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Research Article

Evaluation of HPLC Patterns and Red Cell Parameters in Sickle Cell Anaemia Cases

Dr. Hemangini Patel[1], Dr. Dipti Gajjar[2], Dr. Nayan Koitiya

[1]Senior Resident, GMERS Medical College, Attached Hospital, Rajpipla
[2]Assistant Professor, GMERS Medical College, Attached Hospital, Rajpipla
[3]Associate Professor, GMERS Medical College, Attached Hospital, Rajpipla

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Corresponding Author:

Dr. Nayan Koitiya
Associate Professor, GMERS Medical College, Attached Hospital, Rajpipla.

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ABSTRACT

Background: Sickle cell anaemia (SCA) is an inherited haemoglobinopathy characterized by the presence of abnormal haemoglobin S (HbS). High-performance liquid chromatography (HPLC) provides accurate qualitative and quantitative assessment of haemoglobin variants, enabling reliable diagnosis. Red cell indices such as haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and reticulocyte count help evaluate disease severity and associated haematological changes.
Aim: To evaluate HPLC patterns and red cell parameters in patients with sickle cell anaemia.
Methods: This cross-sectional observational study analysed confirmed SCA cases. Venous blood samples were collected in EDTA tubes. Quantitative fractions of HbS, HbF, and HbA2 were recorded. Complete blood counts were performed using a 5-part haematology analyser, including Hb, RBC count, HCT, MCV, MCH, MCHC, RDW, and reticulocyte percentage.
Results: Most patients showed classical HPLC findings with markedly elevated HbS levels (60–95%), increased HbF (5–25%), and normal or mildly raised HbA2. Red cell parameters showed anaemia in the majority of cases, with Hb levels commonly ranging between 6–10 g/dL. MCV and MCH values were generally low-normal or decreased. Marked elevation in RDW and reticulocyte count was observed, reflecting hemolytic activity. Patients with higher HbF levels demonstrated milder anaemia and fewer clinical complications.
Conclusion: HPLC provides a reliable diagnostic pattern for sickle cell anaemia, characterized by high HbS and increased HbF levels. Red cell indices show significant hemolysis and anisopoikilocytosis. Combined evaluation of HPLC and haematological parameters offers an efficient approach for the diagnosis and monitoring of SCA.

Keywords*: Sickle cell anemia, HbSS; Hemoglobin S, High-performance liquid chromatography (HPLC), Fetal hemoglobin (HbF), Hemolysis; Red cell indices, Reticulocytosis, Peripheral smear, Hemoglobinopathies, Hematological profile, Disease severity..*

INTRODUCTION

Sickle cell anaemia (SCA) is one of the most prevalent monogenic disorders worldwide and is caused by a point mutation in the β-globin gene, resulting in the formation of haemoglobin S (HbS). "Polymerization of deoxygenated HbS leads to RBC sickling, chronic hemolysis, and vaso-occlusion" [1]. The burden is particularly high in India, especially in Gujarat, Maharashtra, Madhya Pradesh, Odisha, and tribal belts, where the prevalence of the sickle cell gene ranges between **8–18%** as reported in large community surveys [2].

High-performance liquid chromatography (HPLC) has emerged as the gold standard for the detection and quantification of haemoglobin variants because of its accuracy and reproducibility [3]. Previous studies have shown that patients with

SCA typically demonstrate **HbS levels >60–90%**, increased HbF levels (5–25%), and absence of HbA [4]. Red cell indices such as Hb, MCV, MCH, reticulocyte count, and RDW reflect hemolysis severity and bone marrow response.

Because of the significant morbidity associated with the disease, comprehensive evaluation using both **HPLC patterns** and **RBC parameters** is essential to characterize disease severity and guide management. This study builds on earlier findings reported by Patel et al. [5] and Fernandes et al. [6] to assess haematological profiles among SCA patients in Western India.

Aim

To evaluate HPLC patterns and red cell parameters in sickle cell anaemia cases.

Objectives

1. To analyse HPLC chromatogram patterns in confirmed sickle cell anaemia (HbSS) cases.
2. To assess red cell parameters (Hb, RBC count, MCV, MCH, MCHC, RDW, reticulocyte count).
3. To correlate HPLC findings with haematological severity.

METHODOLOGY

This cross-sectional study included 100 confirmed sickle cell anaemia (HbSS) patients. The sample size was calculated using the formula $4pq/d^2$, taking a prevalence of 12% from the regional epidemiological survey conducted by Singh et al. [7], which yielded a minimum required sample of 81; however, 100 patients were included for better statistical representation. All HbSS cases confirmed by HPLC were included, while patients with sickle cell trait, other haemoglobinopathies, or recent transfusion were excluded. Blood samples were collected in EDTA vacutainers and analysed using an automated haematology analyser to obtain haemoglobin levels, RBC indices, RDW, and reticulocyte counts. Peripheral smears were evaluated for morphological abnormalities. HPLC was performed using the TOSOH G8 HLC 723 system to quantify HbS, HbF, HbA2, and other variant fractions. Hemolytic markers such as LDH, indirect bilirubin, and haptoglobin were measured when available. All results were compiled, statistically analysed, and correlated with disease severity and existing literature.

RESULTS

A total of **100 confirmed sickle cell anaemia (HbSS)** patients were included in the study. HPLC analysis demonstrated classical chromatographic patterns typical of homozygous sickle haemoglobinopathy. The mean HbS level was **78.5%** (range 60–95%), showing considerable inter-individual variation. Among these, 71% of patients had HbS levels above 75%, consistent with a severe phenotype, whereas 29% had HbS values between 60–75%, often associated with higher HbF levels or co-inherited alpha-thalassemia. HbF values ranged from **5% to 25%**, with a mean of **12.4%**; 36% of patients had HbF >15%, which correlated with milder disease expression. HbA was absent in all samples, and HbA2 was within normal or mildly elevated limits.

Table 1: HPLC Fraction Distribution in SCA Patients (n = 100)

Haemoglobin Fraction	Mean (%)	SD	Range (%)
HbS	78.5	8.4	60–95
HbF	12.4	6.5	5–25
HbA2	3.1	0.7	2–4.5
HbA	0	—	0
Variant Peaks	0.2	—	0–0.5

Patients demonstrated classical HbSS patterns with high HbS and elevated HbF. Haematological parameters revealed significant anaemia and features of chronic haemolysis. The mean haemoglobin level was **8.2 g/dL**, with 82% of patients showing Hb <9 g/dL. Hematocrit averaged **26%**, and RBC count was reduced (mean **3.0 million/µL**). Red cell indices showed microcytic to normocytic patterns with an average MCV of **78.5 fL**. MCH and MCHC were low. RDW was markedly elevated in most cases, and reticulocyte counts averaged **8.5%**, indicating active marrow response.

Table 2: Haematological Parameters in SCA Patients

Parameter	Mean ± SD	Range	Interpretation
Haemoglobin (g/dL)	8.2 ± 1.5	5.6–11.4	Low
Hematocrit (%)	26 ± 3	20–32	Low
RBC Count (million/µL)	3.0 ± 0.6	2.1–4.3	Low

MCV (fL)	78.5 ± 6.2	65–91	Low-normal
MCH (pg)	24.1 ± 2.8	18–31	Low
MCHC (g/dL)	30.5 ± 1.2	28–33	Low
RDW (%)	21.5 ± 3.3	17–28	High
Reticulocyte (%)	8.5 ± 2.1	4–14	High

Elevated RDW (91%) and reticulocytosis (88%) indicate chronic hemolysis and marrow hyperactivity.Hemolytic markers further confirmed ongoing hemolysis. LDH levels were elevated (mean **580 U/L**) and indirect bilirubin (mean **2.1 mg/dL**) was raised in most patients. Haptoglobin was markedly reduced (<10 mg/dL), consistent with increased red cell destruction. Patients with HbF levels greater than 15% displayed lower LDH and bilirubin levels, suggesting reduced hemolysis.

Table 3: Hemolytic Indicators

Indicator	Mean ± SD	Interpretation
Reticulocyte (%)	8.5 ± 2.1	High (Hemolysis)
LDH (U/L)	580 ± 160	Elevated
Indirect bilirubin (mg/dL)	2.1 ± 0.8	Elevated
Haptoglobin (mg/dL)	<10	Low/Absent

Peripheral smear findings supported haematological abnormalities. Sickle cells were present in **96%**, confirming the hallmark morphology. Target cells (74%), polychromasia (82%), and anisopoikilocytosis (89%) were common. Nucleated RBCs (28%) reflected severe erythroid stress.

Table 4: Peripheral Smear Findings

Smear Feature	Frequency (%)
Sickle cells	96%
Polychromasia	82%
Target cells	74%
Tear drop cells	32%
Microcytes	41%
Macrocytes	12%
Anisopoikilocytosis	89%
Nucleated RBCs	28%

A strong relationship was observed between HbF levels and disease severity. Patients with HbF <10% exhibited the most severe anaemia and the highest reticulocyte percentages. Those with HbF 10–20% showed moderate haematological abnormalities, whereas patients with HbF >20% demonstrated milder anaemia and lower hemolytic markers. This highlights the protective role of elevated HbF.

Table 5: Correlation of HbF Levels With Haematological Severity

HbF Category	n (%)	Mean Hb (g/dL)	Mean Retic (%)	Smear Severity	Interpretation
<10%	41	7.5	10.1	Severe	Severe disease
10–20%	33	8.4	8.2	Moderate	Moderate disease
>20%	26	9.6	5.5	Mild	Mild disease

Sickle cell anaemia patients exhibited classical HPLC patterns with high HbS and elevated HbF. Haematological parameters revealed significant anaemia, elevated RDW, and reticulocytosis, confirming chronic hemolysis. Hemolytic markers were markedly abnormal, and peripheral smear findings showed classic sickle morphology. HbF levels played a major modifying role, with higher HbF linked to milder disease severity. The combined interpretation of HPLC data, red cell indices, hemolytic markers, and smear morphology provided a complete assessment of disease severity in this population.

DISCUSSION

In the present study of 100 sickle cell anaemia (HbSS) patients, the HPLC chromatographic patterns showed classical findings consistent with previously published literature. The mean HbS level of **78.5%** is comparable to findings by **Patel et al.**, who reported HbS levels of **76–82%** in Western India [5]. Similarly, **Jain et al.** documented HbS ranging from **70–90%**, indicating similar genetic expression across Indian populations [3]. The complete absence of HbA in all patients in our study aligns with the observations of **Fernandes et al.**, who highlighted that HbA is never present in homozygous SCA [6].

The mean HbF level of **12.4%** corresponds with the findings of **Balgir**, who reported HbF levels between **10–20%** in tribal communities due to hereditary persistence of fetal haemoglobin [4]. In our cohort, **36%** of patients had HbF >15%, correlating with milder anaemia. This trend supports results from the **Cooperative Study of Sickle Cell Disease**, where HbF >15% markedly reduced vaso-occlusive episodes and hemolysis [12]. Similarly, **Steinberg et al.** demonstrated that even a **1% increase in HbF** significantly improves clinical severity and reduces hospitalisation [13].

Our mean haemoglobin value of **8.2 g/dL** is consistent with **Fernandes et al.**, who reported haemoglobin values of **7.5–9.0 g/dL** among Indian SCA patients [6]. Elevated RDW and reticulocyte counts closely resemble findings from **Khodabhai et al.**, who documented reticulocytosis between **6–12%**, reflecting chronic hemolysis and marrow compensation [8]. Microcytic to normocytic MCV changes also matched the findings of **Mohanty et al.**, who reported that α-thalassemia and nutritional deficiencies commonly influence RBC indices in Indian SCA patients [9].

Peripheral smear findings in our study—such as sickled cells (96%), target cells (74%), and polychromasia (82%)—are similar to the results of **Adedeji et al.**, who observed sickle cells in **92–98%** and target cells in **70–80%** of their cohort [10]. The presence of nucleated RBCs in **28%** of cases reflects severe erythroid stress and matches the findings of **Naveen et al.**, who reported NRBC frequencies between **25–30%** [11].

Hemolytic indicators such as elevated LDH, high indirect bilirubin, and low haptoglobin further confirmed ongoing hemolysis. These findings align with reports from **Khodabhai et al.** and the **Cooperative Study Group**, both demonstrating that LDH strongly correlates with disease severity [8,12]. In our study, patients with HbF >15% had lower LDH and reticulocyte counts, supporting the protective effect of HbF described by **Steinberg et al.** [13].

Overall, our study reinforces established evidence that sickle cell anemia demonstrates classic HPLC patterns, significant anemia, hemolysis, and characteristic smear abnormalities. The modifying role of fetal haemoglobin observed in our data is consistent with findings from **Balgir**, **Platt et al.**, and **Steinberg et al.** [4,12,13]. Integrating HPLC results with RBC indices and hemolytic markers provides a comprehensive assessment of disease severity in sickle cell anemia.

CONCLUSION

This study demonstrates that sickle cell anemia shows classical HPLC patterns with high HbS, elevated HbF, and absent HbA, confirming the HbSS genotype. Hematological findings revealed significant anemia, high RDW, and increased reticulocyte counts, indicating chronic hemolysis. Peripheral smear abnormalities supported these findings. Patients with higher HbF levels showed milder disease severity, highlighting the protective effect of HbF. Overall, combining HPLC results with red cell indices provides a reliable and comprehensive assessment of disease severity in sickle cell anemia.

RECOMMENDATIONS

Routine use of HPLC is recommended for accurate diagnosis and monitoring of sickle cell anemia. Regular evaluation of hematological parameters, HbF levels, and peripheral smears should be performed to assess disease severity and guide treatment. Strategies that increase HbF, such as hydroxyurea therapy, may benefit patients with severe disease. Screening and early diagnosis in high-prevalence regions should be strengthened, along with patient education and genetic counselling to improve long-term outcomes.

REFERENCE

1. Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. Lancet. 2010;376(9757):2018–2031.
2. Government of India. Ministry of Health and Family Welfare. National Health Mission: Status of Sickle Cell Disease in India. Annual Tribal Health Report. 2019.
3. Jain D, Chowdhury V, Choudhry VP. High-performance liquid chromatography in diagnosis of haemoglobin opathies. J Pediatr Hematol Oncol. 2010;32(6):e213–e216.
4. Balgir RS. Spectrum of haemoglobin opathies in tribal populations in India. Indian J Med Sci. 2006;60(9):326–333.

5. Patel AP, Naik MR, Shah NM, Sharma N, Parmar P. Haemoglobin opathy in tribal populations of Gujarat. Indian J Hematol Blood Transfus. 2012;28(2):105–109.
6. Fernandes B, Nair S, Satpute S. Hematological profile of sickle cell disease. J Clin Diagn Res. 2015;9(5):EC01–EC04.
7. Singh PJ, Shrivastava AC, Shrikhande AV. Pattern of haemoglobin opathies in central India. Int J Biol Med Res. 2012;3(3):1850–1855.
8. Khodabhai V, Mehta R, Patel P. Hemolytic markers in sickle cell anemia. Int J Med Sci Public Health. 2014;3(2):214–217.
9. Mohanty D, Colah RB, Gorakshakar AC, et al. Prevalence of haemoglobin opathies in India. J Community Genet. 2013;4(1):33–42.
10. Adedeji MO, Cumming V, Thambinayagam J, et al. Peripheral blood morphology in sickle cell anemia. Clin Lab Haematol. 2010;32:410–415.
11. Naveen R, Thomas R, Devi S. Bone marrow stress indicators in sickle cell anemia. Indian J Pathol Microbiol. 2013;56(2):120–124.
12. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality and morbidity in sickle cell disease. N Engl J Med. 1994;330(23):1639–1644.
13. Steinberg MH, Nouraie M, Habara A, et al. Fetal haemoglobin and disease severity. Blood. 2014;123(4):481–485.