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The Spectrum of Haemoglobinopathies: A Tertiary Care Hospital Experience

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ABSTRACT

Background: A scientist, Csaba Harvath first described high-performance chromatography (HPLC). HPLC method is beneficial for detecting different types of hemoglobinopathies, including both quantitative and qualitative disorders of globin chains.This study aimed to explore the significance of HPLC technique in the diagnosis of various hemoglobinopathies. **Methods:** This cross-sectional retrospective study was conducted in the Central Laboratory of Civil Hospital Karachi from 1st Jan 2021 to 31 Dec 2023. A total of 1464 patients were included who came with low hemoglobin levels. Analysis of blood for complete blood count was done on an XN 1000 analyzer and then HPLC was performed on an Arkray analyzer in samples received in our laboratory. Hemoglobinopathies were diagnosed by the more sensitive method, High-Performance Liquid Chromatography (HPLC) on the analyzer ADAMS A1C Model No. HA-8180T Arkray/Japan. **Results:** Out of 1464 cases, 1022 (69.8%) were normal, and 442 (30.1%) cases had abnormal haemoglobin pattern. 688(46.9%) were males and 776 (53.0%) were females. Out of 1022 cases of Anemia, 557 (54.5%) were microcytic hypochromic, 45(4.4%) macrocytic, and the rest 421(41.1%) had a normocytic normochromic picture. Of the total cases, 442 (30.1%) showed abnormal Hb fractions. The major abnormality observed was high HbA2. Other hemoglobinopathies in descending order of frequency were Hb D Disease at 40 (9.0%), sickle cell disease at 29 (6.5%), Hb E at 18(4.0%), Sickle/beta-thalassemia in 06 (1.3%), and Hb C 01 (0.2%). **Conclusion:** Our study revealed a higher frequency of the Beta Thalassaemia trait. It is suggested that detection of HbA2 should be carried out in all the high-risk groups with anaemia. Further larger studies are needed to screen our population to detect thalassaemia carrier state and Iron deficiency Anaemia.

INTRODUCTION

A scientist, Csaba Harvath first described high-performance chromatography (HPLC). HPLC method is beneficial for detecting different types of hemoglobinopathies, including both quantitative and qualitative disorders of globin chains. [1,2]

The most common quantitative defects in hemoglobin synthesis are homozygous beta-thalassemia, and the less common are Alpha thalassemia syndromes. Other defects of globin chains are qualitative during hemoglobin synthesis, known as variant hemoglobin. Hemoglobinopathies are inherited blood diseases that are characterized by decreased hemoglobin synthesis. Thalassemia is prevalent in Italian, Greek, Middle Eastern, South Asian, and African populations[3]. These hemoglobinopathies are inherited in an autosomal recessive manner. The transmission of disease in every offspring is 25% in each pregnancy of carrier parents[4,5]. An estimated 5000-9000 children with β-thalassemia are born per year, although no documentary registry is available in Pakistan. The rate of thalassemia trait rate is estimated around 5-7%, with 9.8 million carriers in the total population [6].

As the awareness of these disorders is increasing more cases are being diagnosed early. Various Hb variants can be screened using a single, highly excellent method of HPLC. It is an automatic system with sample preparation, rapid analysis, better resolution, and accurate identification of Hb variants [7,8].

This study aimed to explore the significance of HPLC technique in the diagnosis of various hemoglobinopathies.

METHODOLOGY

This cross-sectional retrospective study was conducted in the Central Laboratory of Civil Hospital Karachi from 1st Jan 2021 to 31 Dec 2023, following approval from the

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Institutional Review Board (IRB/DUHS/EXEMPTION). This study was conducted in accordance with the principles of the Declaration of Helsinki All suspected cases of thalassemia were included. Analysis of blood for complete blood count was done on an XN 1000 analyzer and then HPLC was performed on an Arkray analyzer in samples received in our laboratory.

A total of 1464 patients were included during a two-year period (2021-2023) who came with low hemoglobin levels.

Hemoglobinopathies were diagnosed by the more sensitive method, High-Performance Liquid Chromatography (HPLC) on the analyzer name ADAMS A1C Model No. HA-8180T Arkray/Japan.

An EDAT anticoagulated tube for blood sampling was used for the testing of complete blood count and HPLC. Blood samples with a history of previous blood transfusion within 3 months were not accepted for this study. Complete blood count was analyzed on an automated hematology analyzer and different red cell parameters were obtained. Hemoglobin (Hb), RBC count, corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and hematocrit (HCT) were analyzed on an automated hematology analyzer by performing CBC. In the suspected cases of sickle cell disease, the sickling test was performed to see the sickling by using metabisulphite.

Arkray analyzer was used for HPLC sample analysis and on the basis of retention time on HPLC analysis helps in the diagnosis of different hemoglobinopathies.

Inclusion criteria

All the patients with Anemia, suspected cases of hemoglobinopathy, and a positive family history of hemoglobinopathy will be included in this study.

Exclusion criteria

All the patients with recent blood transfusion history.

The chi-square test was used for statistical analysis.

RESULTS

Out of 1464 cases, 1022 (69.8%) were normal and 442 (30.1%) cases had abnormal hemoglobin patterns. 688(46.9%) were males and 776 (53.0%) were females. The age range of patients was from 6 months to 88 years. Study participants were grouped according to age, of which the 6 months to 15 years group constituted the predominant one (60.5%) Out of 1022 cases of Anemia, 557 (54.5%) were microcytic hypochromic, 45(4.4%) macrocytic and the rest 421(41.1%) had a normocytic normochromic picture. Of the total cases, 442 (30.1%) showed abnormal Hb fractions. The major abnormality observed was high Hb A2. A cutoff of over 3.5% was taken for the diagnosis of beta thalassemia trait (BTT). Of the 442 abnormal cases, Beta Thalassemia trait 256(57.9%), The RT for Hb A2 was between 3.61- and 3.68-min. ADAMS A1C Model No. HA-8180T Arkray/Japan followed by Beta thalassemia major 69(15.6%). Other hemoglobinopathies in descending order of frequency were Hb D Disease at 40 (9.0%), sickle cell disease at 29 (6.5%), Hb E at 18(4.0%), Sickle/beta-thalassemia in 06 (1.3%), and Hb C 01 (0.2%). We observe that, in the present study, predominant blood findings were microcytosis and hypochromic with raised RBC counts. Most patients are diagnosed with a case of homozygous beta thalassemia during the initial 1-2 years of life. CBC smears of these patients showed severe anemia, anisocytosis, poikilocytosis, and numerous nucleated red cells. Other hemoglobinopathies such as Hb S homozygous and heterozygous, show peaks at the S window ranging from 60-90% and 30-40%, respectively. The sickling test was performed on these cases and found positive in all cases.

HPLC showed a D Window with a Retention Time of 4.07-4.16 min.

The Hb E disease included Hb E homozygous, Hb E heterozygous, and Hb E-BTT double heterozygous. Hb E was detected by a raised peak in the A2 region with Retention Time ranging from 3.56 to 3.68 min.

Table 1 shows the distribution of the hemoglobin pattern. Severe anemia was observed in β-TM (Hb=5.0±1.9), followed by HbE disease (Hb=6.6±3.0). Among all hemoglobinopathies, β-TT showed the highest red blood cell count (5.1±1.1). The lowest mean values of MCV and MCH were observed in β-TT, 64.2 and 18.48 respectively. A significant association was observed between Hb, HCT, MCV, MCH, MCHC, and diagnosis (Table 2).

Table 1

Distribution of Hemoglobin Pattern

Group	Diagnosis	No. of cases
A	Normal hemoglobin pattern	1022
B	Beta thalassemia trait	256
C	Beta thalassemia major	69
D	Sickle cell disease/ sickle beta thalassemia	35
E	Hb D disease	40
F	Hb E disease	18
G	Hb C disease	01
Total		1464

Table 2

Haematological Parameters (Mean & Standard Deviation) in Reported Hemoglobinopathies

Hemoglobinopathy (No. of pts)	Hb(g/dl)	RBCs (x10^12/l)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	TLC (x10^9/l	Platelet (10^9/l)
Beta thalassemia major (BTM)	5.0 ± 1.9	2.4 ± 0.9	16.8 ± 6.3	71 ± 8.2	21 ± 3.6	29.8 ± 3.3	22.8 ± 22.1	371 ± 241
Beta thalassemia trait (BTT)	9.6 ± 2.3	5.1 ± 1.1	32.8 ± 7.5	64.2 ± 8.6	18.8 ± 3.4	29.2 ± 2.4	9.5 ± 5.2	380 ± 190
Sickle cell disease	7.8 ± 3.4	3.3 ± 1.5	24.4 ± 11.2	74.5 ± 8.1	23.8 ± 4.8	31.8 ± 3.5	15 ± 10.6	283 ± 186
Hb D disease	8.1 ± 3.0	4.1 ± 1.5	27.6 ± 9.3	68.5 ± 12.6	20.5 ± 6.0	29.5 ± 4.1	8.4 ± 3.8	332 ± 209
Hb E disease	6.6 ± 3.0	3.4 ± 1.3	22.8 ± 9.5	66.1 ± 8.8	19.2 ± 3.5	28.8 ± 2.4	8.6 ± 4.5	436 ± 406

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Figure 1
Shows the Gender Distribution

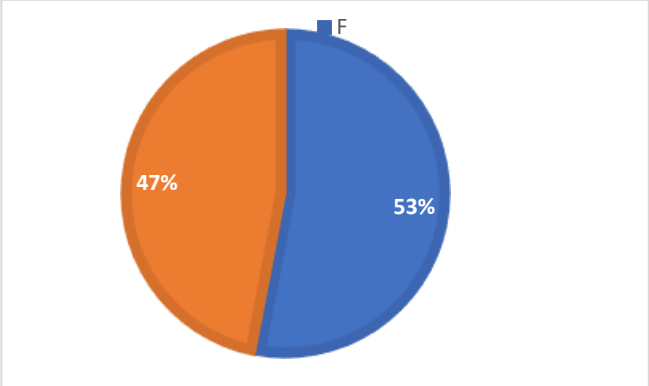
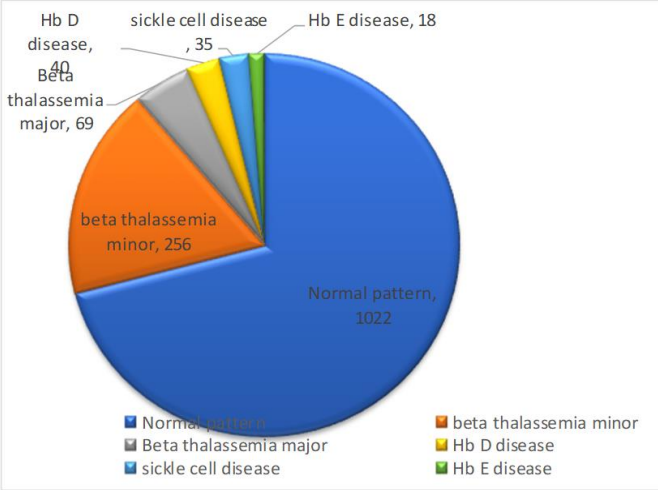


Figure 2
Shows the Frequency of Hemoglobinopathies



DISCUSSION

Thalassemia and its variants are among the most frequent genetic disorders of hematological disorders. In this study, we determined the rate of occurrence of different traits in multiple groups in extensive data. The incidence and prevalence of different types of hemoglobinopathies vary in other regions of the world[9,10].

Anemia could be secondary to many factors including malnutrition, chronic blood loss, or hereditary disorders such as hemoglobinopathies. Disorders of hemoglobin and thalassemia are autosomal recessive genetic disorders, mainly affecting the globin moiety of the Hb molecule. Accordingly, it is important to initiate or continue to monitor national trends, and to identify those hemoglobin variants which are more likely to be diagnosed.

Thalassaemia is a global public health problem, but its burden is overwhelming in the developing world, particularly in Asian countries where fewer resources are available for dealing with the problem.[11] Pakistan is a country that is currently fighting the ever-increasing burden of beta thalassemia major.

The prevalence of beta-thalassemia in the young population represented in the institutes of Central Punjab in the present study is 2.5%. In one study from Nawab Shah (Pakistan), the prevalence of beta thalassemia trait among the students of schools, colleges, and universities was reported to be 4.9%.[12,13]

Awareness of thalassemia prevention is increasing at different platforms in Pakistan and 1 order was approved by the government of Sindh (Pakistan) for the prevention of thalassemia by screening of the thalassemia trait before marriage but no implementation was done according to this bill till date. Our study is an attempt to determine the frequency of various hemoglobinopathies in the Karachi region that can be useful in the prevention and management of various hemoglobinopathies, which may play a vital role in the blood bank as well as in the formulation of transfusion policies. Adequate measures and screening procedures especially prenatal diagnosis should be performed concurrently to reduce the possibility of Hb disorders in offspring, the mental and physical trauma of affected patients, and the socioeconomic burden of the family. Screening is affordable and an accessible way to detect carriers and can be offered in a range of settings in different societies: In high school, before marriage, or in antenatal clinics.

In the present study, the overall frequency of thalassemia and hemoglobinopathies was 30.1% (n=442), which is like the study by Waheed et al. who reported a 28.4% overall frequency of hemoglobinopathies[15]. In our study, the increased frequency of beta thalassemia trait which is like the studies reported from Pakistan stated that the most common cases detected on HPLC was β-thalassemia trait in our region[14]. Another study from Pakistan (KPK province) showed a higher frequency of hemoglobinopathies which was 48.9%[16].

In our country, Pakistan, fighting against the increasing burden of homozygous beta thalassemia due to the lack of a screening system. Available statistics, largely derived from the prevalence of carriers and birth rate, indicate that the country is not successful in decelerating the disease incidence let alone prevention. Hemoglobinopathies do not have definite medical care, thus the World Health Organization (WHO) has recommended a prevention and control program including enlightenment of the public, screening for asymptomatic carriers, antenatal detection, and genetic counselling.[17]

Limitations

The results of this study are biased as only selected samples were tested using conventional methods. Additionally, a major proportion of the study cohort is comprised of samples received from pediatric clinics. Moreover, the high incidence of iron deficiency anemia in the population as well as in the study cohort is another limiting factor that could be a reason for diagnosis.

CONCLUSION

HPLC has the advantage of screening and detecting various hemoglobinopathies over other tests by providing rapid and accurate results. HPLC can detect and measure HbF and HbA2 in a single system. It provides superior resolution, is automated, and internal sample preparation is possible. It is important especially when the incidence of beta thalassemia traits is higher in developing countries like India, where there are limited resources available for early diagnosis.2

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