e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(2); 110-115

Original Research Article

Usefulness of HPLC & Hematological Parameters in Laboratory for Evaluation of Abnormal Hemoglobin Variants in Western-Tribal Part of India

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Received: 29-11-2022 / Revised: 30-12-2022 / Accepted: 29-01-2023
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Conflict of interest: Nil

Abstract

Introduction: Hereditary disorders of abnormal Hemoglobin variants are one of the commonest genetic disorders worldwide. Three broad groups of these disorders are: structural variants (hemoglobinopathies), thalassemias & hereditary persistence of fetal hemoglobin (HPFH). "β thalassaemia belt" where β thalassaemia more coomon include Mediterranean region, Africa, Middle East, some areas of India, Pakistan, and Southeast Asia. In India, average carrier rate for β thalassaemia is 3%.

Objective: The objectives of this study are 1) to find out prevalence of various abnormal hemoglobin (Hb) variants by HPLC method in given period, 2) to compare hematological parameters among various abnormal hemoglobin variants.

Materials and Methods: A Retrospective Study was conducted in a Laboratory in Tertiary care hospital at western-tribal part of India during July 2022 to August 2022. HbA, HbA2, HbF and other abnormal variants of hemoglobin were studied by HPLC (High Performance Liquid Chromatography). Hematological parameters tests were also done.

Results: 2944 patients samples run in HPLC analyzer, and abnormal hemoglobin variants found in 160 cases. These 160 patients had abnormal Hb chromatogram on HPLC. β Thalassemia trait constituted 66 cases followed by 66 cases of HbS trait-heterozygous, 11 cases of HbS disease/anemia-homozygous , 6 cases of Compound HbS homozygous with β thalassemia trait, 6 cases of HbE trait-heterozygous, 3 cases of HbE disease-homozygous and 2 cases of HbD punjab.

Conclusion: Various hereditary Hb disorders identified at Shree Vinoba Bhave Civil-Hospital, DNH (Dadra & Nagar Haveli-Union Territory) situated at western part of INDIA & this study reflectes problem magnitude, which may represent iceberg phenomenon. These hereditary Hb disorders are easily identified on HPLC platform via screening of antenatal women, school & college students. Policy makers, Health care department & various NGOs play important role in preventing these disorders via screening and education programmes.

Keywords: Hemoglobinopathies, Thalassemia,Hematological Parameters, HPLC.

Introduction

Abnormal Hemoglobin variants grouped into three broad categories. 1.Structural Hb variants also known as hemoglobinopathies e.g. HbS, HbE,HbC. 2. Lack or reduced globin chain synthesis e.g. thalassaemias. 3.Failure to complete normal neonatal switch from HbF to HbA e.g. hereditary persistence of fetal Hb (HPFH). Any combination of the above abnormalities, an individual can harbour [1]. These disorders are one of the commonest inherited as well as monogenic diseases in the world. Genetic diseases of hemoglobin (Hb) are usually autosomal recessively inherited. Because most of the times - recessive inheritance, these diseases may occur in children of healthy couple & healthy couple is carrier of that disease at that time. In thalassemic disorders, either lack or reduction in synthesis of globin chains. Examples of thalassemic disorders are: alpha (α) and beta (β) thalassemias. β thalassemias clinically classified on the bases of blood transfusion dependency & severity of anemia in three different groups: Thalassemia major, minor & intermedia. Globin chain synthesis imbalance causes precipitation of unpaired globin chains, ineffective erythropoiesis and haemolysis. While in hemoglobinopathies, there is altered structure of globin chain due to one or more amino acid substitution due to a point mutation in globin chain genes. Common structural Hb variants are HbS, HbE and HbC. Different combinations of above diseases may result in spectrum of disorders like mild microcytic hypochromic anemia, death in utero, hydrops foetalis syndrome, silent carrier state & severe, lifelong, transfusion dependent anemia with multiorgan involvement [2]. The mean prevalence of β thalassaemia heterozygotes in India is reported to be 3.3 % mean carrier rate (range 3-4 %). Every year 10,000 to 11,000 children with thalassaemia major are born in India (10% of all thalassaemics in the world). In India with 1.3 billion population, around 20-30 million Indian people carrying gene for thalassemia [3]. By 32 years of age, Thalasssemia major patient already had received 1250 units of blood transfusions, 20,000 vials of Desferal, 68,000 hours of his life he had needle in his body, patient has to spent 30.2 lakhs INR only for injections. By 50 years of age, Thalasssemia major patient requires 2000 units of blood transfusions, 30,000 vials of Desferal, 3 lakhs hours of his life he had needle in his body, he has to spent 60 lakhs INR only for injections [4] In thalassemia major patient, bone marrow transplant is the hope for cure & transplantation is possible in those patients who got a HLA matched donor [5].

Objective

The objectives of this study are:

1. To find out prevalence of various abnormal hemoglobin variants by HPLC method in given period,
2. Also, to compare hematological parameters of the various Hemoglobinopathies.

Materials and Methods

This Retrospective Study was carried out in Hematology Laboratory situated at Shree VBCH-HOSPITAL, SILVASSA-DNH (a tertiary care hospital at western part of India) from July 2022 to August 2022. Complete blood count (CBC) performed at Hematology Laboratory in all the 2944 patients. Total 2944 persons blood screened by HPLC method to detect abnormal hemoglobin pattern on chromatogram. Out of these 2944 samples, 160 persons had abnormal hemoglobin chromatogram on HPLC. There were 18 male (11.2%) and 142 female patients (88.7%) having abnormal hemoglobin pattern on chromatogram. 1.2 to 53 years is the age range in these patients.

Patients less than one year of age were excluded from the study. About 3 ml of blood was collected in a tube containing EDTA from each patient and the blood samples were sent to our Hematology Laboratory, for CBC & abnormal Hb analysis.

All 2944 blood samples were subjected to full blood count (FBC) using Siemens Advia 2120 - 5 Part Hematology Analyzer/Cell counter. Advia 2120 is bench top analyzer uses a combination of Light scatter, cytochemical staining, & nuclear density methods & also uses Cyanide free Hb method for Hb estimation. The 3-level (High, low & normal) controls were run every day in the cell counter, and the counter was maintained according to the manufacturer's instructions. Variant Hb analysis done by high performance liquid chromatography (HPLC) using Bio-Rad Variant^{TM} Hemoglobin Testing system & control was run every day. This instrument was maintained according to the manufacturer's instructions also. Using HPLC, the normal values for HbA2 are 2.2 – 3.4%, and for HbF <2%. HbA2 cut-off levels of between 4.0 - 8.0% was identified as β - thalassemia trait [6,7].

All the data of this study were analysed using Microsoft Excel.

Results

2944 samples were screened for CBC & HPLC. 160 (5.43%) cases were detected having abnormal hemoglobin patterns on chromatogram. Comparision table between various Hb variant disorders & CBC parameters (mainly RBC parameters) was prepared. (refer Table 1). Table 2 prepared for age wise distribution of various hemoglobinopathies. Table 3 also prepared for gender wise distribution of various hemoglobinopathies. Common hemoglobinopathies chromatograms also shown in image 1.

Table 1: Comparison between Hb variant diseases & CBC parameters mean values

Hb variant disease	Hb	RBC count	MCV	MCH	MCHC	HbA	HbA2	HbF	Others Hb Variants	Number of cases
β Thalassemia trait	9.6	4.3	70.7	22.0	30.2	82.2	5.5	0.8		66
HbS trait	10.4	4.6	73.6	22.8	31.0	61.3	3.2	0.6	HbS-25.7	66
HbS disease	7.8	3	86	27.1	31.4	4.4	2.5	18	HbS-70.2	11
Compound HbS with β thalassemia	7.6	3.2	75.8	23.4	30.8	4.8	5	13.4	HbS-72.3	6
HbE trait	9.9	4.2	72.4	23.3	32.2	63.7	31.2	0.3		6
HbE disease	7	4.04	59	17.1	28.9	7.5	91.4	7.1		3
HbD punjab	10.4	3.72	84.4	28.2	33.4	53.6	2.9	0.8	HbD-35.6	2

Table 2: Age wise distribution of hemoglobinopathies

Age distribution	Number of cases
<10 years	8
10 - 40	147
>40	5

Table 3: Gender wise distribution of Hemoglobinopathies

Gender	Number of cases	In Percentage
Male	17	10.6
Female	143	89.3

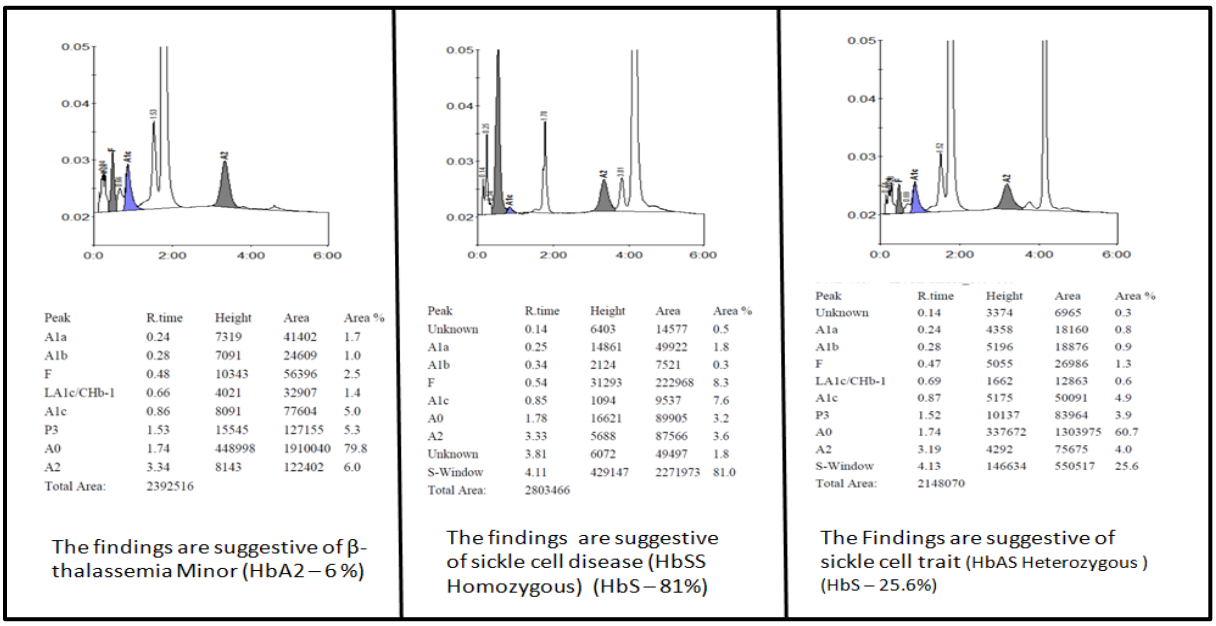

Image 1

Observation

Most common Hb variant disease found in our study is β Thalassemia trait followed by HbS & HbE. Women in the reproductive age group were commonly affected.

These Hb variant diseases were common may be due to DNH is tribally populated area. Compound hemoglobinopathies (two different Hb variants in same person) are also found in our study. Borderline HbA2 levels - one of the problems in routine HPLC diagnosis and need parental HPLC study plus DNA analysis of person. Disease states of these disorders (HbS & HbE disease) associated with severe forms of anemia, while traits associated with mild anemia.

Discussion

Hereditary Hb disorders (mainly β Thalassemia trait, HbS & HbE) are one of the important challenges in tribally populated India. β Thalassemia trait, HbS & HbE genes are variably distributed in Indian tribal populations. HbE is mainly restricted in tribals of North-East, West Bengal, Odisha and those in Andaman and Nicobar Islands. HbS has more extensive distribution in the country (10-40% trait frequency) and the homozygotes and double heterozygotes present with a wide array of morbidities. Study done by Munj V, Dias M *et al*, 1084 patients screened for Hb disorders (our study 2944 patients screened), out of which 130 cases detected having abnormal HPLC chromatograms (our study 160). Diverse Indian population with more than 3000 ethnic groups follow endogamy & hemoglobinopathies are one of the commonest genetic disorders in India mainly in tribal population & important health problem [8]. Thalassemia is the most prevalent hemoglobinopathy with a carrier frequency of 3 to 4% in India [9]. It is observed in certain communities like Muslims, Sindhis, Punjabis, Bengalis and Gujaratis having higher carrier state (3 to 17%) [10]. HbS is prevalent in tribal population of southern, central and western states. HbE is common in North eastern states of India. HbD is seen in Punjab. The cumulative gene frequency for sickle cell, HbE and HbD is 5.35% in India. Due to migration of ethnically diverse group of patients, there is mixing of people causing

increase in hemoglobinopathies and it will be responsible for major clinically significant hemoglobinopathies in the coming decades [11]. Our study carried out in Shree VBCH-Hospital, DNH - laboratory shows a spectrum of different Haemoglobinopathies which reflects the presence of different hemoglobin variants. Traits or heterozygous conditions are not important health problems but when they occur with other traits or other variant Hb then they are responsible for severe clinical health problems. Cross or Consanguineous marriages or mixing of trait populations from differant areas of country may lead to serious clinical forms in coming years [11]. The aim of the study was to detect various Haemoglobinopathies by HPLC which has advantage of quantifying HbF, HbA2 along with detection of variants in a single test. HPLC is less labor intensive, has rapid turnaround time, is sensitive, specific and a reproducible test, ideal for clinical laboratory which can replace alkaline and acid electrophoresis which cannot detect all abnormal variants of hemoglobin [9,12,13]. Quantification of HbA2 and HbF levels by HPLC along with CBC was of importance in this study which helped to arrive at a conclusive diagnosis as genetic studies facilities are not available [14,15]. As confirmatory diagnostic facilities are not easily available, Parental screening with iron studies and HPLC may be done before referring the patient for genetic testing [12,16]. Refractory microcytic hypochromic anemia with borderline or reduced HbA2 on HPLC may be investigated for alpha Thalassemia, which is the commonest hemoglobinopathy in India. It is diagnosed by molecular genotyping, thus preventing unnecessary iron therapy [16]. HbA2 reduced in iron deficiency anemia, according to some studies [17]. So, if we do iron studies along with HPLC, it can be useful for borderline cases and can guide us to perform genetic testing [18-20]. HPFH and Delta-beta Thalassemia, these two disorders have differant RBC indices & have similar HPLC findings & diagnosis done by DNA analysis [12].

Conclusion

The study done by us, it provides data of various Hb disorders at Shree VBCH-HOSPITAL, DNH. It also reflects hemoglobinopathy is one of big challenges in diagnosis for that we require the help of HPLC & genetics. These disorders are just tip of iceberg requiring proper screening of whole population, so that we can prevent severe hemoglobinopathies by screening carrier state. Policymakers & health department can also take measures according to study for prevention of disorders. Reproductive age group women affected most commonly in this study, so they (Reproductive age group women & children) are the target population screening. We can also make general public aware about these hemoglobinopaties. RBC indices, HPLC, parental or family screening & ethnic data guides us in identifying these disorders. Clinician awareness also required regarding limitations & problems in routine diagnosis of these disorders. Genetic testing laboratory & other abnormal peaks on HPLC require help of reference Labs, so reference lab set up required in state.

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