Original article:

Study on Spectrum of Hemoglobinopathies in A Tertiary Care Hospital in Haryana, India

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Abstract

Background: Hemoglobinopathies are the most common inherited disorders and are major public health problem in many parts of the world. According to WHO, 5% of the world population is carrier for hemoglobinopathies. High Performance Liquid Chromatography (HPLC) is a sensitive, specific and rapid method aiding in detection of hemoglobinopathies. ***Objective:*** To evaluate the spectrum of various hemoglobinopathies by using HPLC in a rural tertiary care hospital in Haryana, India. ***Methods:*** This prospective study was conducted for detection of hemoglobinopathies using BioRad D-10 for patients visiting various OPDs in Bhagat Phool Singh Government Medical College for Women, Haryana, India. ***Results:*** A total of 160 patients were included in the study. 25 (15.6%) cases were detected to have hemoglobinopathies. Most common hemoglobinopathy was beta thalassemia trait found in 9 cases (36%) followed by beta thalassemia major in 4 cases (16%), Hemoglobin D Punjab heterozygous in 3 cases (12%), Hemoglobin E heterozygous in 2 cases (8%). ***Conclusion:*** Our study provides an overview on the spectrum of hemoglobinopathies in Haryana region of India. Hemoglobinopathies pose considerable economic and psychosocial burden on the affected individuals, society, and the country. It is concluded that HPLC is a versatile, reproducible technique for the estimation of hemoglobinopathies.

Keywords: Hemoglobinopathies, high performance liquid chromatography, public health

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Introduction

In the entire world, hemoglobinopathies are the most prevalent inherited diseases. In many regions of the world, especially Southeast Asia, it is posing a serious threat to public health. The ethnic composition of the population in these countries is quite diverse and malaria is prevalent in the majority of these regions, which likely exerts selection pressure and increases the occurrence of hemoglobinopathies (such as thalassemia and variant hemoglobin (Hb)) in these places.[1] As hemoglobinopathies are one of the major public health problems in India and can be quantitative (thalassemia syndrome) or qualitative (variant hemoglobinopathies), of these beta thalassemia is the commonest monogenic disorder.[2] According to WHO, 5% of the world population is carrier of Hb disorders.[3]

Hemolytic anemia, erythrocytosis, cyanosis or vase occlusive stigmata are a few presenting symptoms of hemoglobinopathies.[4] Mild or no anemia in beta thalassemia trait is seen which is characterized by low mean corpuscular volume (MCV), low mean corpuscular Hb (MCH).[5] Beta thalassemia major and a small number of cases of intermedia cause dreadful complications that necessitate blood transfusions.[6] Thalassemia has a serious impact on public health due to its high prevalence.[1] The early and accurate diagnosis of various hemoglobinopathies helps avoid the development of more severe conditions like thalassemia major in infants.

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The serious thalassemia syndromes, particularly beta thalassemia major and some types of alpha thalassemia, constitute a significant contributor to morbidity.[7] The birth of a thalassemic kid places a great deal of physical, financial and emotional pressure on the child, family, society and country as a whole. Therefore, the focus should change from therapy to future birth prevention strategies.[8] Prevention strategies including public health education, mass screening, genetic counselling and prenatal diagnosis may be practical and effective for resolving this issue.[9]

Hemograms and electrophoresis techniques are used in screening programmes.[10] High performance liquid chromatography (HPLC) and electrophoresis are used for detection these hemoglobinopathies.[11] It provides with a more sensitive, specific and rapid screening capacity for early and accurate detection which further assists in the management and prevention of different hemoglobinopathies.[12,13] HPLC technique is used more commonly to screen Hb variants and hemoglobinopathies because it has the advantage of quantifying Hb F and Hb A2 along with Hb variants in a single, highly reproducible system. This methodology is appropriate for even small clinical laboratories due to the ease of the automated system with internal sample preparation, superior resolution, quick assay time and precise measurement of Hb fractions.[14] Therefore, the present study was done to evaluate the spectrum of various hemoglobinopathies by using HPLC in a rural tertiary care hospital in Haryana, India.

Methods

This prospective study was conducted in the Department of Pathology, Bhagat Phool Singh Government Medical College for Women, Haryana, India. The duration of the study was one year, from 2021 to 2022. Blood samples for detection of hemoglobinopathies were received in the Department of Pathology from patients in whom presence of hemoglobinopathy was suspected clinically and their hemoglobin was less than 10 grams/dL. Patients with a history of blood transfusion in the past three months were excluded from the study. Venous blood was collected in EDTA vacutainer from each patient under aseptic precautions. HPLC was done using Hb A_2 -Hb F program of D10 (BIO-RAD) based on the principle of cation ion-exchange chromatography. The column comprises a small cation exchange cartridge, with a requirement of 2 ml of the blood sample and red cell morphology was observed in peripheral blood film stained with Leishman's stain.

Data was collected through high scrutiny and interpretation was went under high validation and adjustment. Data was entered using Microsoft Excel 2016 and imported to statistical package for social sciences Version 21.0 for analysis. Data scrutinizing, cleaning and inconsistency checking was done through the execution of range and influential/outlier value identification. Data was presented through different tables and graphs.

Results

In the present study, a total of 160 cases were included. The age of the cases from whom samples were obtained ranged from 6 months-83 years. Females and males constituted (57.5%) and (42.5%) respectively. Hemoglobinopathy was present in 25 (15.60 %) cases and the rest 135 (84.40%) cases showed absence of hemoglobinopathy. The majority of cases with hemoglobinopathy were found to be in 0-30 years of age group 21(84%). Most cases were constituted by beta thalassemia related hemoglobinopathies either alone or in combination with other hemoglobinopathies 16 (64%) cases in total out of which beta thalassemia alone (including beta thalassemia trait (Figure 1&2), beta thalassemia major (Figure 3&4), while beta thalassemia intermedia constituted 14 cases (56%). Hb D was found in 4 cases (16%) i.e., 3 cases of Hb D Punjab heterozygous and 1 case of Hb D Punjab-beta thalassemia heterozygous (Figure 5). Hb S constituted 3 (12%) cases: 1 each of Hb S heterozygous, Hb S Homozygous and Hb S - Beta Thalassemia heterozygous (Figure 6&7). Hb E heterozygous was detected in 2 (8%) cases (Figure 8). Our results indicate that beta thalassemia is the commonest hemoglobinopathy in our region followed by Hb D (Table 1). None of the cases with hemoglobinopathy showed macrocytic blood picture. Microcytic hypochromic picture was more common in hemoglobinopathies than normocyctic normochromic picture. All cases of thalassemia intermedia 1 (100%) case and thalassemia major 4 (100%) cases were associated with microcyctic hypochromic picture. Cases of beta thalassemia trait 2 (22.2%) cases were associated with normocyctic normochromic picture. Cases with Hb S heterozygous and homozygous 1 case each

(100% each) were associated with normocytic normochromic picture. This suggests that presence of normocytic normochromic picture does not rule out the possibility of hemoglobinopathy. (Table 2). Morphological observations of red blood cells (RBC) revealed features of anisocytosis, poikilocytosis, polychromasia and anisochromasia. Anisocytosis was the most common variety followed by poikilocytosis (Table 3).

Table 1: Distribution of various hemoglobinopathies (n=25)

HPLC interpretation	No. of cases (Percentage)
Beta Thalassemia Trait	9 (36%)
Beta Thalassemia Major	4 (16%)
Beta Thalassemia Intermedia	1 (4%)
Hb D Punjab Heterozygous	3 (12%)
Hb D Punjab-Beta Thalassemia Heterozygous	1 (4%)
Hb E Heterozygous	2 (8%)
Hb D Iran Heterozygous	1 (4%)
Hb S Heterozygous	1 (4%)
Hb S Homozygous	1 (4%)
Hb S- Beta Thalassemia Heterozygous	1 (4%)
Hb Lepore	1 (4%)
Total	**25 (100%)**

Table 2: Distribution of cases according to peripheral blood film (PBF) findings in hemoglobinopathies (n=25)

	PBF - RBC			Total
HPLC - interpretation	**M/H**	**MACRO**	**N/N**	
Beta Thalassemia Trait	7 (77.8%)	0	2 (22.2%)	9 (100%)
Beta Thalassemia Major	4 (100%)	0	0	4 (100%)
Beta Thalassemia Intermedia	1 (100%)	0	0	1 (100%)
Hb D Punjab Heterozygous	3 (100%)	0	0	3 (100%)
Hb D Punjab-Beta Thalassemia Heterozygous	1 (100%)	0	0	1 (100%)
Hb E heterozygous	1 (50%)	0	1 (50%)	2 (100%)
Hb D Iran Heterozygous	1 (100%)	0	0	1 (100%)
Hb S Heterozygous	0	0	1 (100%)	1 (100%)
Hb S Homozygous	0	0	1 (100%)	1 (100%)
Hb S- Beta Thalassemia heterozygous	1 (100%)	0	0	1 (100%)
Hb Lepore	1 (100%)	0	0	1 (100%)
Total	**20**	**0**	**5**	**25**

M/H- Microcytic Hypochromic, MACRO – Macrocytic, N/N- Normocytic Normochromic

Table 3: RBC morphology wise distribution of hemoglobinopathies (n=25)

PBF-RBC	HPLC Hemoglobinopathy present
Anisochromasia	8 (32%)
Polychromasia	4 (16%)
Anisocytosis	12 (48%)
Poikilocytosis	9 (36%)

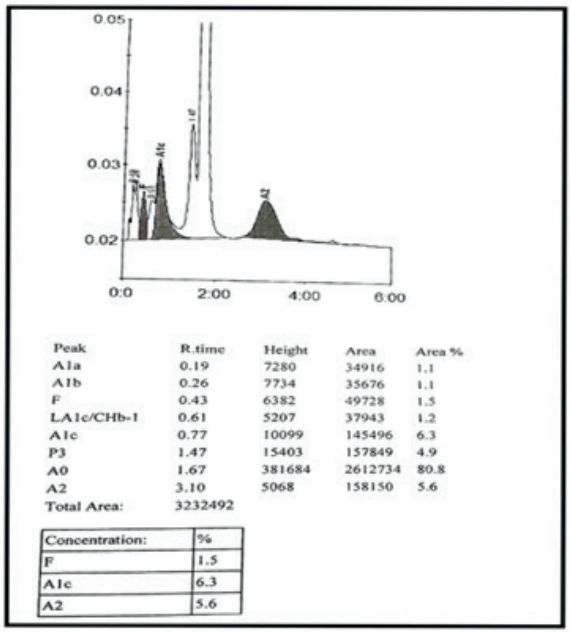


Figure 1: Chromatogram showing beta thalassemia trait.

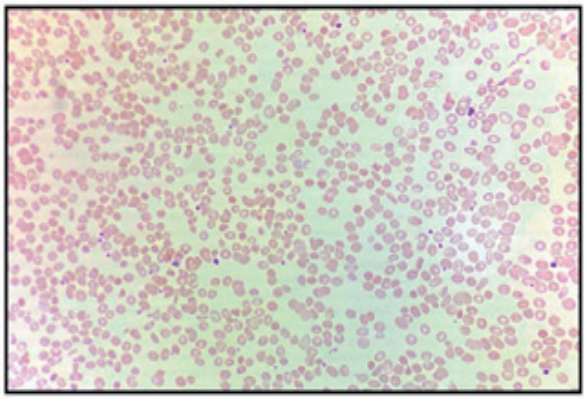
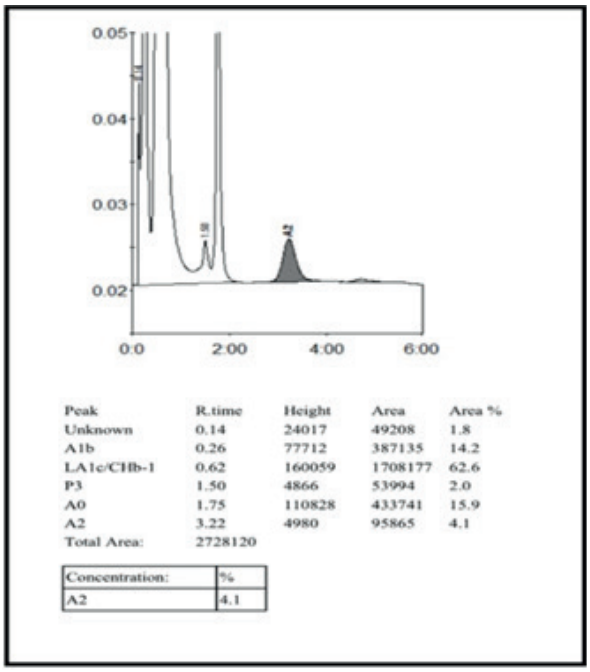


Figure 2: Microphotograph of peripheral blood film of a beta thalassemia trait showing anisopikilocytosis with microcytic hypochromic blood picture (Leishman stain, ×400).



Peak	R.time	Height	Area	Area %
Unknown	0.14	24017	49208	1.8
A1b	0.26	77712	387135	14.2
LA1c/CHb-1	0.62	160059	1708177	62.6
P3	1.50	4866	53994	2.0
A0	1.75	110828	433741	15.9
A2	3.22	4980	95865	4.1
Total Area:			2728120	

Concentration:	%
A2	4.1

Figure 3: Chromatogram showing beta thalassemia major as Hb F is elevated, which is eluting in LA1c and A1b in the picture.

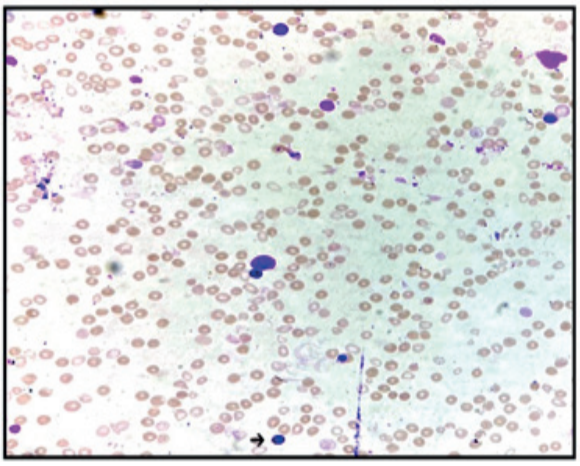
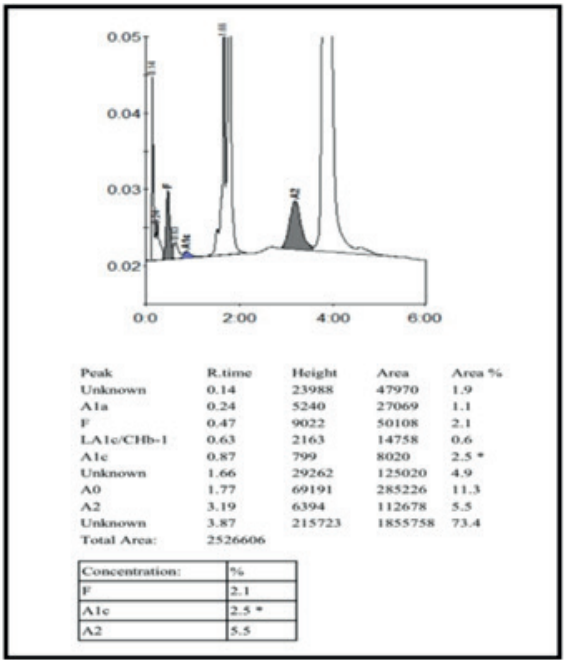


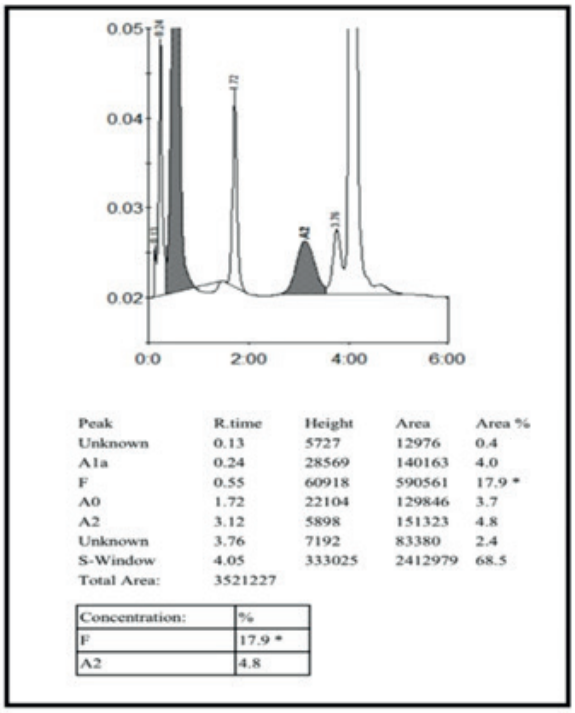
Figure 4: Microphotograph of peripheral blood film of beta thalassemia major showing anisopikilocytosis with microcytic hypochromic blood picture with few nRBC (arrow) and polychromasia (Leishman stain, ×400).



Peak	R.time	Height	Area	Area %
Unknown	0.14	23988	47970	1.9
A1a	0.24	5240	27069	1.1
F	0.47	9022	50108	2.1
LA1c/CHb-1	0.63	2163	14758	0.6
A1c	0.87	799	8020	2.5 *
Unknown	1.66	29262	125020	4.9
A0	1.77	69191	285226	11.3
A2	3.19	6394	112678	5.5
Unknown	3.87	215723	1855758	73.4
Total Area:			2526606	

Concentration:	%
F	2.1
A1c	2.5 *
A2	5.5

Figure 5: Chromatogram showing Hb D Punjab-Beta Thalassemia Heterozygous as unknown peak is eluting at 3.90 seconds corresponding to D-Punjab window with elevated Hb A2.



Peak	R.time	Height	Area	Area %
Unknown	0.13	5727	12976	0.4
A1a	0.24	28569	140163	4.0
F	0.55	60918	590561	17.9 *
A0	1.72	22104	129846	3.7
A2	3.12	5898	151323	4.8
Unknown	3.76	7192	83380	2.4
S-Window	4.05	333025	2412979	68.5
Total Area:			3521227	

Concentration:	%
F	17.9 *
A2	4.8

Figure 6: Chromatogram showing Hb S-beta thalassemia heterozygous as Hb-S is elevated which is eluting in S-window and Hb A2 is elevated.

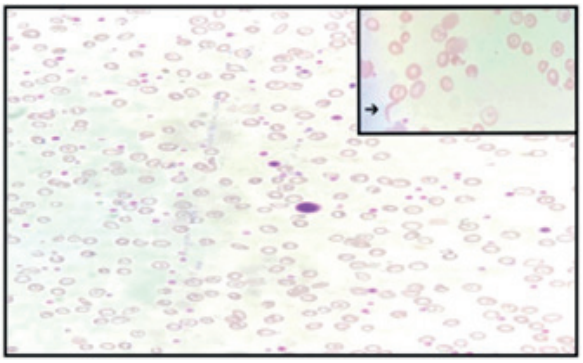
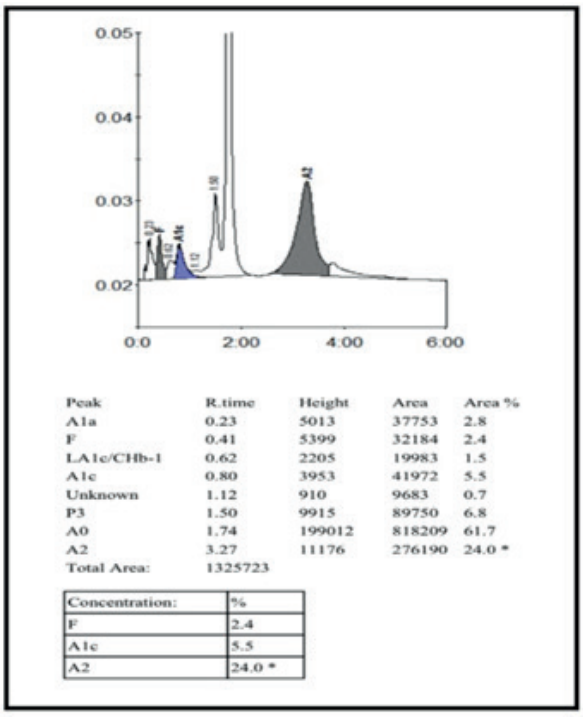


Figure 7: Microphotograph of peripheral blood film of Hb-S beta thalassemia trait showing anisopikilocytosis with microcytic hypochromic blood picture with target cells. Inset shows sickle cell (arrow) (Leishman stain, ×400).



Peak	R.time	Height	Area	Area %
A1a	0.23	5013	37753	2.8
F	0.41	5399	32184	2.4
LA1c/CHb-1	0.62	2205	19983	1.5
A1c	0.80	3953	41972	5.5
Unknown	1.12	910	9683	0.7
P3	1.50	9915	89750	6.8
A0	1.74	199012	818209	61.7
A2	3.27	11176	276190	24.0 *
Total Area:		1325723		

Concentration:	%
F	2.4
A1c	5.5
A2	24.0 *

Figure 8: Chromatogram showing Hb E Heterozygous as HbA2 is elevated.

Discussion

Hemoglobinopathies are the most common inherited disorders worldwide. These hereditary disorders are a major public health problem in many parts of the world including Southeast Asia.[1] Hemoglobinopathies are characterized by the presence of structurally defective Hb due to abnormalities in the formation of globin moiety of the molecule.[15]

For the evaluation of patients with suspected hemoglobinopathies multiple laboratory techniques and tests are utilized based on available resources. Some of these include commonly available tests like complete blood count, peripheral blood smear examination, osmotic fragility test like NESTROF (naked eye single tube red cell osmotic fragility test), sickling test and sickle solubility test. Hb F estimation by slide method using Betke Keilhauer method. Other tests available at some centres are Hb electrophoresis by acid and alkali method, HPLC and genetic testing.[7,16]

HPLC is a preferred diagnostic method for the evaluation of Hb disorders now a days as it lends itself easily to automated analysis thus significantly reducing the time required for evaluation of suspected hemoglobinopathies, in multiple studies have shown good results in detecting hemoglobinopathies. Hb fraction analysis by cation-exchange HPLC has the advantage of quantifying Hb F and Hb A2 along with Hb variant screening in a single, automated system with internal sample preparation, superior resolution, rapid assay time, high sensitivity, increase throughput and accurate quantification of Hb fractions makes this an ideal methodology and excellent technology for the routine clinical laboratory to screen for hemoglobinopathies.[17]

In our study, hemoglobinopathies were present in 15.60% of cases, similar findings have been observed in other studies done by Mandal et al.[18] Sachdev et al.[7] However, in some studies, as done by Patel et al.[19] and Ghosh et al.[6] reported higher percentages of hemoglobinopathies. This might be due to different referral criteria for HPLC testing in different institutes. Also, thalassemias and other hemoglobin variants are restricted to some particular geographical areas, caste, tribes and religion especially where marriages were confined to the same community and regions. However, now they are prevalent throughout the globe. The probable explanation to this is following increased migration of people from one place to other.[20]

In the present study, 64% of cases were constituted by beta thalassemia related hemoglobinopathies. In majority of other studies from Northern and Western India comparable findings have been observed.[19, 21, 22] In some studies done in Eastern India show higher proportion of sickle cell related hemoglobinopathies.[23,24] This high rate was possibly because study was done in high prevalence zone for sickle cell hemoglobinopathies.[23] This

increased occurrence of sickle cell disease in tribal populations in Eastern India as has been documented in such literature.[23,24]

In the present study, the microcytic hypochromic picture was most common in hemoglobinopathies and none of the hemoglobinopathies showed macrocytic picture. In a study done by KC et al.,[25] there was an almost equitable distribution of hemoglobinopathies with microcytic hypochromic and normocytic normochromic picture. In our study we did not get any cases with macrocytic anemia in contrast to study by KC et al.[25] In their study, there were cases with macrocytic picture this might be related to the higher prevalence of megaloblastic anemia and requires further study.

However, we could not come across any study in English literature which compared anisocytosis, anisochromasia, poikilocytosis and polychromasia RBC parameters on PBF. This could be because now-a-days most of the hematological studies are based on parameters obtained by CBC on automated hematology analyzers. However, what we observed in our study is that RBC morphological parameters did not differ substantially in cases with or without hemoglobinopathy (did not reach statistical significance) except for anisocytosis.

Conclusion

To conclude, we observed that there is a substantial presence of hemoglobinopathies in Haryana. The antenatal and pediatric diagnosis of hemoglobinopathies offers the opportunity to detect hemoglobinopathies in other siblings of the affected patient as well as the option of genetic counselling thereby preventing their occurrence in subsequent child births. It is very important that the population is screened so that carriers can be detected and informed about the various complications and reproductive risks. The usage of automated HPLC based techniques for the diagnosis of hemoglobinopathies makes it very helpful in this work and widespread adoption of HPLC techniques along with genetic counselling and more advanced techniques can lead to decreased occurrence of hemoglobinopathies thus promoting the wellbeing of the population.

Conflict of interest: None declared.

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Ethical approval: This study was approved by the Institutional Ethical Committee of Bhagat Phool Singh Government Medical College for Women, Haryana, India (Ref: BPSGMCW/RC/640/IEC/2021, Dated 26/02/2021).

Authors' contribution: Conceptualization: KS, P, RA; Design, investigation and data collection: KS, P, RA, MR; Data analysis: KS, P, RA, NC; Supervision, validation and visualization: SK, PR; Manuscript writing, editing and finalizing: KS, P, RA, MR, NC, SK, PR.

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