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Original article

A HOSPITAL-BASED STUDY ON UTILISING HPLC TO DETECT HB-VARIANTS AND HEMOGLOBINOPATHIES: A RETROSPECTIVE DESCRIPTIVE STUDY.

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ABSTRACT

Background

Hemoglobinopathies are a growing global health issue. These hereditary diseases are most common worldwide. WHO says the Middle East and India have the highest incidence. HPLC is a good way to screen hemoglobin variants such HbF and HbA2. The present study was carried out to diagnose hemoglobinopathies and thalassemias by the use of HPLC in Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India.

Materials and Methods

A retrospective descriptive research assessed 550 patients for thalassaemia or other hemoglobin structural abnormalities. This investigation included all cases of microcytic hypochromic anemia (MCV< 80 fl, MCH< 27 pg) with a clinical suspicion of hemoglobinopathy that did not respond to usual treatment. The trial excluded patients who had blood transfusions within three months.

Results

This study included 550 cases, 310 boys and 240 females, aged 2 months to 40 years. HPLC analysis revealed abnormal hemoglobin fractions in 96 cases. One person had delta thalassemia, one had delta with beta-thalassemia, one had hemoglobin D Iran, two had HbE with beta, six had HbE trait, eight (1%) had beta major, and less than 1% had HbE homozygous. Of the cases, 72 (13%) had beta thalassemia. The HPLC pattern was normal in 48%. In all cases, peripheral blood smears showed target cells, microcytosis, and hypochromia. The majority of patients had increased RBCs.

Conclusion

Hemoglobinopathies can be quickly, accurately, and effectively diagnosed with HPLC. It is a valuable diagnostic technique for beta thalassemia characteristics, particularly in impoverished nations like India where hemoglobinopathies are difficult to identify. Prompt diagnosis could aid in appropriate and targeted treatment.

Recommendation

This study emphasizes the need of HPLC for hemoglobinopathy diagnosis, especially in high-burden countries like India. HPLC screening and diagnosis can help combat hemoglobinopathies' global health issues by enabling early detection and focused treatment.

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INTRODUCTION

Liquid chromatography was first utilized for the purpose of segregating pigmented compounds in the course of the 20th century [1]. The nomenclature HPLC, denoting High-Performance Liquid Chromatography, was originally introduced by Csaba Horvath and subsequently adopted as the universally recognized appellation for this analytical technique [1]. HPLC is utilized across a diverse spectrum of clinical diagnostic applications, with a particular emphasis on the identification and detection of diseases pertaining to hemoglobinopathies. This encompasses conditions such as thalassemias and disorders characterized by aberrant glandular secretions [2].

Hemoglobinopathies represent the most commonly encountered genetic disorders on a global scale, exhibiting the highest documented prevalence in regions such as the Middle East and the Indian subcontinent. As per the World Health Organization (WHO), the global prevalence of hemoglobin disorders is estimated to be approximately 269 million individuals [3]. Annually, an estimated 300,000 neonates manifest severe hemoglobin (Hb) disorders, with a predominant 80% of these instances observed within developing nations [4]. Thalassemia major and hemoglobin E exhibit a notably elevated prevalence within the Indian subcontinent [3, 4]. Moreover, it is worth noting that there is a notable prevalence of alpha thalassemia in regions

located in South East Asia and the Mediterranean [2, 3]. The increasing recognition of these disorders has resulted in a higher prevalence of timely diagnoses.

HPLC has been recognized as a notably efficient technique for the screening of diverse Hb variants. The present automated system serves to optimize the workflow, providing efficient sample preparation, expedited analysis, enhanced resolution, and precise identification of Hb variants [4].

The principal aim of this investigation was to ascertain the presence of hemoglobinopathies and thalassemias through the utilization of the HPLC technique within a tertiary healthcare facility.

MATERIAL AND METHODS

Study design

A retrospective descriptive study was conducted.

Study setting

This study was conducted at the Hematology Department of Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, in a duration of one year (July 2021 to May 2022).

Participants

A total of 550 cases underwent screening in order to identify the presence of thalassemia or other structural hemoglobin variants.

Inclusion criteria

1) Individuals presenting with microcytic hypochromic anemia, characterized by mean corpuscular volume (MCV) values below 80 femtoliters (fL) and mean corpuscular hemoglobin (MCH) levels below 27 picograms (pg). 2)Screening for hemoglobinopathy in pregnant females coming for routine antenatal check-up. 3)Spouse of pregnant women, whose HPLC was found abnormal.

Exclusion criteria

Participants who had undergone a blood transfusion within a three-month period prior to the collection of samples were excluded from the study.

Data Collection and Analysis

A blood sample of 2 milliliters was collected from the patient using an ethylenediaminetetraacetic acid (EDTA) anticoagulant. The collected sample was then subjected to analysis using an automated hematology analyzer, specifically the siemens advia 2120i model. The purpose of this analysis was to obtain comprehensive information regarding the patient's complete blood counts (CBC) as well as red blood cell (RBC) indices. Hemoglobin separation was successfully accomplished via HPLC methodology. The Bio-Rad D10 instrument was employed in accordance with established ion-exchange conditions specific to high-performance liquid chromatography. This process facilitated the distinct separation of HbA2, HbF, and various other hemoglobin variants.

Variables

The HPLC chromatograms exhibited the Hb fractions, their corresponding retention times, peak areas, and respective quantitative values. The concentrations of Hemoglobin A2 (Hb A2) and Hemoglobin F (HbF) were subjected to analysis, with the assistance of Variant II CDM software, to facilitate the interpretation of the obtained data. In the case of pediatric patients with blood samples measuring less than 500mL, a manual pre-dilution procedure was conducted prior to subjecting the samples to high-performance liquid chromatography (HPLC) analysis.

Hemoglobin A2/F calibrators, as well as normal and abnormal controls, were run prior to each sample analysis. Complete blood count (CBC), red blood cell (RBC) indices, and peripheral blood examinations were performed in all cases. A diagnostic threshold of Hb A2 levels exceeding 3.5% was employed for the identification of Beta thalassemia trait. The retention time of Hb A2 fell within the range of 3.30-3.90 minutes.

The chromatogram exhibited distinct peaks corresponding to HbA0, A2, and HbF, as well as C, D, and S windows. Additionally, minor peaks P2 and P3 were observed. The diagnosis of hemoglobin variants was established by considering factors such as ethnicity, retention time, and peak areas. In the context of beta-thalassemia major, it is noteworthy to consider hemoglobin F (Hb F) levels ranging from 30% to 90% or higher. The diagnosis of delta/beta thalassemia was predicated upon the assessment of HbF concentration, which typically accounts for 5-15% of the total hemoglobin. Notably, individuals with hemoglobin S (HbS) beta zero-thalassemia exhibited elevated levels of HbF concentration.

Bias

There was a chance that bias would arise when the study first started, but it was avoided by giving all participants the identical information and hiding the group allocation from the nurses who collected the data.

Study limitations

The study has several limitations: its retrospective design may introduce biases and hinder causal relationship establishment; the inclusion criteria may lead to selection bias; the sample size, though 550 cases, may limit generalizability; its single-center nature restricts broader applicability, and the one-year duration may overlook seasonal or long-term trends in hemoglobinopathies.

Ethical considerations

Informed consent was obtained from all participants.

RESULTS

This study included 550 cases, ages ranging from 2 months to 40 years, including 310 males and 240 females. Ninety-six of these instances showed aberrant hemoglobin fractions upon HPLC analysis. For females, the age bracket most impacted was 20-31, whereas for men, it was under 15 years old. Table 1 summarizes the distribution of hemoglobin patterns.

Table 1: Hemoglobin Pattern Among Study Subjects

Hemoglobin Pattern	Participant numbers	Percentage
β-Thalassemia Trait (TT)	72	13%
β-Thalassemia Major (TM)	8	1%
Hemoglobin E trait	6	<1%
HbE with β-thalassemia	2	<1%
Delta thalassemia	1	<1%
Sickle homozygous	3	<1%
HbE homozygous	2	<1%
Hemoglobin D Iran	1	
Delta β-thalassemia	1	

Table 2: Hemoglobin and RBC Indices in Patient with Beta Thalassemia Trait

Hemoglobin value	Percentage (%)
< 7 gm/dl	34.71%
7-9 gm/dl	28.57%
9-10 gm/dl	18.04%
> 10 gm/dl	15.66%
RCB indices	
MCV	
< 80 fl	100%
80-92 fl	0
>92 fl	0
MCH	
<27 pg	100%
27-32 pg	0
>32 pg	0
MCHC	
<32 %	15.66%
32-37%	82.34%
RBC Count	
<3.8 million/cumm	8.5%
3.8-4.8 million/cumm	32.33%
>4.8 million/cumm	56.14%

One participant had delta thalassemia, one had delta beta-thalassemia, one had hemoglobin D Iran, two patients had HbE with beta thalassemia, six had HbE trait, eight patients (1%) had beta thalassemia major, and less than 1% of the cases had HbE homozygous. Of the cases, 72 participants (13%) exhibited beta thalassemia trait. Furthermore, 48% showed a typical HPLC pattern. Target cells were present in every instance, as well as microcytosis and hypochromia, according to peripheral blood smears. The majority of patients had higher RBC counts. Sixty-two percent of those with beta-thalassemia trait had Hb levels less than 9 g/dl, and thirty-three percent had Hb values between 9 and 11 g/dl. MCHC exceeded 31% in 82.34% of cases, and RBC counts exceeded 4.7 million in 56.14% of cases.

Severe anemia, noticeable anisopoikilocytosis, microcytic hypochromic RBCs, polychromasia, and nucleated RBCs in peripheral smears were all found in beta thalassemia major patients. In most cases, the Hb levels were less than 7g/dl. Our inclusion criteria were met by all patients when their

MCV was continuously less than 80 fL. MCH was always less than 27. In 82.34% of instances, the MCHC levels were primarily between 32 and 37%, while in 15.66% of cases, they were below 32%. Additionally, in cases of beta thalassemia major, HbF levels exceeded 75%, and 56.14% of individuals had RBC counts greater than 4.7 million/cumm.

DISCUSSION

Anemia, which is defined as a disease with reduced hemoglobin (Hb) levels, can have many different causes, including persistent bleeding, malnourishment, and genetic disorders like hemoglobinopathies. These abnormalities are often inherited in an autosomal recessive manner and mostly affect the globin component of the Hb molecule [5]. The two most common hereditary hemoglobin diseases worldwide are beta and alpha thalassemia. Originally, the prerequisites indicated above were limited to specific geographic areas, castes, tribes, and religious groups that practiced endogamous marriages. Increased migration and intercaste marriages have been linked to an increase in the prevalence of hemoglobinopathies worldwide [6].

Ninety-six cases with ages ranging from two months to forty years had abnormal hemoglobin fractions as identified by HPLC in this retrospective cohort research. Of the cases that were observed, 72 people (13%) were found to have β-thalassemia trait, and 8 people (1%) were found to have beta thalassemia major. Furthermore, two of the cases had beta thalassemia along with HbE, one case had delta thalassemia, one had delta with beta-thalassemia, one had hemoglobin D Iran and less than 1% of the cases had homozygous HbE. There are slight differences in the observed prevalence between this study and other research, which can be attributed mostly to regional differences. People who are heterozygous Hb S gene carriers- those with one copy of the Hb S gene in their genetic makeup- have been reported to suffer from a disorder known as HPFH [1, 2, 7]. There was a regional concentration in the prevalence of the HbE trait and disease, especially in Assam, West Bengal, Nagaland, and Manipur [8].

Compared to other studies, we found that homozygous sickle-cell disease is more common, occurring at a rate of 3%, and that homozygous HbE was less common, occurring at a rate of 2%. The observed discrepancy could be explained by the diverse genetic makeup and geographic differences among the participants in our study [9].

The population under study exhibits several hemoglobin variations, highlighting the significance of hemoglobinopathies as a global health concern. The observed differences in hemoglobinopathy patterns are partly explained by the presence of awareness-related and regional factors.

South East Asians are known to have high blood levels of HbE. Heterozygotes, or people with one copy of the HbE gene, usually have HbE levels below 40%, which is regarded as normal. When a person has the HbE gene mutation homozygously and has HbE levels higher than 70%, anemia, microcytosis, hypochromia, and target cell presence are among the clinical symptoms that they express. People with a double heterozygous genotype for both beta thalassemia characteristics and HbE present with clinical signs that are similar to those of thalassemia major [2].

When treating anemia in patients with thalassemia condition or other hemoglobin variations, it is crucial to prevent unnecessary iron therapy [10]. The practice of pre-marital screenings is still not widely followed in India, especially in Bihar. The probability of homozygous inheritance of hemoglobinopathies can be effectively reduced by promoting the practice of screening among people requesting medical or hematological outpatient care, pregnant populations, and extended family members of patients with known thalassemia. When effective screening programs are put in place in high-risk countries, the prevalence of these disorders has significantly decreased.

The current study demonstrates the powerful diagnostic potential of HPLC in precisely identifying hemoglobin variations. The technique demonstrates remarkable diagnostic efficacy by distinguishing between hemoglobin variations that are very similar by examining retention duration and hemoglobin fraction proportion.

Hemoglobinopathies represent a complex group of disorders with various genetic and regional factors contributing to their prevalence and distribution. This comprehensive retrospective cohort study sheds light on the diversity of hemoglobin variations observed in a population with ages ranging from two months to forty years. The study highlights the significance of hemoglobinopathies as a global health concern, emphasizing the need for increased awareness and regional considerations when addressing these disorders. The observed differences in hemoglobinopathy patterns can be attributed to factors such as genetic makeup, regional concentrations, and awareness-related issues. Effective screening programs, especially in high-risk regions, play a crucial role in reducing the prevalence of these disorders. Furthermore, the study underscores the diagnostic potential of High-Performance Liquid Chromatography (HPLC) in accurately identifying and distinguishing between various hemoglobin variations, providing valuable insights for clinical diagnosis and management.

CONCLUSION

Anemia can arise due to nutritional deficiencies as well as abnormal hemoglobin levels, leading to significant complications in individuals with homozygous hemoglobinopathies. The etiology of anemia can be elucidated through comprehensive examination, encompassing premarital and antenatal screening, thereby facilitating the detection of aberrant hemoglobin disorders and individuals harboring diverse hemoglobinopathies. The implementation of a comprehensive strategy encompassing both primary and secondary prevention measures has demonstrated notable cost-effectiveness and efficacy in mitigating the occurrence of genetic homozygous

inheritance disorders among neonates. Furthermore, it facilitates the identification and appropriate handling of hemoglobinopathies and their respective variants. HPLC confers distinct advantages in the screening and detection of hemoglobinopathies owing to its expeditious and accurate outcomes. The system possesses the capability to quantify levels of HbF and HbA2 concurrently, presenting enhanced resolution, automated processes, and the provision for internal sample preparation. The significance of this matter is particularly pronounced in regions such as India, where there exists a notable prevalence of beta thalassemia traits and a scarcity of resources for timely detection.

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Conflict of interest

The authors have no competing interests to declare.

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