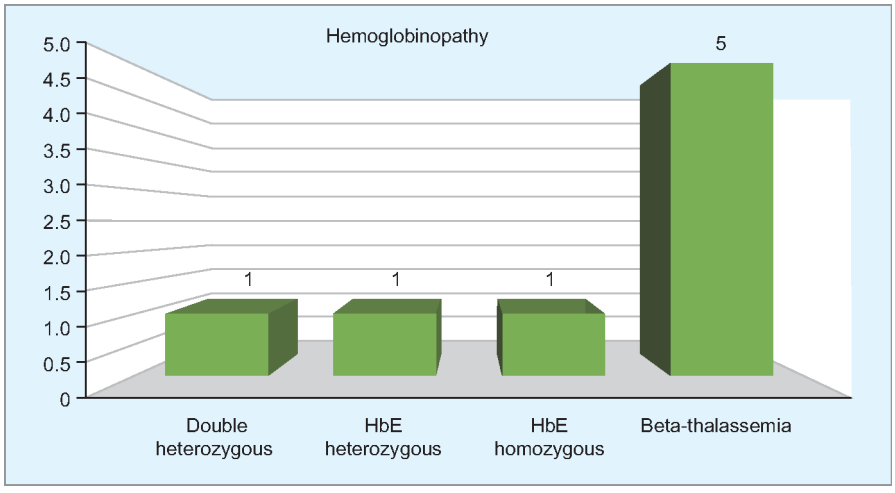

Fig. 2: Distribution of thalassemia and variants on Hb electrophoresis

On evaluating the peripheral blood smears, 508 (97.69%) antenatal females had microcytic hypochromic anemia, and 12 (2.31%) had normocytic normochromic anemia. This significant predominance of microcytic hypochromic findings underscores the commonality of iron deficiency in the study population.

The obstetric history of the study subjects showed G2P1L1 as the most common category, representing 188 cases (36.15%), indicating women with one pregnancy and one live birth. On Hb electrophoresis, the majority of the cases, that is, 511 out of 520 (98.26%), showed a normal pattern, while 9 cases (1.7%) showed abnormal results. Among these, there were eight cases (1.55%) identified with thalassemia, and only one case (0.19%) was sickle cell trait (SCT) as depicted in Table 1. This data indicates a relatively low prevalence of hemoglobinopathies in the study population. Among the cases with hemoglobinopathies, beta-thalassemia was the most prevalent type, with five out of eight cases (62.50%). The remaining types included double heterozygous (12.50%), HbE heterozygous (12.50%), and HbE homozygous (12.50%). This suggests that beta-thalassemia is the most common hemoglobinopathy in this study population (Fig. 2).

The mean complete blood count (CBC) parameters were found to be significantly lower than normal. The RBC indices were found to be statistically significantly across different types of hemoglobinopathies as depicted in Table 2. The analysis of variance (ANOVA) test *p*-value for Hb, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) parameters was 0.0001, indicating significant differences across hemoglobinopathy types as depicted in Table 2.

Discussion

In our study, the age distribution showed a nearly equal division between the third- and fourth-decade age-groups, with a slight majority in the older group (50.77 %). A study by Udho et al.[6] showed anemia in women aged ≥30 years age-group (37%). A study by Siddiqui et al. in New Delhi also highlighted a high prevalence of anemia (70%) among women older than 35 years.[7]

The reason for this slight predominance in the older age-group may be attributed to cumulative nutritional deficits and higher parity in this demographic, factors that have been well-documented in literature.

Table 2: Mean CBC parameters in different types of hemoglobinopathy on Hb electrophoresis

Type of hemoglobinopathy	*Hb*	*RBC*	*MCV*	*MCH*	*Mentzer's index*
Double heterozygous	6.8	5.1	55.3	29.6	55.3/5.1 = 10.84
HbE heterozygous	5.50	5.8	48.60	32.1	48.60/5.8 = 8.38
HbE homozygous	7.90	6.2	66.2	24.8	66.2/6.2 = 12.29
Beta-thalassemia: mean ± SD	7.75 ± 1.31	6.5	61.15 ± 8.83	25.0 ± 0.70	61.15/6.5 = 9.21
Case 1	9.8	6	56.3	25.6	
Case 2	7.8	5.8	57.8	23.8	
Case 3	6.2	5.3	76.3	25.3	
Case 4	7.2	6.2	54.2	25.3	
SCT	6.5	4.9	54.8	26.8	54.8/4.9 = 11.18
ANOVA test *p*-value	0.0001*	0.0001	0.0001*	0.0001*	0.0001*

*Indicates clinical significance

Our study found that 65% of antenatal women were anemic, indicating a high prevalence of anemia, which may be attributed to multiple contributing factors, including nutritional deficiencies (especially iron and folic acid), poor dietary intake, increased physiological demands during pregnancy, and socioeconomic factors.

Furthermore, the 35% of antenatal females without anemia in our study indicate that a fraction of the population has access to proper nutrition, iron supplementation, and overall better healthcare facilities.

Udho et al.[6] highlighted a slightly lower prevalence of anemia (24.7%).

In our study, moderate anemia (hemoglobin levels between 7.1 and 9.9 gm/dL) was the most prevalent, observed in 54.62% of the cases. This is comparable to the findings of Toteja et al.,[8] highlighting the prevalence of moderate anemia to be 60.1% in pregnant women. Severe anemia was less common in our study (4.42%).

A study from Uganda by Udho et al.[6] indicated the prevalence of severe anemia to be significantly lower (2.2%), indicating successful preventive strategies in Uganda.

The overwhelming majority of cases in our study exhibited a microcytic hypochromic pattern (97.69%), indicative of iron deficiency anemia (IDA). This finding is consistent with studies by Patra et al.[9] who found microcytic hypochromic anemia to be the commonest type, found in 49% of the severely anemic women.

There were no cases of megaloblastic anemia found in our study.

In contrast, Khanduri and Sharma[10] reported cobalamin deficiency in 78 patients (65%) out of 120 women diagnosed with megaloblastic anemia.

The most common obstetric history in our study was G2P1L1, accounting for 36.15% of the cases. This pattern reflects the reproductive behavior in the population studied. Balarajan et al. stated that anemia is more prevalent in women with multiple pregnancies.[11] High parity is a known risk factor for anemia, as repeated pregnancies can deplete iron stores and other essential nutrients, exacerbating the risk of anemia. Severe anemia was observed mainly in G2P2L1 females.

The Mentzer index calculated in our study was <13 for all types of hemoglobinopathies. A study conducted at Sri Guru Ram Das Charitable Hospital in Amritsar evaluated the efficacy of the Mentzer index in screening for beta-thalassemia trait (BTT) among 130 pregnant women with anemia. The findings revealed that the Mentzer index had a sensitivity of 80% and specificity of 95.65% for detecting BTT. For IDA, the sensitivity was 95.33% and the specificity was 86.96%.[12] These results suggest that the Mentzer index is a useful, cost-effective tool for distinguishing between IDA and BTT in resource-limited settings.

Among the cases with hemoglobinopathies, beta-thalassemia was the most prevalent type, consistent with findings from studies such as Verma et al.,[13] which reported beta-thalassemia as the most common single gene disorder hemoglobinopathy in India. The mean CBC parameters varied significantly across different types of hemoglobinopathies, with beta-thalassemia cases showing the lowest mean Hb levels, which aligns with global findings on the hematological profile of thalassemia patients.

In another study from Uttarakhand by Singh et al.,[14] the prevalence of BTT and HbD-Punjab trait among pregnant women was found to be 2.6 and 0.2%, respectively. In contrast, a study done in Central India by Balgir[15] depicted 12.26% prevalence of hemoglobinopathies, with SCT being the most common (7.45%).

Kate and Lingojwar screened major communities from Maharashtra state and found high prevalence among SC, ST, and OBC with an overall carrier frequency of approximately 10%. Highest rates are reported from the eastern region (Vidarbha), parts of north Maharashtra, and the Marathwada belt. Among tribal communities, several groups exhibit alarmingly high frequencies. The Otkar tribe in Gadchiroli reports a prevalence of 35%, followed by the Pardhan tribe in Gadchiroli, Chandrapur, and Yavatmal at 32%, and the Pawara tribe in Nandurbar and Jalgaon at 25%. Other tribes with significant prevalence include the Bhill and Madia (each at 20%), Halbi (13%), Rajgond (11%), Korku (10%), and both Kolam and Warli tribes.[16]

It is estimated that Maharashtra has approximately 2.5 million carriers of the sickle cell gene and about 1,25,000 sufferers (HbSS), based on 2001 census data. The disease is especially concentrated in rural areas where awareness and medical resources are limited.[16]

In the case of BTT, a study conducted by Satpute et al. in South-western Maharashtra (covering Satara, Sangli, and Kolhapur districts) analyzed 126 beta-thalassemia carriers. The most common mutation found was intervening sequence (IVS) I-5 (G–C), present in 65.07% of cases, making it the dominant variant in the region. The second most prevalent was IVS I-1 (G–T), found in 9.52% of subjects. Other mutations included codon 8/9 (+G) and codon 15 (G–A), both occurring in 6.34% of the sample, followed by codon 41/42 (–TCTT) at 3.96% and 619 bp deletion at 2.38%. Additionally, 6.34% of the subjects had uncharacterized mutations. This data emphasizes the regional genetic variation in beta-thalassemia mutations and the importance of targeted screening and counselling strategies in these districts.[17]

All the above findings reinforce the significant geographical variation of hemoglobinopathies across India as well as within individual states. Such high regional figures justify the implementation of screening protocols as public health mandates.

As per the National Health Mission (NHM) and WHO recommendations, hemoglobinopathy screening during

antenatal care is particularly essential in regions with high carrier frequency. The Indian Council of Medical Research (ICMR) also recommends early identification and counselling of carriers in high-prevalence zones to curtail the burden of severe inherited hemoglobin disorders.

The limitations of this study were that the study was conducted with a sample size of 800 antenatal females from a single center, which may limit the generalizability of the findings to broader populations. A larger, multicentric study would provide more representative data. Further molecular studies may be needed in order to detect certain other hemoglobinopathies which may have been missed.

Conclusion

An observational cross-sectional study conducted on antenatal females revealed a high prevalence of anemia. The majority of participants were in their second or third decade of life, with nearly equal distribution between the two age-groups. Most cases were of moderate severity, with peripheral blood smear findings predominantly indicating microcytic hypochromic anemia, suggestive of iron deficiency. A significant proportion of the participants belonged to lower-middle-income backgrounds, highlighting the socioeconomic influence on maternal health. Additionally, obstetric history analysis showed that the most common parity status was among women with one previous pregnancy and one living child. These findings emphasize the need for targeted interventions to address anemia in antenatal care.

This study assessed the prevalence of hemoglobinopathies among antenatal females in a specific region, revealing a low prevalence (1.55%), with beta-thalassemia being the most common type identified. The high rate of microcytic hypochromic anemia underscores the need for targeted interventions to address iron deficiency and improve maternal health outcomes.

Acknowledgment

We extend our sincere thanks to Dr DY Patil Medical College, Hospital and Research Centre, Pimpri, Pune, for providing resources, facilities, and funding for the project.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Mallika Agarwal (MA), upon reasonable request.

Declaration of Patient Consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the females have given their consent for the clinical information to be reported in the journal. The females understand that their name and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

References

1. Galanello R. Screening and diagnosis for haemoglobin disorders. Prevention of Thalassaemias and Other Haemoglobin Disorders: Volume 1: Principles [Internet], 2nd edition. Thalassaemia International Federation; 2019.
2. Angastiniotis M, Lobitz S. Thalassemias: an overview. Int J Neonatal Screen 2019;5(1):16.
3. ACOG Committee on Obstetrics. ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. Obstet Gynecol 2007;109(1):229–237.
4. Alpha-thalassemia. [internet]. Wikipedia, The Free Encyclopedia. [Cited March, 2025].
5. Thalassemia. [internet]. Wikipedia, The Free Encyclopedia; 2025. [Cited March, 2025].
6. Udho S, Nankumbi J, Namutebi M, et al. Prevalence of anaemia in pregnancy and associated factors in northern Uganda: a cross-sectional study. S Afr J Clin Nutr 2022;36(4):136–141.
7. Siddiqui MZ, Goli S, Reja T, et al. Prevalence of anaemia among pregnant, lactating, and non-pregnant non-lactating women in India. SAGE Open 2017;7(3).
8. Toteja GS, Singh P, Dhillon BS, et al. Prevalence of anemia among pregnant women and adolescent girls in 16 districts of India. Food Nutr Bull 2006;27(4):311–315.
9. Patra S, Pasrija S, Trivedi SS, et al. Maternal and perinatal outcome in patients with severe anemia in pregnancy. Int J Gynaecol Obstet 2005;91(2):164–165.
10. Khanduri U, Sharma A. Megaloblastic anaemia: prevalence and causative factors. Natl Med J India 2007;20(4):172–175.
11. Balarajan Y, Ramakrishnan U, Özaltin E, et al. Anaemia in low-income and middle-income countries. Lancet 2011;378(9809):2123–2135.
12. Kaur S, Pahwa S, Kakkar S, et al. Evaluation of efficacy of the Mentzer index for screening of beta-thalassemia trait in antenatal women. Indian J Obstet Gynecol Res 2024;11(4):591.
13. Verma IC, Saxena R, Thomas E, et al. Regional distribution of beta-thalassemia mutations in India. Hum Genet 1997;100(1):109–113.
14. Singh N, Chowdhury N, Bahadur A, et al. Thalassemia and hemoglobinopathy screening in women attending antenatal clinic at a tertiary care center in Uttarakhand, India: a re-look at the laboratory parameters mandating high-performance liquid chromatography workup. Cureus 2023;15(6):e40667.
15. Balgir RS. Prevalence of hemolytic anemia and hemoglobinopathies among the pregnant women attending a tertiary hospital in central India. Thalass Rep 2015;5:4644.
16. Kate SL, Lingojwar DP. Epidemiology of sickle cell disorder in the state of Maharashtra. Int J Hum Genet 2002;2(3):161–167.
17. Satpute SB, Bankar MP, Momin AA. The prevalence of β-thalassemia mutations in South Western Maharashtra. Indian J Clin Biochem 2012;27(4):389–393.