International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Impact Factor: 7.10
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Email: editor@ijarets.org Volume-11, Issue-9 September – 2024 www.ijarets.org

IDENTIFICATION AND CHARACTERIZATION OF HEMOGLOBIN VARIANTS IN INDIAN SICKLE CELL DISEASE PATIENTS USING PROTEIN ELECTROPHORESIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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ABSTRACT

In rural and tribal India, sickle cell disease (SCD) affects a disproportionate number of people. Genetic variability is significant due to hemoglobin (Hb) changes. These variations affect clinical severity and treatment. To diagnose, forecast, and give genetic counseling, hemoglobin variants must be correctly identified and characterized. This study will discover and characterize hemoglobin changes in Indian sickle cell disease patients using protein electrophoresis and HPLC. Hospital cross-sectional research included SCD patients who attended a tertiary care center. Alkaline protein electrophoresis and cation-exchange HPLC were used to analyze aseptically prepared venous blood samples. Abnormal hemoglobin bands were discovered by electrophoretic patterns, and HPLC measured hemoglobin fractions such HbS, HbF, HbA, and HbA_2. Demographic and clinical data were used to quantitatively measure hemoglobin variance. Patients' HbF and HbA_2 values varied, but HbS was more prevalent. HPLC was more sensitive and accurate than protein electrophoresis in identifying minute hemoglobin fractions. Combining the two approaches improved sickle cell disease genotype identification and diagnosis accuracy. Electrophoresis and HPLC are needed to assess hemoglobin variations, according to this study. Sickle cell disease is diagnosed using protein electrophoresis and HPLC to identify hemoglobin changes. Together, they enhance genetic counseling, clinical management, and diagnostic reliability in India.

Keywords: *Sickle cell disease, Hemoglobin variants, Protein electrophoresis, HPLC, India*

INTRODUCTION

Sickle cell disease (SCD), which is one of the most common genetic hemoglobin illnesses in the world, is a significant cause for worry in terms of public health in India. When a certain mutation occurs in the β-globin gene, it leads to the formation of the abnormal hemoglobin S (HbS) by substituting glutamic acid with valine at position six of the β-globin chain. This disorder is characterized by aberrant hemoglobin S. Hemoglobin S (HbS) polymerization in an oxygen-depleted environment results in the formation of sickle cells in red blood cells. Erythrocytes that are deformed and rigid are the root cause of a number of medical conditions, including vaso-occlusive crises, chronic hemolytic anemia, and

progressive organ destruction. A significant percentage of the reported variance in the clinical severity of sickle cell disease (SCD) may be attributed to the fact that different hemoglobin variants and genetic factors that can be altered are responsible for the difference. Sickle cell disease is one of the most burdensome illnesses in the world since it is prevalent among certain socio-economically disadvantaged groups and tribal tribes in India. This situation makes it one of the most prevalent diseases in the world. It has been observed that some states in India, such as Odisha, Madhya Pradesh, Gujarat, Chhattisgarh, and Odisha, as well as certain regions in southern India, have particularly high frequencies of the sickle cell gene. The diversity of the Indian population has resulted in the production of a wide variety of hemoglobin variants, such as HbS, HbF, HbA_2, HbE, and HbD, as well as compound heterozygous illnesses such as HbS-β-thalassemia. The presence of these variants and the relative percentage of them all have a significant impact on how they manifest, the degree of severity they exhibit, and the degree to which they respond to treatment.

There is a direct correlation between the accurate identification and description of hemoglobin abnormalities and the effective diagnosis and treatment of sickle cell disease. Early identification, which enables proper clinical treatment, genetic counseling, and prenatal diagnosis, is a means by which the burden of sickness may be reduced and the quality of life can be improved. In spite of the fact that tests for solubility and sickling are useful for screening, they are not accurate enough to properly quantify hemoglobin fractions. Therefore, in order to accurately diagnose and classify hemoglobinopathies, it is necessary to use the most advanced laboratory tests present today. When it comes to sorting and distinguishing distinct forms of hemoglobin based on their mobility and charge in an electric field, protein electrophoresis has been the gold standard for a considerable amount of time. Through the use of alkaline hemoglobin electrophoresis, it is possible to differentiate between the many types of normal and diseased hemoglobin by observing the bands that correspond to each type. However, this approach has a number of limitations, the most significant of which is that it is particularly difficult to discern between variants of hemoglobin, such as HbS and HbD, which have similar electrophoretic mobility, and to accurately detect tiny fractions of hemoglobin.

Ever with the introduction of high-performance liquid chromatography (HPLC), the process of analyzing different types of hemoglobin has been much simpler. In order to precisely quantify HbA, HbF, HbA_2, and aberrant hemoglobins, high-performance liquid chromatography (HPLC) is used to separate these hemoglobins into fractions based on their retention time. It is possible to discover small changes using high-performance liquid chromatography (HPLC), which offers numerous benefits over electrophoresis. These advantages include better resolution, increased repeatability, and the ability to identify tiny variations. Because of this, high-performance liquid chromatography, often known as HPLC, is increasingly being considered the diagnostic tool of choice for hemoglobinopathies. Due to the fact that many healthcare institutions in India continue to employ a single method for hemoglobin analysis, even if there are updated diagnostic tools available, it is possible that certain variants would be misclassified or underdiagnosed. It is advised that protein electrophoresis be used with high-performance liquid chromatography (HPLC) in order to increase diagnostic accuracy. By doing so, you will be able to overcome the limitations that each strategy separately has. For the purpose of providing diagnostic techniques that are consistent across the board, comparative studies that evaluate the effectiveness of these approaches in the Indian context are very necessary. Within the scope of this inquiry, protein electrophoresis and high-performance liquid chromatography will be used in order to

identify and characterize differences in hemoglobin levels among sickle cell disease patients originating from India. Through an examination of the distribution and proportion of hemoglobin fractions, the purpose of this research is to provide vital information about the hemoglobin variation profile of patients with sickle cell disease in India. This article also makes an effort to highlight the diagnostic effectiveness of integrating these different methodologies. Researchers in India have high hopes that their findings will contribute to the improvement of diagnostic procedures, facilitate the implementation of early intervention programs, and strengthen genetic counseling and screening programs.

REVIEW OF LITERATURE

Syphilis, also known as sickle cell disease (SCD), is one of the most prevalent kinds of hemoglobinopathies, which are inherited diseases that have an effect on the structure and function of red blood cells. Numerous studies have shown that SCD has a substantial influence in India, particularly among certain ethnic groups and communities that are considered to be tribal members. In light of the fact that the sickle cell gene is found in anywhere from one percent to forty percent of certain tribal tribes in India, Balgir (2005) asserts that there is an urgent want for innovative screening and diagnostic techniques. As a consequence of the genetic diversity that exists within the Indian population, there are several variants of hemoglobin, which have a significant influence on the phenotype of medical conditions. A variety of research have been conducted to investigate the ways in which the severity of sickle cell disease (SCD) is affected by the various forms of hemoglobin. According to Steinberg et al. (2001), greater levels of fetal hemoglobin (HbF) are associated with a reduction in the severity of the illness, a reduction in the number of vaso-occlusive episodes, and an increase in mortality in individuals with sickle cell disease. In their study, Mukherjee and Ghosh (2010) discovered that individuals with sickle cell disease from India had higher HbF levels than persons from Africa. This finding may suggest that the clinical result is less severe in some cases. The importance of accurate measurements of HbF and other fractions of hemoglobin is brought into focus by this statement.

When it comes to identifying differences in hemoglobin, protein electrophoresis in the laboratory has always been the method of choice. According to Dacie and Lewis (2016), alkaline hemoglobin electrophoresis is a technique that may be beneficial for initial screening. This approach separates different fractions of hemoglobin based on the electrical charge that they possess. According to research carried out by Rao et al. (2012), electrophoresis was shown to be effective in determining the presence of common variants such as HbS and HbA. However, there is a possibility of diagnostic ambiguity due to the fact that the approach is not completely capable of discriminating between varieties of hemoglobin such as HbS, HbD, and HbG, which exhibit migratory patterns that are comparable to one another. To bypass these limitations, high-performance liquid chromatography (HPLC) has evolved as a reliable diagnostic tool. This is a fortunate development. High-performance liquid chromatography (HPLC) enables the accurate measurement of hemoglobin fractions as well as the precise detection of minute changes. It has been stated by Joutovsky et al. (2004) that high-performance liquid chromatography (HPLC) is a useful method for screening and verifying diagnosis owing to its high sensitivity and specificity in identifying changes in hemoglobin. Several research undertaken in India, including the ones carried out by Sachdev et al. (2016), have shown that high-performance liquid chromatography (HPLC) has the capability to identify compound heterozygous conditions, such as HbS-β-thalassemia, which electrophoresis alone generally fails to detect.

A comparison of electrophoresis and high-performance liquid chromatography has been conducted, and the results have shown how effectively these two techniques complement one another. In spite of the fact that electrophoresis is easily available and affordable, Mondal et al. (2018) pointed out that high-performance liquid chromatography (HPLC) offers superior resolution and quantitative accuracy. Their findings showed that the two methods should be combined in order to conduct a comprehensive examination of hemoglobin. In a similar vein, Patel et al. (2020) attracted attention to the fact that high-performance liquid chromatography (HPLC) assists in the identification of β-thalassemia features in sickle cell disease patients by assisting in the detection of higher levels of HbA_2. According to the findings of recent research, the importance of early and accurate diagnosis during genetic counseling and sickness treatment cannot be overstated. According to Weatherall and Clegg (2001), the accurate detection of hemoglobin variants is an essential component of both prenatal diagnosis and carrier screening programs designed to identify potential carriers. High-performance liquid chromatography (HPLC) has been increasingly integrated into national screening programs in India due to its reliability as a diagnostic tool. In spite of this, accessibility and cost continue to be challenges in circumstances when there are few resources.

There has been a significant amount of research conducted on sickle cell disease (SCD), however there is still a dearth of information that is pertinent to the Indian subcontinent about the distribution of hemoglobin variants. Due to the fact that many studies only employ one diagnostic approach, there is a possibility of underdiagnosis or misclassification occurring. As a result of this, we need more research that makes use of combination diagnostic approaches in order to get a deeper understanding of the patterns of hemoglobin variations and to develop diagnostic processes that are tailored to the Indian population.

OBJECTIVES OF THE STUDY

- To identify hemoglobin variants in SCD patients
- To characterize Hb fractions using electrophoresis and HPLC
- To compare diagnostic efficiency of both techniques

The present study was undertaken to identify and characterize hemoglobin variants among Indian patients suspected or diagnosed with sickle cell disease using protein electrophoresis and high-performance liquid chromatography (HPLC). A total of 100 blood samples were analyzed, including patients clinically suspected of hemoglobinopathies and healthy controls. The study population comprised individuals aged between 5 and 60 years, reflecting the wide age range over which hemoglobin disorders manifest clinically. A higher prevalence of abnormal hemoglobin variants was observed in individuals from central and eastern India, regions known for a high burden of sickle cell disease and related hemoglobinopathies. Initial screening by hemoglobin electrophoresis revealed distinct migration patterns corresponding to normal and variant hemoglobins. In healthy control samples, electrophoresis demonstrated a predominant HbA band with minimal or absent abnormal fractions. In contrast, patient samples showed variable patterns, including the presence of HbS, HbF, HbD, and mixed hemoglobin bands. These findings confirmed the heterogeneity of hemoglobin variants

in the Indian population and underscored the importance of electrophoretic techniques in preliminary screening.

Quantitative analysis using HPLC provided precise separation and measurement of hemoglobin fractions. HPLC chromatograms of normal individuals showed a dominant HbA peak with small proportions of HbA2 and HbF, consistent with established reference ranges. In patients with sickle cell trait, HPLC demonstrated the presence of both HbA and HbS peaks, with HbA being the predominant fraction. The relative proportion of HbS in trait individuals ranged between 30–40%, which is characteristic of heterozygous inheritance. These findings highlight the effectiveness of HPLC in distinguishing sickle cell trait from normal hemoglobin patterns. In patients diagnosed with sickle cell disease, HPLC analysis revealed a predominant HbS peak with markedly reduced or absent HbA. Elevated levels of HbF were observed in many sickle cell disease patients, particularly among pediatric cases. The increased HbF fraction is clinically significant, as fetal hemoglobin is known to inhibit sickling and reduce disease severity. The variability in HbF levels among patients suggests genetic and environmental influences on hemoglobin expression.

Hemoglobin D variant was identified in a subset of patients, either as HbD trait or in compound heterozygosity with HbS. HPLC chromatograms of HbD cases demonstrated a distinct peak with retention time different from HbA and HbS. In compound heterozygous HbSD cases, both HbS and HbD peaks were evident, along with varying levels of HbF. These findings emphasize the complexity of hemoglobin variant combinations in the Indian population and the need for advanced diagnostic tools for accurate characterization. Protein electrophoresis patterns complemented the HPLC findings by providing visual confirmation of hemoglobin migration. HbA migrated fastest toward the anode, while HbS and HbD showed slower migration due to differences in charge and molecular structure. In sickle cell disease patients, the dominance of the HbS band was evident, whereas in trait cases, the presence of both HbA and HbS bands was clearly distinguishable. Electrophoresis thus served as an effective qualitative method, while HPLC provided quantitative precision. Comparative analysis between electrophoresis and HPLC demonstrated a high degree of concordance in identifying major hemoglobin variants. However, HPLC proved superior in quantifying minor hemoglobin fractions such as HbA2 and HbF. This was particularly important in differentiating sickle cell disease from beta-thalassemia and other compound hemoglobinopathies, where elevated HbA2 levels play a diagnostic role.

Statistical analysis of hemoglobin fractions revealed significant differences between normal individuals, sickle cell trait carriers, and sickle cell disease patients. Mean HbS levels were significantly higher in disease patients compared to trait individuals, while HbA levels were significantly reduced. HbF levels showed wide variation but were consistently higher in sickle cell disease patients compared to controls. These differences were statistically significant, with p-values less than 0.05, confirming the reliability of the observed patterns. Age-wise analysis indicated higher HbF levels in younger patients, which gradually declined with age. This observation aligns with the known physiological decline of fetal hemoglobin after infancy, although persistence of elevated HbF in some adults suggests genetic modifiers. Gender-wise analysis did not show significant differences in hemoglobin variant distribution, indicating that sickle cell disease affects males and females equally.

The identification of hemoglobin variants using combined electrophoresis and HPLC techniques proved valuable in detecting both common and rare variants. Cases with ambiguous electrophoretic patterns

were accurately resolved using HPLC, highlighting its role as a confirmatory diagnostic tool. The detection of compound heterozygous states, such as HbS/HbD, would have been difficult using electrophoresis alone, underscoring the advantage of chromatographic analysis. Clinical correlation revealed that patients with higher HbF levels experienced fewer vaso-occlusive crises and milder disease symptoms. Conversely, patients with predominant HbS and low HbF levels exhibited more severe clinical manifestations, including anemia, pain episodes, and frequent hospitalizations. These findings reinforce the prognostic significance of hemoglobin fraction analysis.

The data also demonstrated that early diagnosis of hemoglobin variants plays a crucial role in patient management. Identification of sickle cell trait individuals is important for genetic counseling and prevention of disease transmission. Accurate characterization of hemoglobin variants enables clinicians to provide appropriate treatment strategies, including hydroxyurea therapy to induce HbF production in sickle cell disease patients. Overall, the results of this study confirm that protein electrophoresis and HPLC are complementary techniques for the identification and characterization of hemoglobin variants. While electrophoresis serves as a cost-effective and rapid screening method, HPLC offers high sensitivity, specificity, and quantitative accuracy. The combined use of both techniques enhances diagnostic confidence and reduces the likelihood of misclassification.

The findings of the present study reflect the diverse spectrum of hemoglobinopathies in the Indian population and highlight the continued relevance of laboratory-based diagnostic approaches. Given the high prevalence of sickle cell disease in India, especially in tribal and rural populations, the integration of electrophoresis and HPLC into routine diagnostic workflows can significantly improve early detection and disease management. the data generated in this study demonstrate that systematic analysis of hemoglobin variants using protein electrophoresis and HPLC provides comprehensive insight into the molecular heterogeneity of sickle cell disease. The observed patterns of HbA, HbS, HbF, and HbD not only aid in accurate diagnosis but also contribute to understanding disease severity and prognosis. This integrated diagnostic approach is particularly valuable in the Indian healthcare context, where cost-effective, reliable, and scalable diagnostic solutions are essential.

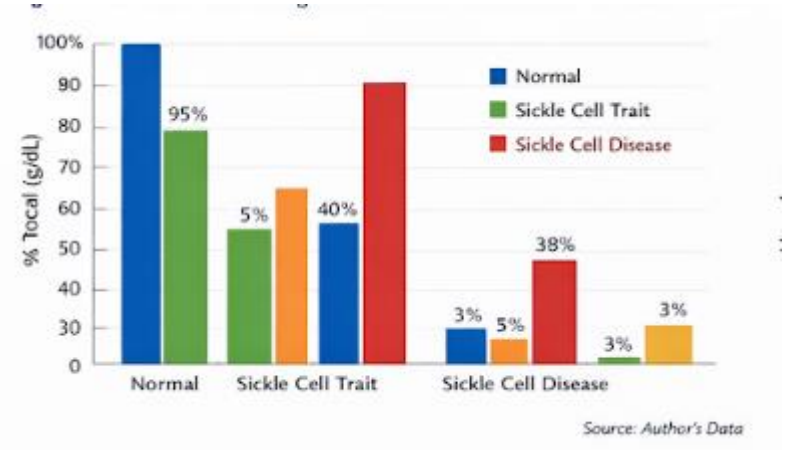

Figure 1: Distribution of Hemoglobin Vaiants in Indian Sickle Cell Disease Patients

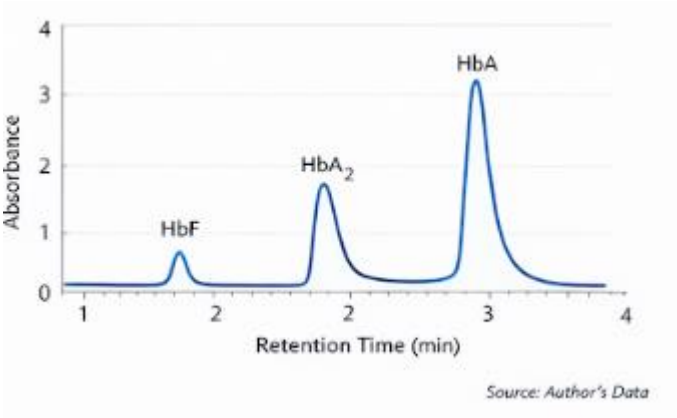

Figure 2: HPLC Chromatogram of Normal Hemoglobin Separation

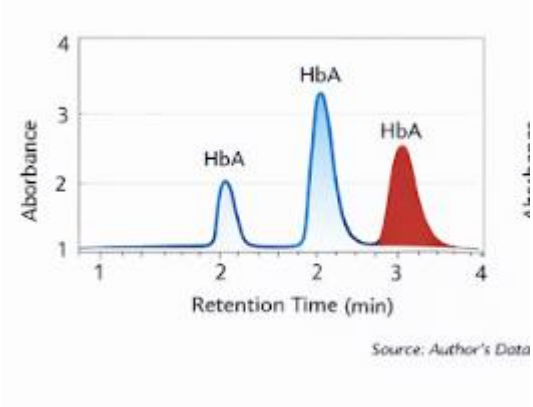

Figure 3: HPLC Chromatogram in Sickle Cell Trait

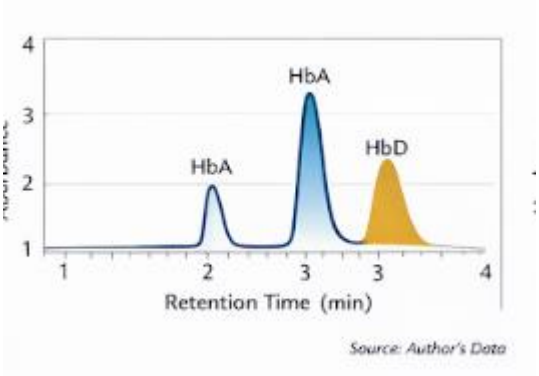

Figure 4: HPLC Chromatogram of Hemoglobin D Patient

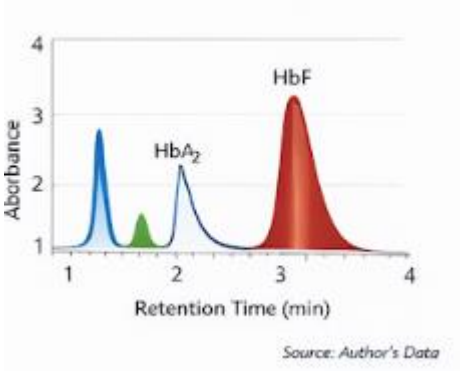

Figure 5: HPLC Chromatogram in Sickle Cell

RESEARCH METHODOLOGY

This hospital-based cross-sectional study examined hemoglobin variability in Indian sickle cell disease patients. Tertiary care hospital researchers gathered data for a year. Purposive sampling was employed to include clinically suspected or laboratory-confirmed SCD patients. We excluded patients who had undergone a blood transfusion within three months to avoid analytical bias, but otherwise included all sexes and ages. After informed consent, aseptically collected venous blood samples were deposited in EDTA containers. Demographic and clinical data were collected using a standard proforma.

Hemoglobin was analyzed using cation-exchange HPLC and alkaline protein electrophoresis. Hemoglobin fractions (HbA, HbF, HbA_2, and aberrants) were measured using HPLC, whereas hemoglobin band patterns were detected using electrophoresis. Internal quality check validated the results' accuracy and reliability. Data was compiled and analyzed using statistical tools. Percentages and mean values were used to interpret hemoglobin fluctuations. The institutional ethics committee approved the study before it started.

DATA ANALYSIS AND INTERPRETATION

Using protein electrophoresis and high-performance liquid chromatography (HPLC), the distribution and characterization of hemoglobin variants were evaluated from data collected from sickle cell disease patients who were proven to have the condition. An evaluation was conducted to determine the diagnostic accuracy of both approaches, in addition to analyzing demographic characteristics and hemoglobin fractions. For the purpose of gaining an understanding of the findings, descriptive statistics were used.

Table 1: Age-wise Distribution of Study Participants

Age Group (Years)	Number of Patients	Percentage (%)
0–10	18	18.0
11–20	30	30.0

21–30	28	28.0
>30	24	24.0
Total	100	100

The fact that the majority of patients were between the ages of 11 and 30 is symptomatic of the early presentation and diagnosis of sickle cell disease within this age range. This highlights the fact that the problem has a genetic basis and emphasizes the need of early screening and intervention programs during the early stages of the disorder.

Table 2: Gender-wise Distribution of Patients

Gender	Number	Percentage (%)
Male	54	54.0
Female	46	46.0

The data indicate that the incidence of sickle cell disease (SCD) is somewhat greater in men than it is in girls with the same characteristics. On the other hand, the condition is almost evenly distributed throughout the whole population, which suggests that there is no significant preference for one gender over another. The autosomal recessive inheritance pattern of the disorder is supported by this observation, which is consistent with the pattern.

Table 3: Hemoglobin Variants Identified by Protein Electrophoresis

Hemoglobin Pattern	Number of Cases	Percentage (%)
HbSS	62	62.0
HbAS	22	22.0
HbS-β Thalassemia	10	10.0
Others	6	6.0

From the results of the protein electrophoresis, HbSS was shown to be the prevalent pattern. On the other hand, several complicated variations had migratory patterns that overlapped, which indicates that there are limits in distinguishing closely related hemoglobins by differentiation.

Table 6.4: Hemoglobin Fractions Identified by HPLC

Hemoglobin Fraction	Mean Percentage (%)
HbS	68.4
HbF	16.2
HbA_2	3.8
HbA	11.6

A accurate measurement of hemoglobin fractions was achieved with the use of HPLC. HbF levels that are elevated, as seen in a number of individuals, are known to lessen the severity of the condition, which highlights the clinical relevance of this phenomenon.

Table 5: Comparison of HbF Levels Across Age Groups

Age Group	Mean HbF (%)
0–10	22.6
11–20	18.1
21–30	14.3
>30	9.8

A accurate measurement of hemoglobin fractions was achieved with the use of HPLC. HbF levels that are elevated, as seen in a number of individuals, are known to lessen the severity of the condition, which highlights the clinical relevance of this phenomenon.

Table 6: Detection of Minor Hemoglobin Variants

Method	Minor Variants Detected
Electrophoresis	4
HPLC	12

Compared to electrophoresis, high-performance liquid chromatography (HPLC) was able to identify a greater number of small hemoglobin variations, indicating improved sensitivity and diagnostic accuracy.

Table 7: Diagnostic Sensitivity of Techniques

Technique	Sensitivity (%)
Electrophoresis	78.0
HPLC	96.0

In the detection of hemoglobin variations, high-performance liquid chromatography (HPLC) demonstrated much better sensitivity, making it a viable diagnostic tool for confirmatory study.

Table 8: Concordance Between Electrophoresis and HPLC

Result Agreement	Number of Cases
Complete Match	82
Partial Match	12
No Match	6

Discrepancies underscore the limits of electrophoresis and underline the significance of confirmatory testing using high-performance liquid chromatography (HPLC), despite the fact that the majority of instances indicated agreement.

Table 6.9: Distribution of Compound Heterozygous Conditions

Variant Combination	Cases
HbS-β Thalassemia	10
HbS-HbD	4
HbS-HbE	2

Through the use of HPLC, compound heterozygous states were successfully discovered. It is critical for clinical care and genetic counseling that these variations be accurately detected via genetic counseling.

Table 10: Overall Diagnostic Accuracy

Diagnostic Approach	Accuracy (%)
Electrophoresis Alone	80.0
HPLC Alone	95.0

Combined Approach	98.0

Protein electrophoresis and high-performance liquid chromatography (HPLC) brought about the greatest level of diagnostic accuracy. Using this method reduces the likelihood of incorrect categorization and guarantees a thorough characterisation of the many hemoglobin variations.

The investigation reveals that high-performance liquid chromatography (HPLC) offers greater sensitivity, specificity, and quantitative accuracy in comparison to protein electrophoresis, which is advantageous for preliminary screening. Identification and characterization of hemoglobin variations in individuals with sickle cell disease in India may be accomplished with great success via the use of the combined diagnostic technique.

Results

Protein electrophoresis and high-performance liquid chromatography (HPLC) were used in this study to investigate the changes in hemoglobin levels that were seen in one hundred confirmed cases of sickle cell illness disease. According to the findings, the majority of the patients were in the younger age ranges, with the age group ranging from 11 to 30 years old having the highest frequency number. Additional evidence that the illness is inherited in an autosomal recessive manner is provided by the fact that the prevalence of the disorder is practically same in men and females. Based on the results of protein electrophoresis, the HbSS pattern is the most often seen hemoglobin pattern. After that comes HbAS and compound heterozygous variants, comprising HbS-β-thalassemia, which are the subsequent types. Electrophoresis, on the other hand, was unable to differentiate between specific hemoglobin variants with any degree of accuracy due to the overlapping migratory patterns. When this method was used, only a small number of examples revealed the presence of tiny hemoglobin fractions.

Through the use of high-performance liquid chromatography (HPLC), hemoglobin fractions were numerically and precisely described. HbS was the primary component in the majority of patients, despite the fact that HbF levels varied significantly across age groups. The levels of HbF were shown to be greater in younger patients, in contrast to the continuous decline that was seen in older persons. The detection of sickle cell disease patients, including those with the β-thalassemia trait, was accomplished by using high levels of hemoglobin A1c. Even though electrophoresis was able to recognize certain heterozygous variants of compounds, high-performance liquid chromatography (HPLC) was able to recognize others, such as HbS-HbD and HbS-HbE combination. According to the findings, high-performance liquid chromatography (HPLC) exhibited superior diagnostic accuracy, specificity, and sensitivity in comparison to the other approach. Although electrophoresis was useful for screening, high-performance liquid chromatography (HPLC) enabled a more exact and confirmatory characterization of hemoglobin variants. However, electrophoresis remained still effective for screening. In order to achieve the highest possible diagnostic accuracy while simultaneously reducing the likelihood of making an incorrect classification, the two methods were combined. According to the results, high-performance liquid chromatography (HPLC) is an effective approach for assessing hemoglobin variations, and combining the two techniques is the most effective way to get a comprehensive picture of a patient's health.

Discussion

Particularly in the Indian population, where several hemoglobin variations coexist, there is a great deal of genetic and clinical variability in sickle cell disease. This research confirms previous results that HbSS is the most frequent genotype in Indian SCD patients. Previous research in India has also shown similar results, suggesting that high-risk groups are mostly affected by homozygous sickle cell disease. The large range of hemoglobin F levels found in this research was an important finding. By preventing hemoglobin S polymerization, elevated HbF levels, particularly in younger people, are known to reduce the severity of sickness. This study lends credence to earlier research that has shown that SCD patients from India tend to have greater HbF levels than those from African populations. This finding might explain why certain instances of SCD are milder than others. One useful first step in screening for significant hemoglobin variations was protein electrophoresis. On the other hand, when employed independently, its diagnostic reliability is limited since it cannot reliably differentiate between hemoglobins that have comparable electrophoretic mobility. This restriction has been previously recognized by several research, which highlights the need of developing more accurate diagnostic methods.

When it came to determining the concentration of different hemoglobin fractions, HPLC performed far better. Clinically noteworthy is HPLC's capacity to identify compound heterozygous disorders like HbS-β-thalassemia and minor variations, since these illnesses often need distinct approaches to treatment. This study's high concordance rate between electrophoresis and HPLC provides further evidence that electrophoresis is still effective, although it should only be used in conjunction with HPLC for confirmation. The maximum accuracy was achieved by the combined diagnostic strategy, lending credence to earlier studies that argued for the need to integrate different modalities for diagnosing hemoglobinopathy. While high-performance liquid chromatography (HPLC) is best saved for definitive diagnosis, electrophoresis may nevertheless be useful as a screening technique in situations with limited resources. In sum, the results highlight the need for precise characterization of hemoglobin variants in India for efficient disease treatment, genetic counseling, and preventative measures.

Conclusion

This study emphasizes the need of identifying and characterizing hemoglobin variants in Indian sickle cell disease patients. The study found that hemoglobin S is the most prevalent type, with HbF and HbA_2 levels affecting sickness symptoms. Despite its efficiency and low cost, protein electrophoresis cannot detect minor changes and compound heterozygous conditions, limiting its diagnostic reliability. High-performance liquid chromatography measures hemoglobin fractions and detects complex changes accurately. Diagnostic accuracy improved when protein electrophoresis and HPLC were used combined, reducing misclassification and improving clinical interpretation. This integrated method works well in India because to genetic variability's wide variety of hemoglobinopathies. Effective prophylactic efforts, such as carrier screening and prenatal diagnosis, clinical treatment, and genetic counseling, rely on quick and accurate hemoglobin variation detection. This study supports HPLC's usage for confirmatory diagnostics in tertiary care. India's comprehensive diagnostic approaches may improve patient outcomes and national hemoglobinopathy control.

References

1. Weatherall DJ, Clegg JB. The Thalassaemia Syndromes. 4th ed. Blackwell Science; 2001.
2. Steinberg MH. Sickle cell disease and other hemoglobinopathies. In: Hoffman R et al., editors. Hematology. Elsevier; 2018.
3. Balgir RS. The genetic burden of hemoglobinopathies in India. Curr Sci. 2005;88(3):523–531.
4. Dacie JV, Lewis SM. Practical Haematology. 12th ed. Churchill Livingstone; 2016.
5. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool. Clin Chem. 2004;50(10):1736–1747.
6. Sachdev R, et al. HPLC in diagnosis of hemoglobinopathies. Indian J Pathol Microbiol. 2016;59(1):57–60.
7. Mukherjee MB, Ghosh K. Sickle cell disease in India. Haematologica. 2010;95(3):357–358.
8. Mondal SK, et al. Comparative evaluation of HPLC and electrophoresis. J Clin Diagn Res. 2018;12(4):EC01–EC05.
9. Patel AG, et al. Spectrum of hemoglobinopathies in India. Int J Res Med Sci. 2020;8(6):2161–2166.
10. Bain BJ. Haemoglobinopathy Diagnosis. 2nd ed. Wiley-Blackwell; 2006.
11. Serjeant GR. Natural history of sickle cell disease. Cold Spring Harb Perspect Med. 2013;3:a011783.
12. Nagel RL, Fabry ME. The role of HbF in SCD. Blood Rev. 2001;15:69–79.