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Original Research Article

STUDY ON HEMOGLOBINOPATHIES IN ANEMIC PATIENTS IN A TERTIARY HOSPITAL OF JHARKHAND

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ABSTRACT

Background: Hemoglobinopathies represent a group of genetic disorders characterized by abnormal hemoglobin production, posing significant clinical and diagnostic challenges. High-Performance Liquid Chromatography (HPLC) has emerged as a reliable tool for the characterization of hemoglobin variants, offering insights into the clinical implications of different genetic mutations.
Objectives: This study aims to utilize HPLC to characterize hemoglobin variants and analyze their clinical significance in various hemoglobinopathies.
Materials and Methods: Patients presenting with suspected hemoglobinopathies were recruited for the study. Hemoglobin variants were characterized using HPLC, and their clinical implications were assessed. Data were analyzed using appropriate statistical methods to determine the prevalence and clinical significance of different hemoglobin variants.
Results: Distinct hematological profiles were observed in patients with different hemoglobinopathies. β-thalassemia major/intermedia exhibited significantly lower hemoglobin levels and altered RBC indices, while sickle cell disease manifested moderate anemia and characteristic abnormalities in RBC morphology. β-thalassemia trait showed microcytic hypochromic features, whereas Hb D Punjab Sickle cell variant exhibited elevated hemoglobin levels alongside abnormal RBC indices.
Conclusion: The findings of this study highlight the clinical significance of precise hemoglobin characterization using HPLC in various hemoglobinopathies. Accurate diagnosis and tailored management strategies are essential for improving patient outcomes in hemoglobinopathy care. Continued research in this area is warranted to further enhance our understanding of hemoglobinopathies and optimize diagnostic and therapeutic approaches.
Keywords: Hemoglobinopathy, High-Performance Liquid Chromatography, Hemoglobin Variants, β-thalassemia, Sickle Cell Disease.

INTRODUCTION

Hemoglobin disorders, encompassing a spectrum of genetic anomalies affecting the structure or production of hemoglobin molecules, pose a significant public health challenge globally, particularly in regions like India.[1] Among these disorders, beta (β)-thalassemia and sickle cell disease emerge as predominant concerns, with diverse clinical presentations ranging from asymptomatic carriers to severe conditions necessitating regular transfusions and intensive medical management.[2] These disorders not only impose a substantial burden on affected individuals and families but also strain healthcare resources, especially in resource-limited settings.

In India, where the burden of anemia is notably high, accounting for 58% of children in the age range of 6-59 months and affecting 53% of females and 22.7% of males aged 15-49 years, the prevalence of hemoglobinopathies adds another layer of complexity to public health interventions.[3,4] The

World Health Organization (WHO) estimates that approximately 5% of the global population carries genes for hemoglobin disorders, emphasizing the widespread impact of these conditions.[5]

Accurate diagnosis of hemoglobinopathies is pivotal for effective management and prevention strategies. Blood testing, including comprehensive hematologic evaluations and specialized techniques such as high-performance liquid chromatography (HPLC), plays a crucial role in screening and diagnosing patients.[6] HPLC, in particular, offers a rapid and reliable method for detecting various hemoglobin variants, enabling clinicians to tailor treatment plans accordingly.

Furthermore, early identification of hemoglobin disorders through blood testing facilitates timely interventions, including genetic counseling and targeted therapies. As curative treatments such as bone marrow transplantation remain costly and inaccessible to many, the emphasis shifts toward preventive measures through population screening and genetic counseling.

This study aims to contribute to the understanding of common hemoglobin disorders among patients presenting with anemia in a tertiary care hospital in Jharkhand, India. By elucidating the prevalence and clinical profiles of these disorders, the findings will inform the development of context-specific diagnostic, preventive, and therapeutic strategies, addressing the unique challenges posed by hemoglobinopathies in the region.

MATERIAL AND METHODS

This retrospective study involved the analysis of data from 100 subjects who presented to the Department of Pathology Shahid Nirmal Mahato Medical College and Hospital, Dhanbad India, over a period of one years from October 2022 to September 2023.Patients included in the study were those who visited the Department of Pathology for various reasons, including suspicion of coexisting hemoglobinopathy in cases of microcytic hypochromic anemia based on red cell indices. Subjects with a history of blood transfusion within the last month were excluded. Detailed clinical histories, including family histories and history of blood transfusions, were obtained from each patient. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vials for hematological analysis and high-performance liquid chromatography (HPLC) testing.

Complete blood counts were performed using an automated cell counter (Sysmex) to obtain hemoglobin levels, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC) count, and red cell distribution width (RDW). Peripheral blood smears were prepared and stained with Leishman stain for examination. High-performance liquid chromatography (HPLC) was conducted using a Bio-Rad Variant II machine. HPLC utilizes the exchange of charged groups on an ion exchange material for charged groups on the hemoglobin molecule. Hemoglobins were identified based on retention time, and quantification was done by determining the area under the corresponding peak in the elution profile.

The data obtained from hematological analyses and HPLC testing were compiled and analyzed using appropriate statistical methods to determine the prevalence of common hemoglobin disorders in the study population.The study was conducted following the principles outlined in the Declaration of Helsinki, and ethical approval was obtained from the Institutional Review Board of the tertiary care teaching medical institution.

RESULTS

Among 100 cases in the study was determined to be 64% males and 36% females. This distribution reflects a balanced representation of both genders within the sample, with a slightly higher prevalence of males. Such proportionate allocation ensures adequate representation of both sexes, which is crucial for maintaining sample integrity and avoiding gender bias in research outcomes. [Table 1]

The distribution of age groups among the 100 cases in the study was as follows: 46 cases (46%) were in the age range of 0-15 years, 30 cases (30%) were in the age range of 16-45 years, and 24 cases (24%) were in the age range of over 45 years. This distribution indicates a varied representation of age groups within the sample, with a significant proportion falling within the younger age bracket. [Table 2]

The hemoglobin variants identified through High-Performance Liquid Chromatography (HPLC) analysis revealed distinct hematological profiles associated with various hemoglobinopathies. Normal hemoglobin levels exhibited a mean of 7.9 g/dl with standard deviation (±SD) of 3.2, accompanied by typical red blood cell (RBC) counts and packed cell volume (PCV) percentages. β-thalassemia major/intermedia presented notably lower hemoglobin levels (mean 3.4 g/dl, ±SD 2.1) alongside reduced RBC indices, reflecting the severity of this condition. Sickle cell disease manifested moderate anemia (mean hemoglobin 6 g/dl, ±SD 2.1) with characteristic abnormalities in RBC morphology. β-thalassemia trait exhibited slightly higher hemoglobin levels (mean 7.5 g/dl, ±SD 3.4) but distinct microcytic hypochromic features. Hb D Punjab Sickle cell variant showed elevated hemoglobin levels (mean 8.2 g/dl, ±SD 1) with characteristic abnormalities in RBC indices. These distinct hematological profiles provide valuable insights for accurate diagnosis and

management strategies tailored to specific hemoglobinopathies. [Table 3]

Table 1: Gender Distribution of Subjects

Gender	No. of Cases (Proportionate)	Percentage (Proportionate)
Male	64	64%
Female	36	36%
Total	100	100%

Table 2: Age distribution of subjects

Age (In Years)	Number of Cases	Percentage (%)
0-15 yrs.	46	46
16-45 yrs.	30	30
>45 yrs.	24	24
Total	100	100

Table 3:

HPLC Dx	Hb (g/dl) ±SD	RBC ±SD	PCV (%) ±SD	MCV (fl) ±SD	MCH (pg) ±SD	MCHC (g/dl) ±SD	RDW± SD	Hb A	HbA2/E	HbF	Hb D	HbS	Others
Normal	7.9±3.2	3.5±1.3	28.1±10.8	78 ± 12.5	27.8±9.1	32.1±3.0	16.3±4.2	87±4.8	2.4±0.5	0.8±0.7			9.5±2.0
β (beta) thalassaemia Major/intermedia	3.4±2.1	1.8±1.1	12.3±6.7	66.2±8.5	20.3±3.4	32.2±3.5	26.2±3.6	6.9±2.5	4.5±1.5	88±6.2			4.5±3.4
Sickle cell disease	6±2.1	2.6±2.7	19.3±6.6	83.1±12.4	26.1±3.1	31.6±4.1	21.7±4.4	3.3±4.3	3.3±0.7	19.3±7.3		72±8.3	6.1±3.1
β (beta) thalassaemia trait	7.5±3.4	3.5±1.5	25±8.3	71.7±11.1	22.4±4.3	32.5±2.7	18±4.9	83.2±5.3	5.1±1.0	1.5±1.3			9±2.3
Hb D Punjab Sickle cell	8.2±1	3.2±0.4	34.2±5.7	79±8.5	24.7±3.1	32.3±2.5	14±2.1	60±3.2	2.2±.1	1.8±.2	30±5.2		7.2±.4

DISCUSSION

The findings from the HPLC analysis of hemoglobin variants in this study provide valuable insights into the hematological profiles associated with different hemoglobinopathies. The discussion of these results highlights the clinical significance and diagnostic implications of the observed differences.

Firstly, the identification of distinct hematological profiles associated with various hemoglobinopathies underscores the importance of accurate diagnostic techniques such as HPLC. This method enables the precise characterization of hemoglobin variants based on their unique retention times, allowing for the differentiation between normal and abnormal hemoglobinpatterns.[7]

The significantly lower hemoglobin levels observed in individuals with β-thalassemia major/intermedia align with previous research highlighting the severe anemia characteristic of this condition.[8] Similarly, the microcytic hypochromic features seen in β-thalassemia trait are consistent with the expected hematological abnormalities associated with this genetic disorder.[9]

The moderate anemia observed in sickle cell disease patients is indicative of the chronic hemolytic process and vaso-occlusive crises characteristic of this condition.[10] The presence of abnormal RBC indices further supports the diagnosis of sickle cell disease and underscores the importance of comprehensive hematological evaluation in its management.

Interestingly, the Hb D Punjab Sickle cell variant exhibited elevated hemoglobin levels compared to other hemoglobinopathies, which may reflect the co-inheritance of two hemoglobin variants. This finding highlights the complexity of hemoglobinopathies and underscores the need for careful interpretation of HPLC results in clinical practice.[11]

Overall, the discussion of these results emphasizes the clinical utility of HPLC in accurately diagnosing and characterizing hemoglobinopathies based on their distinct hematological profiles. By providing insights into the pathophysiology of these disorders, these findings contribute to the development of personalized management strategies aimed at improving patient outcomes.

CONCLUSION

In conclusion, the comprehensive analysis of hemoglobin variants using High-Performance Liquid Chromatography (HPLC) in this study has provided valuable insights into the hematological profiles associated with various hemoglobinopathies. The distinct patterns observed in different conditions, including β-thalassemia major/intermedia, sickle cell disease, β-thalassemia trait, and Hb D Punjab Sickle cell variant, underscore the clinical significance of accurate diagnostic techniques in hemoglobinopathy

management. These findings highlight the importance of tailored management strategies based on precise characterization of hemoglobin variants. Furthermore, the study reinforces the utility of HPLC as a reliable tool for the diagnosis and characterization of hemoglobinopathies, contributing to improved patient care and outcomes. Moving forward, continued research in this area is essential for advancing our understanding of hemoglobinopathies and enhancing diagnostic and therapeutic approaches for individuals affected by these conditions.

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