Section: Pathology

Original Research Article

SPECTRUM OF HAEMOGLOBIN VARIANTS IN ANTENATAL SCREENING AT A TERTIARY CARE CENTRE IN MARATHWADA

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ABSTRACT

Background: Haemoglobinopathies constitute a significant group of inherited red cell disorders globally. With an estimated 7% of the world population carrying haemoglobin disorders, antenatal screening is a critical preventive measure. Data regarding the spectrum of haemoglobin variants and patterns in the Marathwada region of Maharashtra remains scarce. **Aims:** To determine the spectrum of haemoglobin patterns diagnosed by Cation Exchange-High Performance Liquid Chromatography (CE-HPLC) in antenatal (ANC) patients and to evaluate complete blood count (CBC) findings across various haemoglobinopathies.

Materials and Methods: An retrospective observational study was conducted over three years (2019–2021) at a tertiary care centre. A total of 2,067 ANC patients underwent screening. CBC was performed using a fully automated DXH 800 cell counter, and CE-HPLC was conducted using the Bio-Rad D-10 analyzer.

Results: Out of 2,067 ANC patients, abnormal or borderline haemoglobin patterns were detected in 103 (4.98%) cases. The most common abnormality was the β-thalassemia trait (3.10%), followed by sickle cell heterozygous (0.53%), Hb D Punjab heterozygous (0.29%), Hb E heterozygous (0.19%), Hereditary Persistence of Foetal Haemoglobin (HPFH) (0.15%), and δβ-thalassemia trait (0.10%).

Conclusion: CE-HPLC is an effective, rapid, and reproducible tool for antenatal screening. The detection of carrier states is particularly relevant to prevention of severe, transfusion-dependent haemoglobinopathies. The 4.98% prevalence of abnormal haemoglobin patterns observed in this antenatal cohort underscores the importance of routine screening to help identify carrier states, partner testing, genetic counselling and, where indicated, prenatal diagnosis.

Keywords: Haemoglobinopathies; Antenatal screening; HPLC; β-thalassemia trait; Haemoglobin variants; Marathwada.

INTRODUCTION

Haemoglobinopathies encompass a wide group of inherited red cell disorders that result in the production of structurally abnormal haemoglobin. According to the World Health Organization, approximately 7% of the global population is a

carrier for haemoglobin disorders. Consequently, screening for haemoglobin variants is an important part of antenatal care, and helps in identifying couples at risk of having children with significant haemoglobinopathies.[1,2]

Rapid screening and presumptive identification of these genetic variations is effectively achieved utilizing Cation Exchange-High Performance Liquid Chromatography (CE-HPLC). This diagnostic modality is highly reproducible, offers operational simplicity through automation, and yields rapid results. While the national burden of haemoglobinopathies is widely acknowledged, specific regional data detailing the prevalence and spectrum of these disorders in the Marathwada region of Maharashtra remain scarce. This study aims to describe the spectrum of haemoglobin patterns diagnosed by CE-HPLC in ANC patients and to analyse the associated CBC findings across various observed variants.[2-4]

MATERIALS AND METHODS

A retrospective observational study was conducted at Department of Pathology, R.K. Damani Medical College & Dr. Hedgewar Hospital, Chhatrapati Sambhajinagar, Maharashtra, which is a tertiary care health centre.

Study spanned a period of three years, from 2019 to 2021. The study cohort comprised 2,067 ANC patients.

Inclusion Criteria: All consecutive ANC patients undergoing routine screening for haemoglobinopathies via HPLC.

Exclusion Criteria: Patients with a recent history of blood transfusion and those providing inadequate sample volumes.

Whole blood samples were collected in EDTA vacutainers. Initial haematological evaluation was performed by subjecting the samples to a complete blood count (CBC) using a fully automated DXH 800 cell counter. Subsequently, the samples were analysed in batches via CE-HPLC using the extended Hb A2/F/A1c program in Bio-Rad D-10 analyser. The principle of this automated system relies on the chromatographic separation of analytes across a cartridge using a buffered gradient, which elutes various haemoglobin fractions at specific retention times. The flow cell utilizes a filter photometer to measure changes in absorbance at 415 nm, generating a detailed chromatogram for interpretation. HbA2 values of approximately 2.0–3.5% were considered within the laboratory reference range; values above 4%, in conjunction with the haematological findings, were interpreted as suggestive of β-thalassemia trait. Hb A2 value of 3.6 to 4% was defined as borderline Hb A2. CBC and CE-HPLC parameters were analysed by describing means, ranges and percentages.[2,4] Ethics committee approval was taken before conducting the study (EC/Approval/08/2026/02).

Table 1: Manufacturer Assigned Retention Time & Windows,[4]

Peak name	Retention time (min)	Window (min)
F	0.48	0.38-0.58
A1c	0.87	0.70-1.04
A0	1.70	1.55-1.85
A2	3.25	3.00-3.50
S-window	4.16	4.02-4.30
C-window	4.65	4.55-4.75

RESULTS

A total of 2,067 ANC patients were screened during the study period. Abnormal haemoglobin patterns were detected in 103 cases, yielding an overall regional prevalence of 4.98%.

β-thalassemia trait was the predominant haemoglobinopathy pattern, accounting for 64 cases (3.10% of the screened population). This was followed by 11 cases of sickle cell heterozygous (0.53%), 6 Hb D Punjab heterozygous (0.29%), and 4 Hb E heterozygous (0.19%). Rarer findings included 3 HPFH (0.15%) and the 2 δβ-thalassemia trait (0.10%). One case (0.05%) with an HPLC pattern suggestive of Hb Lepore was also identified.

Table 2: % Distribution of Haemoglobin patterns in CE-HPLC

	No. (%)
β Thalassemia trait	64 (3.10)
Sickle cell heterozygous	11 (0.53)
Hb D Punjab heterozygous	6 (0.29)
Hb E heterozygous	4 (0.19)
HPFH	3 (0.15)
δβ Thalassemia trait	2 (0.10)
Borderline HbA2 (3.5–4.0%)	6 (0.29)
Raised HbF (1–5%)	5 (0.24)
HPLC pattern suggestive of Hb Lepore	1 (0.05)
Unidentified peak	1 (0.05)
Total	103 (4.98)

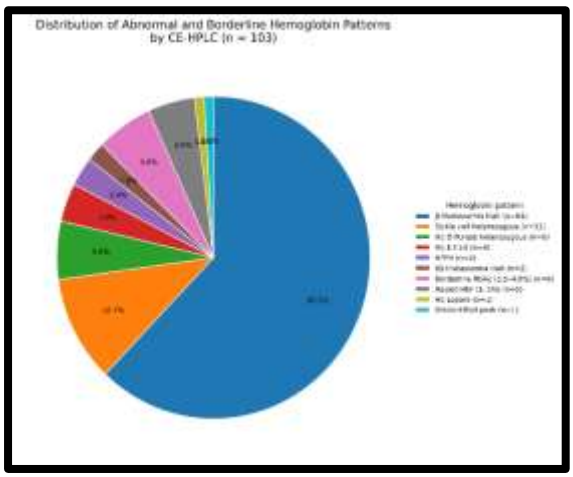
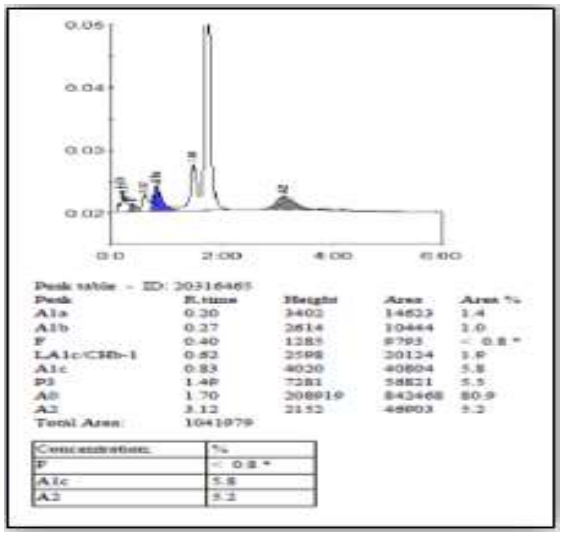


Figure 1: % Distribution of Haemoglobin patterns Cases in ANC Patients

The other 13 included 6 cases of Borderline Hb A2, 5 increased Hb F, one case with an HPLC pattern suggestive of Hb Lepore and one unidentified peak.

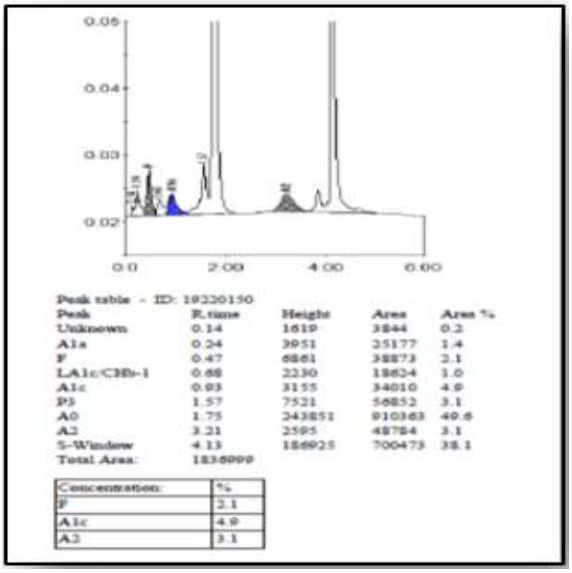


Peak table - ID: 20316465

Peak	R.time	Height	Area	Area %
A1a	0.20	3402	14633	1.4
A1b	0.27	2614	10444	1.0
F	0.40	1285	8793	< 0.8 *
LA1c/CHb-1	0.62	2598	20124	1.9
A1c	0.83	4020	40804	3.8
P3	1.49	7281	58821	5.5
A0	1.70	208919	843468	80.9
A2	3.12	2152	48903	5.2
Total Area:		1041979		

Concentration:	%
F	< 0.8 *
A1c	5.8
A2	5.2

Figure 2: β thalassemia trait

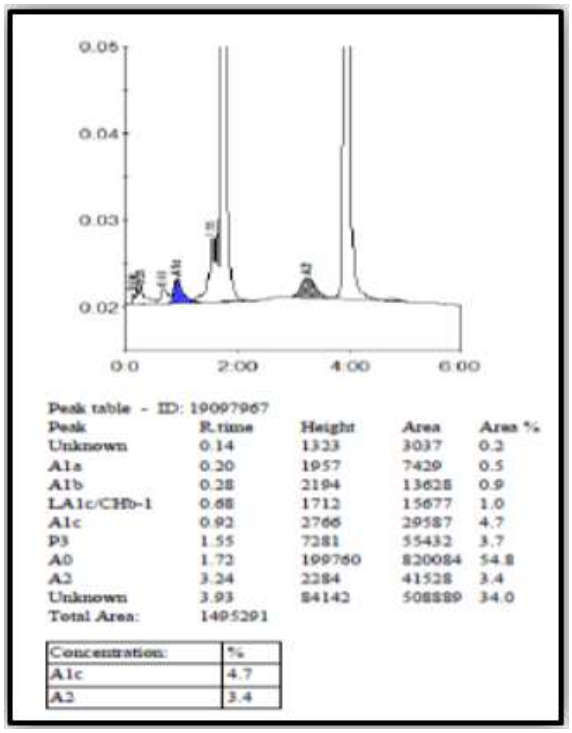


Peak table - ID: 19220150

Peak	R.time	Height	Area	Area %
Unknown	0.14	1619	3844	0.2
A1a	0.24	3951	25177	1.4
F	0.47	6861	38873	2.1
LA1c/CHb-1	0.68	2230	18624	1.0
A1c	0.93	3155	34010	4.9
P3	1.57	7521	56852	3.1
A0	1.75	243851	910363	40.6
A2	3.21	2595	48784	3.1
S-Window	4.13	186925	700473	38.1
Total Area:		1836099		

Concentration:	%
F	2.1
A1c	4.9
A2	3.1

Figure 3: Sickle cell heterozygous

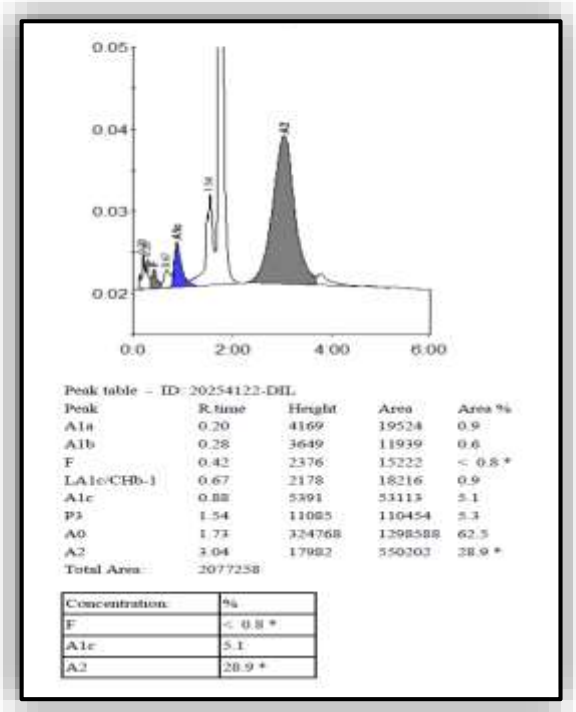


Peak table - ID: 19097967

Peak	R.time	Height	Area	Area %
Unknown	0.14	1323	3037	0.2
A1a	0.20	1957	7429	0.5
A1b	0.28	2194	13628	0.9
LA1c/CHb-1	0.68	1712	15677	1.0
A1c	0.92	2766	29587	4.7
P3	1.55	7281	55432	3.7
A0	1.72	199760	820084	54.8
A2	3.24	2284	41528	3.4
Unknown	3.93	84142	508889	34.0
Total Area:		1495291		

Concentration:	%
A1c	4.7
A2	3.4

Figure 4: Hb D Punjab heterozygous



Peak table - ID: 20254122-DIL

Peak	R.time	Height	Area	Area %
A1a	0.20	4169	19524	0.9
A1b	0.28	3649	11939	0.6
F	0.42	2376	15222	< 0.8 *
LA1c/CHb-1	0.67	2178	18216	0.9
A1c	0.88	5391	53113	5.1
P3	1.54	11085	110454	5.3
A0	1.73	324768	1298588	62.5
A2	3.04	17982	550203	28.9 *
Total Area:		2077258		

Concentration:	%
F	< 0.8 *
A1c	5.1
A2	28.9 *

Figure 5: Hb E heterozygous

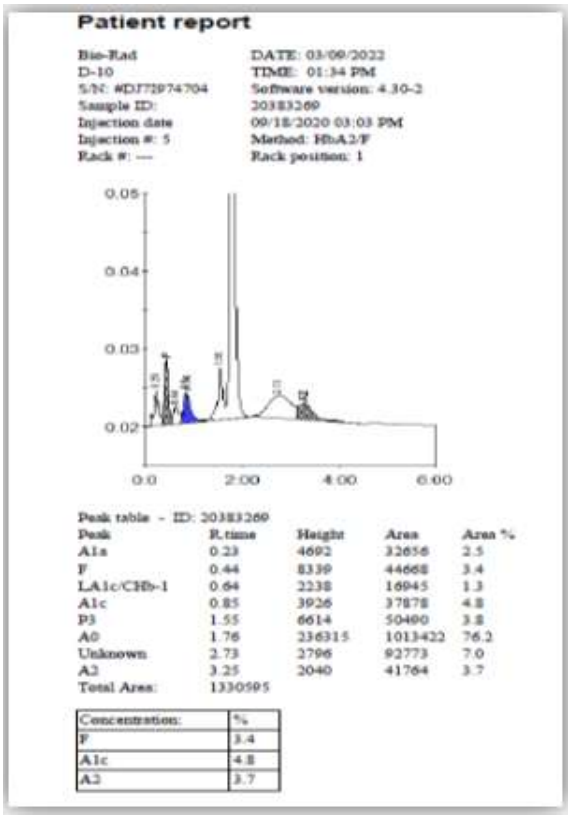


Figure 6: HPLC pattern suggestive of Hb Lepore

Analysis of the haematological indices demonstrated distinct profiles for the identified variants. Patients with the β-thalassemia trait exhibited microcytic hypochromic indices, detailed in Table 3, while those with the sickle cell heterozygous trait displayed milder alterations (Table 4).

An unidentified chromatographic peak was observed in one case, associated with marked microcytosis but normal HbA2 (2.8%) and HbF (1.0%). The finding was therefore reported as an unknown peak without assigning a specific haemoglobinopathy diagnosis.

Table 3: CBC Findings & % Hb Fractions in β Thalassemia Trait

	% Hb A2	% Hb F	RBC	HB	MCV
Mean	5.24	1.4	4.94	9.74	62.60
Min	3.8	0.8	3.36	7.5	53.9
Max	6.7	5.6	5.79	12.2	79.6

Table 4: CBC Findings & % Hb Fractions in Sickle Cell Heterozygous

	% Hb S	% Hb F	% Hb A2	RBC	HB	MCV
Mean	33.75	1.56	3.2	4.75	11.85	76.36
Min	30.1	0.8	2.8	4.15	10.2	58
Max	38.2	4.5	3.5	5.58	12.7	88

DISCUSSION

Haemoglobinopathies impose a significant medical and economic burden in India, making the implementation of effective antenatal screening programs a critical public health priority. The present study evaluated 2,067 antenatal women in the Marathwada region, revealing an overall abnormal haemoglobin pattern in 4.98% (103 cases). In this study, most ANC patients reported in their first trimester of pregnancy, allowing sufficient time for the screening of respective partners and prenatal diagnosis. [2,10]

The prevalence and distribution in our study were comparable with those reported in other Indian studies (Table 5).[1,5-7,9-11] However, variation in reported prevalence may reflect differences in geographic region, ethnicity, referral patterns, inclusion criteria, anaemia status, and whether the study population consisted of all antenatal women or selected patients with suspected haemoglobinopathy.[9,11]

Table 5: Comparative Study of Haemoglobinopathies

Study name	Kelkar et al[5]	Kaul et al[6]	Sawaimul et al[7]	Shukla et al[1]	Our Study
Total No. Of Cases	674	234	500	2000	2067
Cases with Abnormal Haemoglobin patterns	58 (8.6%)	12 (5.12%)	20 (4%)	63 (3.15%)	103 (4.98%)
β Thalassemia Trait	45 (6.68%)	7 (2.99%)	11 (2.2%)	59 (2.95%)	64 (3.10%)
Sickle Cell heterozygous	8 (0.75%)	-	6 (1.2%)	1 (0.05%)	11 (0.53%)
Hb E heterozygous	-	3 (1.28%)	-	1 (0.05%)	4 (0.19%)
Hb D Punjab heterozygous	-	1 (0.43%)	-	1 (0.05%)	6 (0.29%)

Consistent with national epidemiological data, the β-thalassemia trait was the most frequently encountered abnormality in our cohort, accounting for 3.10% of total cases. As shown in Table 6 and Table 7, the haemoglobin fractions and CBC parameters for β-thalassemia trait carriers in this study were highly comparable to existing literature. In our study, mean Hb A2 was 5.24% and mean MCV was 62.6 fL in the cases of β-thalassemia trait. The average Mentzer index in the β-thalassemia trait cohort was 12.67, which was consistent with the conventional threshold suggestive of β-thalassemia trait.[1,5-7,12]

A pilot study from Pune in Western Maharashtra reported abnormal haemoglobin patterns in 5.05% of individuals investigated, with β-thalassemia trait accounting for 3.7%; however, the study population consisted of patients referred for suspected haemoglobinopathy and therefore was not directly comparable with an unselected antenatal cohort. Six women demonstrated borderline HbA2 values (3.5–4.0%). Such values may occur in mild β-thalassemia alleles and require interpretation in conjunction with red-cell indices, HbF, iron status, family studies and, where clinically indicated, further investigation.[2,8]

Table 6: Comparison of Haemoglobin Fractions in β Thalassemia Trait

%	Kelkar et al[5]	Kaul et al[6]	Sawaimul et al[7]	Shukla et al[1]	Our Study
Hb A2	4.2	4.8	5.4	5.3	5.3
Hb F	0.95	1.3	0.3	1	1.4
Hb A	84.2	93.9	94.6	93.7	93.3

Table 7: Comparison of CBC Findings in β Thalassemia Trait with Other Studies

Mean	Kelkar et al5	Kaul et al6	Shukla et al1	Our Study
MCV	64	61.8	-	62.6
HB	9.03	10.4	9.96	9.74
RBC Count	4.53	5.05	-	4.94

Sickle cell trait (Hb AS) was the second most common variant, observed in 0.53% of the screened population. We also identified rare structural variants, including Hb D Punjab (0.29%) and Hb E heterozygous (0.19%).[5-7,9,10]

Five cases showed increased HbF levels ranging from 1–5% without a diagnostic pattern sufficient to classify them as HPFH or δβ-thalassemia. Raised HbF has a broad differential diagnosis and may require correlation with red-cell indices, clinical context, family studies and, where indicated, molecular investigation.[2]

Although HPLC is highly useful for antenatal screening, identification of haemoglobin variants based solely on chromatographic retention characteristics remains presumptive. The BSH guideline recommends confirmation of abnormal screening results using a different technique appropriate to the suspected variant. Definitive characterization of unusual variants may require molecular or protein-based methods. This consideration is particularly relevant to the unidentified peak and the cases with raised HbF in the present study.[2]

The identification of these 103 abnormal/borderline HPLC patterns during early pregnancy underscores why HPLC is a useful tool to generate epidemiological data where it is currently scarce. Because the lifelong management of transfusion-dependent haemoglobinopathies places an immense socio-economic strain on families, prevention remains decidedly better than treatment due to associated disabilities and costs.[2,9,10]

CONCLUSION

The present study, with 2,067 ANC cases, is one of the largest in the region to describe the spectrum of haemoglobinopathies which demonstrated abnormal haemoglobin patterns in 4.98% cases. The β-thalassemia trait was the most common abnormality. Minor proportions of sickle cell heterozygous, Hb D Punjab trait, Hb E heterozygous, an HPLC pattern suggestive of Hb Lepore and δβ-thalassemia trait were also seen. The findings in the study were comparable with other similar studies in India. Data relating to the prevalence of haemoglobin variants is obscure in various regions of India, and HPLC serves as a useful tool to generate such data. Detection of haemoglobin variants by HPLC can prompt further investigations for prenatal diagnosis in high-risk cases where prospective parents are carriers. The findings in this antenatal cohort highlight the importance of routine screening to help identify carrier states, partner testing, genetic counselling and, where indicated, prenatal diagnosis.

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