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IMPORTANCE OF FAMILY STUDIES WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN HEMOGLOBIN DISORDERS: AN ANALYSIS OF 34 FAMILIES

Pathology

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ABSTRACT

Background: Hemoglobinopathies are most common hereditary disorders in India and poses a major health problem. The present study is useful to generate detailed data of role of family study in haemoglobin disorders. In developing countries like India due to paucity of funds and resources, family studies on HPLC could be alternative tool. So that carrier identification in family would be useful to create awareness and offer them like partner selection, prenatal diagnosis, and contraception.

Aims And Objectives:

1) The aim of the study will be to analyze laboratory aspects, namely, hematological profile, and HPLC findings of the hemoglobin variants detected.
2) Detection of spectrum of haemoglobin disorders in study population comprising of index cases and family study.
3) The aim of the study is to identify asymptomatic individuals who carry variants associate with hemoglobinopathies.

Materials & methods: This is retrospective study carried out in the department of pathology, M. P. Shah Govt medical college and hospital, Jamnagar over a period of 2 years from January 2021 to December 2022 **Inclusion Criteria:** All patients having particular hemoglobinopathy along with their parents and siblings are included in the study. **Exclusion Criteria:** Patients whose blood sample was not accompanied by family members' blood were not included in the study. **Conclusion:** HPLC family studies is cheap, easily accessible, and equally efficacious compared to genetic studies. To detect "asymptomatic traits", for early diagnosis of variants presenting late in life, for accurate diagnosis of complicated heterozygotes in absence of molecular studies and prevention of propagation of hemoglobinopathies in families by prudent premarital and prenatal studies, family studies are indispensable.

KEYWORDS

Family studies, HPLC, Hemoglobinopathies

INTRODUCTION:

Hemoglobinopathies are most common hereditary disorders in India and poses a major health problem. In India, Beta Thalassemia is prevalent across the country, with an average frequency of carriers being 3-4%. A higher frequency has been observed in certain communities, such as Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas and Gaurs. HbS is highly prevalent in the tribal populations of Southern, Central and Western states reaching as high as 48% in some communities. HbE is common in the North Eastern states, and has a carrier frequency as high as 50%, in some areas. It is found in lower frequencies in the Eastern states of West Bengal, Bihar and Uttar Pradesh, while HbD is present in about 2% of people in Punjab.[1]

Inherited abnormalities of hemoglobin (Hb) include a myriad of disorders ranging from thalassemia syndromes to structurally abnormal Hb variants/hemoglobinopathies. Detailed clinical history, complete hematologic evaluation along with newer ancillary techniques including High-performance liquid chromatography (HPLC), when used systematically can achieve laboratory diagnosis of thalassemia syndromes and hemoglobinopathies. [2]

The DNA based tests identify the defect at the gene level and provide final confirmation of the defect but, in developing countries like India due to paucity of funds and resources, family studies on HPLC could be alternative tool. The present study is useful to generate detailed data of role of family study in hemoglobin disorders. So that health care resources are often successfully targeted at them. Carrier identification in family would be useful to create awareness and offer them like partner selection, prenatal diagnosis, and contraception.

AIMS AND OBJECTIVES:

1). The aim of the study will be to analyze laboratory aspects, namely, hematological profile, and HPLC findings of the hemoglobin variants detected.
2). Detection of spectrum of hemoglobin disorders in study population comprising of index cases and family study.
3). The aim of the study is to identify asymptomatic individuals who carry variants of hemoglobinopathies.

MATERIALS &METHODS:

This is retrospective study carried out in the department of pathology, M. P. Shah Govt medical college and hospital, Jamnagar over a period of 2 years from January 2021 to December 2022

Type of study: Retrospective

Patients Selection

All patients having particular hemoglobinopathy along with their whole family members are the study population.

Patients Sample For Laboratory Testing

EDTA blood sample were collected from the patients with the detailed personal history, family history and geographic location of the patients.

Haematological parameters: RBC Count, Hb, Hct, MCV and MCH were obtained using 5 part automated haematology analyser horriba penta XLR blood cell counter. Mentzer index was counted using obtained haematological parameter. Sickling test, Solubility test, Acid elusion test and reticulocyte count were performed. Peripheral blood smear examination was done to evaluate red cell morphology.

Standard Method

Primary screening for hemoglobinopathy was done using - arkray ADAMSA_{1C}HA-8180T.

Principle: The -8180T measures HbA1c, HbA2 and Hb F in blood using reversed- phase cation exchange chromatography. Blood sample diluted with the hemolysis washing solution is sent to the column, which fractionates the sample into several hemoglobin components based on high performance liquid chromatography (HPLC). Each component eluted from the column is measured by the dual-wavelength (420 nm/500 nm) colorimeter, and the result is process by a computer to obtain peak identification and content. Guidelines to assure acceptable results-

1. The instrument has passed calibration
2. The instrument can give acceptable QC result
3. Total area od A0 should be:20,000 – 60,000

4. Base line should be clear
5. The reportable ranges (based on the measurements range): HbA1c (3%-20% or 9-195mmol); HbF (0-100%)

Reagents: 1. ELUENTS 80A
2. ELUENTS 80B
3. ELUENTS 80CT
4. HEMOLYSIS WASHING SOLUTION 80H

The graph generated was interpreted according to the elusion times T model given as reference literature with the machine.

Inclusion Criteria:
All patients having particular hemoglobinopathy along with their parents and siblings are included in the study.

Exclusion Criteria:
Patients whose blood sample was not accompanied by family members' blood sample due to distance, remarriage, death, and reluctance in parents, lack of awareness, misconceptions, and psychosocial reasons were not included in the study.

RESULT:
In the present study we have selected 34 families for study of hemoglobinopathies which comprises of total 120 family members. Out of 120 members 100 members were found to have hemoglobinopathies and 20 of them were normal.

Classification Of Cases
The cases are grouped according to complete hematological workup, HPLC study and family study cases are classified as: Group-A Normal, Group-B Sickle Cell Anemia, Group-C Sickle Cell Trait, Group-D Sickle Cell Disease, Group-E Beta Thalassemia Major, Group-F Beta Thalassemia Trait, Group-G Sickle Beta Thalassemia, Group -H Beta Thalassemia Major with Hb Variant HBH, Group -I Delta Beta Thalassemia, Group- J Hb D Trait, Group-K HbF High for Age, Group-L Beta Thalassemia Major with Hb Lepore.

Table 1: Cases are grouped as follows with the percentage (N = 120).

Group	Haemoglobin variants	Number (%)
A	Normal	20(16)
B	Sickle Cell Anemia	7(5.8)
C	Sickle Cell Trait	18(15)
D	Sickle Cell Disease	5(4.1)
E	Beta Thalassemia Major	9(7.5)
F	Beta Thalassemia trait	44(0.8)
G	Sickle Beta Thalassemia	4(3.3)
H	Beta Thalassemia Major with Hb Variant HBH	1(0.8)
I	Delta Beta Thalassemia	3(2.5)
J	Hb D Trait	2(1.6)
K	HbF High for Age	6(5.0)
L	Beta Thalassemia Major with Hb Lepore	1(0.8)
	Total	120

Age And Sex Wise Distribution Of Cases Among All The Groups
Maximum cases (44) of group F (Beta thalassemia trait) of which 21 cases are seen in 1-10 years of age followed by 9 cases in 21-30 years of age, 5 cases in 31-40 years of age, 5 cases in 41-50 years of age, 3 cases in 11-20 years of age and single case in >50 years of age followed by group C (Sickle cell trait) 18 cases are seen in which more cases in 21-30 years of age, 4 cases in1-10 years of age,2cases in 11-20 years of age, single case in 31-40 years of age group and group E (Beta thalassemia major) in which 09 cases of which 5 cases are seen in 1-10 years of age,2 cases in 11-20 years of age group and single case in each group in 21-30 and 31-40 years of age followed by group B (Sickle cell anemia) 2 cases are seen in 1-10 years,2 cases are seen in 21-30 years of age, 2 case in 31-40 years of age and single case in 11-20 years of age group.

In Group-E i.e. (Beta thalassemia major) No. of male patient are 07 and female patient 02 with M: F ratio 3.5:1, followed by in Group-F i.e. (Beta Thalassemia Trait) No. of male patient 26 and female patient 17 with M: F ratio 1.5:1, in Group-C i.e. (Sickle cell trait) No. of male patient 07 and female patient 11 with M: F ratio 0.6:1, in Group-B i.e. (Sickle cell Anemia) No. of male patient 02 and female patient 05 with M: F ratio 0.4:1.

In the study out of 34 family's history of consanguinity was present in 07 (20.58%) families. Caste wise distribution of families, Schedule cast (59%) was the most common ethnic background among all the groups followed by Muslims (41%). In HbD Trait cases, most common ethnic background was Sindhi followed by Muslim.

Majority of the cases presented clinically with Pallor i.e 39 cases (32.5%) next common presentation was abdominal pain. Other clinical features were jaundice, hemolytic facies, and abdominal pain, bone pain.

Distribution of index cases with splenomegaly and hepatomegaly
Out of 37 cases 15 cases presented with Splenomegaly. Out of 15 cases 08 cases were found in 0-10 years age group followed by 07 cases in 11-20 years age group. So, the maximum cases of Splenomegaly were found in first decade. Maximum cases of hepatomegaly were found in first decade of life of which maximum No. of patients with hepatomegaly were found in Group-E (Beta thalassemia major).

Mean hematological parameters in all age groups
Lowest Hb was found in Group E i.e. Beta thalassemia major and highest level of Hb was found in Group C i.e. Sickle cell trait. Lowest RBC count was found in Group E i.e. Beta thalassemia major and highest RBC count was found in Group C i.e. Sickle cell trait. Lowest MCV was found in Group E i.e. Beta thalassemia major and highest MCV was found in Group B i.e. Sickle cell trait. Lowest MCH was found in Group C i.e. Beta thalassemia trait and highest MCH was found in Group B i.e. Sickle cell anemia

DISCUSSION:
In the present study of total 120 members of 34 families, the average age of presentation of sickle cell disease was 11.28 years, β-thal major was 2.31 years. Similar findings were observed by R.S Balgir and Tyagi, et al. [4,5].

Out of 6 cases of sickle cell disease, 4 were male and 2 was female, 9 cases of β-thal major, 7 were male and 2 were female. 04 cases of sickle β thal 02 were male and 02 were female. Similar observation was made by R.S Balgir & Tyagi, et al. [4,5].

Pallor is the most common clinical presentation observed in sickle cell disease patients (100%), Beta thal major (100%) in this study. Abdominal pain was the next most common presentation in sickle cell disease (49.48%), Beta thal major (50.52%) similar findings were seen by R.S Balgir & Tyagi, et al. [4,5], except they observed vaso-occlusive crisis 31% and 81.3% respectively.

In the present study the most commonly sickle cell disease affected ethnic group is Buddha followed by banjara and muslim. Most common Beta thalassemia affected ethnic group is Buddha followed by muslim. Most common Sickle beta thalassemia affected ethinic group is Buddha followed by muslim. Kate SL reported high prevalence amongst SC, ST and OBC in State of Maharashtra. Bhatia and Rao found prevalence of sickle cell disorder is very high amongst tribal and scheduled caste population group where carrier frequencies range between 5-40% or more. Dangi, et al., found Muslims, Sindhis and Tribal population the major carriers of sickle gene [6,7].

Patel DKhad quoted that, in India the practice of endogamy and consanguineous marriages are very common, and since β-thalassemia and hemoglobin S (HbS) are relatively frequent in India, compound heterozygosity for these two disorders (βS / β-thalassemia), usually expressed in a severe type of disease, is not rare [8].

Tariq HA narrated that, the main factor, which may increase or decrease the risk of having an affected child, is the parents' relationship. The risk of hemoglobinopathies increases with consanguineous marriages. This is because all the hemoglobinopathies are transmitted only through heredity [3]. In the present study there were 07 families out of 34 (20.58%) showing history of consanguinity. Consanguinity is commonly practiced in Buddha and Muslim communities.

In the present study splenomegaly was found in 2 (40%) patients of sickle cell disease. In the Beta thalassemia major patient's splenomegaly was found in 5 (55.5%) patients. In the Sickle beta thalassemia patient's splenomegaly was found in 2 (50%) patients. Tyagi, et al. in their study on sickle cell syndrome, found that pallor

(62.5%), jaundice (75%), painful crisis (62.5%) and leg ulceration (25%) were more frequent in sickle cell disease than in double heterozygous Sβ Thal patients [5]. They also found that the frequency of splenomegaly was significantly higher (75%) in patients with Sβ Thal than Sickle cell disease. Shah SJ, et al. reported - 35 out of 35 cases with pallor (100%), 31 cases with splenomegaly (88.6%), 25 cases with hepatomegaly (71.4%) and 8 cases with icterus (22.9%) [10]. RS Balgir, et al. found splenomegaly in 17.1% of cases. Splenomegaly is higher in patients with Sβ thalassemia than SS disease [4]. Patel DK stated that persistent gross splenic enlargement is peculiarity of Indian sickle disorder patients [8].

Hematological Parameters

Present study means hematological parameters in all Age groups: In the study of various authors, it is observed that low hemoglobin levels in thalassemia major are < 7 g%. Similar was the finding in present study with thalassemia major group showing low hemoglobin levels (mean 4.6 g%) as compared to other groups. Similarly, hemoglobin levels in sickle beta thalassemia were < 10.1 g%. In the present study mean hemoglobin levels in sickle beta thalassemia were 7.1 g%. Hemoglobin level. [5,9-12]

In the present study the HbF level in Beta thalassemia major was high > 90% as compared to the other studies in which it was quite variable < 90%. This may be due to the reason that the cases included in the present study did not receive any blood transfusion [12].

In the study of Ambekar SS, Rao S, et al. C Vani and S Mamta and in the present study the Hb S levels were > 60% (except in Dangi, et al. study). In all the above studies the average Hb F levels were high > 15%, in the present study it was 17.91%. The average HbA2 level in present study was 5.44% [07].

Our findings thus support that HPLC family studies is cheap, easily accessible, and equally efficacious compared to genetic studies. To detect "asymptomatic traits", for early diagnosis of variants presenting late in life, for accurate diagnosis of complicated heterozygotes in absence of molecular studies and prevention of propagation of hemoglobinopathies in families by prudent premarital and prenatal studies, family studies are indispensable. Community based targeted family studies along with HPLC can be an effective tool in reduction of disease burden.

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