

# Examination of the primary fraction of hemoglobin in individuals having sickle cell disease using high-performance liquid chromatography



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## ABSTRACT

**Background:** Hemoglobinopathies refer to a range of diseases, in which the hemoglobin (Hb) molecule's synthesis or structure is aberrant. It is passed down through the generations (inherited). Hb C illness, Hb S-C disease, sickle cell anemia, and thalassemia are all part of this group. Hemoglobinopathies are the most frequent genetically inherited red blood cell condition on the planet. Hb variant screening and precise identification have become more crucial in the antenatal diagnosis and prevention of Hb diseases. **Aims and Objectives:** For Hb analysis, cation exchange high-performance liquid chromatography (HPLC) has emerged as the method of choice. This research was brought up to diagnose numerous hemoglobinopathies and also to measure distinct Hb fractions. **Materials and Methods:** Four hundred and seventy participants were examined for hemoglobinopathies in the investigation. Different fractions of Hb were compared to patients with normal Hb after diagnosis. **Results:** Normal HPLC, SCA (Hb SS), sickle cell trait (SCT), and  $\beta$ -thalassemia trait were discovered in 110 (23.4%), 130 (27.6%), 210 (44.6%), and 20 (4.2%) individuals, respectively, out of 470 patients. Rare hemoglobinopathies such as tenacious fetal Hb (PFH), Hb E, Hb D-Punjab, and Hb Q India accounted for 15 (3.1%) of the sufferers. Patients with SCA had a mean percent of Hb S, Hb F, Hb A, and Hb A2 of  $81.6 \pm 74.6$ ,  $22.9 \pm 13.9$ ,  $3.1 \pm 6.1$ , and  $2.5 \pm 1.02$ , respectively. Hb F was  $22.9 \pm 13.9\%$  on average, which was much higher than normal and SCT. A high HbA2 level ( $>3.5\%$ ) indicated the existence of the  $\beta$ -thalassemia trait. **Conclusion:** In the included section of the population, SCD is the most common kind of hemoglobinopathy, both homozygous and heterozygous. Hb F concentrations are observed to be elevated in SCA patients, and it is thought to be a key element in protecting patients from problems. Raised Hb A2, which indicates the presence of the  $\beta$ -thalassemia trait, was also discovered in this area, and it is suspected when the value is  $>3.7\%$ . Cation exchange HPLC is ideal for routine hemoglobinopathies research due to its high resolution, short test time, and precise quantification.

**Key words:** Fetal hemoglobin; Hemoglobin A2; Hemoglobinopathy; high-performance liquid chromatography; Sickle cell disease

## INTRODUCTION

The most recurrent genetically inherited illnesses are hemoglobinopathies. According to the World Health Organization (WHO) estimates, about 5% of the world's

population is genetically predisposed to hemoglobin (Hb) abnormalities. Over 44 million people are carriers each year, and over 13,000 babies take birth with a severe and clinically significant hemoglobinopathy. Hemoglobinopathies have a collective gene frequency

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of roughly 4.2% in India. Two types of inherited Hb problems include hemoglobinopathy and thalassemia. The former is caused by a structural flaw in Hb, whereas the latter is caused by a defect in globin synthesis, and both are transmitted in an autosomal recessive way.<sup>1</sup> Sickle cell disease (SCD), characterized by the exchange of valine for glutamic acid at position 6 of the  $\beta$  chain of globin, is one of the most recurrent hereditary diseases on the planet.<sup>2</sup> Sickle cell anemia (SCA) is caused by homozygous SCD (Hb SS), while sickle cell trait (SCT) is caused by heterozygous SCD (Hb SS) (SCT). SCD is defined by Hb SS or Hb S coupled with additional Hb variations, as well as clinical characteristics such as shortened RBC life span, persistent hemolytic anemia, and different organ damage.<sup>3</sup>

The two most common kinds of thalassemia are alpha ( $\alpha$ ) and beta ( $\beta$ ). They appear as a result of a slower rate of synthesis of the matching chain and chain. Due to the affinity, caste, and geographical endogamy, the ubiquity of  $\beta$ -thalassemia trait and sickle cell disease in several parts of India ranges from 3% to 17% and 1% to 44%, respectively.<sup>4</sup>

The concentrations of fetal Hb (HbF) and fetal Hb (HbA2) are critical determinants in the survival advantage of sickle cell disease.<sup>5</sup> It has been proven that patients with SCA have a higher concentration of Hb F, which causes the condition to be less severe.<sup>6</sup> The quantity of distinct fractions of Hb varies in hemoglobinopathies, impacting the clinical course of the disease.<sup>1</sup> Adult blood includes mostly Hb A, a tiny percentage of Hb A2, and a minimal amount of Hb F.

Cation exchange high-performance liquid chromatography (CE-HPLC) is one of the most effective procedures for screening, detecting, and identifying a heterogeneity of hemoglobinopathies with quick, repeatable, and precise findings. It offers the advantage of being able to quantify Hb F and Hb A2 as well as screen for Hb variants in a single, highly repeatable method. Molecular techniques can be used to definitively identify mutations, especially in the case of rare Hb variants. Our study's goals were to estimate the generality of various hemoglobinopathies in our investigative population and to characterize each hemoglobinopathy that was discovered. Furthermore, our research intended to discover any unusual Hb variants using DNA sequencing whenever possible.

### Aims and objectives

For Hb analysis, cation exchange high-performance liquid chromatography (HPLC) has emerged as the method of choice. This research was brought up to diagnose numerous hemoglobinopathies and also to measure distinct Hb fractions.

## MATERIALS AND METHODS

From January 2018 to December 2020, the present study was undertaken in the Department of Medicine, VSS Institute of Medical Sciences and Research, Burla, and the Gupta Diagnostic and Research Centre, Burla. The study was pre-approved by the Institutional Ethics Committee of Gupta Diagnostics for the final permission. This study involved 470 patients who went to the Medicine OPD and Gupta Diagnostic Center for sickle cell disease screening. Following an in-depth clinical examination, blood was drawn for a thorough hematological analysis. In all cases, a sickling test and Hb electrophoresis on an agar gel were performed. HPLC was applied in each case for hemoglobinopathy screening and measurement of main Hb components. It was carried out using the Bio-Rad variant Hb testing system and the variant  $\beta$ -thalassemia short program from Bio-Rad Laboratory's in the United States. A vacuum blood collection tube containing EDTA as an anticoagulant was used to collect whole blood for this study.

5  $\mu$ L of blood were pipetted into a 1.5 mL sample vial. 1 mL hemolytic reagent was added to it. Inversion was applied to cover and mix each sample vial. The program was started after the sample vial was placed in the variation sample tray. When a batch of 15 samples was ready, the blood was extracted and stored at  $-10^{\circ}\text{C}$  for the test. At the beginning and conclusion of each group of patient specimens, a set of normal (Hb F 1–2%, Hb A2 1.8–3.2%) and pathological (Hb F 5–10%, Hb A2 6%) controls were conducted. The percentage of each Hb fraction is estimated and examined based on the chromatographic analysis.<sup>7</sup>

## RESULTS

A total of 470 patients were examined for hemoglobinopathies, and all major Hb fractions were determined using HPLC. The bulk of the patients were between the ages of 21 and 30. The ratio of males to females was 1.4:1. Normal HPLC, SCA (Hb SS), SCT, and  $\beta$ -thalassemia trait were discovered in 110 (23.4%), 130 (27.6%), 210 (44.6%), and 20 (4.2%) cases, respectively, out of 470 patients. Rare hemoglobinopathies such as recurrent fetal Hb (PFH), Hb E, Hb D-Punjab, and Hb Q India accounted for 15 (3.1%) of the sufferers.

Patients with normal Hb were used as controls, while patients with unusual Hb variations ( $n=15$ ) were eliminated from the research due to the small unit of patients. Hb S, Hb A0, and Hb A2 mean values in SCA were  $81.6\pm 74.6$ ,  $3.1\pm 6.1$ , and  $2.5\pm 1.02$ , respectively (Table 1). The mean Hb F was  $22.9\pm 13.9\%$ , which was significantly higher than

usual, and the SCT was 22.9±13.9%. Further, investigation revealed that Hb F levels of <10%, 10–20%, 20–30%, and 30–40% were detected in 17 (13%), 44 (33.8%), 53 (40.7%), and 15 (11.5%) of SCA patients, respectively (Table 2). Hb F was between 20 and 30% in 41.1% of patients, with a mean of 25.5±3.1. In 20 cases, the β-thalassemia trait was discovered. HbA2 levels were elevated (>3.7%) in 26 (of 129), 62 (212) instances of SCD and SCT patients, and associated β-thalassemia trait was found (Table 3).

## DISCUSSION

HPLC is a sensitive and fast technology for detecting hemoglobinopathies and quantifying different Hb fractions. Unlike electrophoresis and column techniques, HPLC analysis of Hb integrates three processes in a single assay: Separation, identification, and area percentage measurement of distinct Hb fractions.<sup>7,8</sup> Retention time windows, such as S, C, and D windows, are used to identify distinct Hb variations and are considered an ideal method for Hb analysis.<sup>7</sup>

Hb F is more in this series than the African haplotype and is similar to Saudi Arab patients, who are classified as Arab-Indian haplotype. The Hb F molecule prevents Hb S molecules from polymerizing and so protects against early life-threatening conditions, and high levels are associated with a benign course.<sup>9</sup> A threshold level

of 20% Hb F has also been discovered to be required for a positive effect.<sup>10</sup>

Higher Hb F levels in the order of 25% are found in Eastern Arabian and Indian SCA populations who are announced to have a relatively mild course of the ailment. In the current investigation, around 68% of patients had Hb F levels higher than 20%. An earlier study from this center revealed that the average Hb F was 16.8%.<sup>4,6</sup> It is 22.9±13.9%, according to our research, and this is related to HPLC estimation. Low Hb F was identified among Africans when compared to Arab-Indian (Asian) haplotypes, which has been linked to the disease’s malignant course.<sup>11</sup>

### Limitations of the study

The limitation of this study is that molecular analysis was not conducted which would have conclusively excluded the diagnosis of β-thalassemia. We recommend that in cases where a possible diagnosis of Hb S/0 thalassemia is suspected, the Hb profile be conducted by HPLC instead, or that if it is, a second confirmatory test be performed. In addition, if there are any questions, a molecular investigation should be performed to discover deletions or other mutations, which will confirm the clinical diagnosis and lead genetic counselling.

## CONCLUSION

The most common single-gene Hb diseases in the world are α and β thalassemia. These illnesses, which were once isolated to a specific region, religion, caste, or tribe, are now found all over the world. Global human migration and marriages of both diverse groups and endogamous norms have had a substantial impact on the global growth in cumulative instances of hemoglobinopathies.

HbF is quite low in normal people, accounting for <0.6% of total Hb. In this series, a higher Hb F concentration among SCA has been recognized as a key component that protects patients from problems and hence increases survival. The legacy of genes that determine high Hb F levels is a mystery. A gene well removed from the β globin locus, presumably on a distinct autosome, the X-chromosome in Asian haplotype (Dover et al.),<sup>12</sup> is one possible genetic component that may contribute to the elevation of Hb F levels.

In this section of the population, SCD, both homozygous and heterozygous, is the most common form of a hemoglobinopathy, according to the current study. Hb F concentrations are observed to be higher in SCA patients, and it is thought to be a key element in protecting patients from problems. Raised Hb A2, which indicates the presence of the β-thalassemia trait, was also discovered in this area,

**Table 1: Age and sex distribution**

Age group	Male	Female	Total No.	%
<5 years	21	16	37	7.8
6–10	42	26	68	14.4
11–20	65	41	106	22.4
21–30	76	57	133	28.2
31–40	50	42	92	19.4
41–50	22	14	36	7.6
	276	196	472	

M: F=1.4:1

**Table 2: Fractions of hemoglobin**

Group	A0	A2	F	S	C
Normal 110	85.5±4.8	3.2±1.1	1.3±1.5	-	-
SCA 130	3.1±6.1	2.5±1.02	22.9±13.9	81.6±74.6	-
SCT 210	36.5±36.2	3.3±2.3	3.7±1.9	35.5±10.2	-
PFH 5	19.6±11.4	7.1±2.5	79.6±20.1	-	-

**Table 3: Foetal Hb in SCA**

Foetal Hb (%)	No. of patients	Mean Hb F (%)
<10	17 (13.2%)	5.2±3.4
10.1–20	44 (34%)	15.4±2.8
20.1–30	53 (41.1%)	25.5±3.1
30.1–40	15 (11.6%)	35.1±3.4

and it is suspected when the value is >3.7%. HPLC has been used to detect Hb variations quickly and precisely, as well as sensitively quantify HbA.

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### Authors Contribution:

**MKM**- Prepared manuscript and revision; **PKB**- Concept and design of the study, reviewing the past literature; **KT**- Statistical analysis, coordinating, and interpreting findings; **NKJ**- Preparing first draft of manuscript

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