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Family Study of Different Hemoglobin Disorders and Variants in North-Western India in Tertiary Center

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ABSTRACT

Hemoglobin-related disorders are among the most common inherited genetic disorders in the world. They are posing a serious health burden to the global health system. As per WHO, the highest incidence of hemoglobinopathies is in the Middle East and Indian subcontinent. Screening methods like High-Performance Liquid Chromatography (HPLC) help in determining values of HbA, HbA2, and HbF and diagnosing hemoglobinopathies at the initial stages.The present study aims to determine the role of family studies using HPLC in hemoglobin-related disorders.A retrospective study of 48 months between January 2019 and January 2024, comprising 137 patients was conducted. Patients attending the Outpatient Department (OPD) and admitted to the Inpatient Department (IPD) with anemia and abnormal values of different hemoglobin (HbA, HbA2,HbF, etc.) along with family members were included in this study. Patients with less than 3 months of history of blood transfusion and less than 6 months of age were excluded from the study. A total number of 572 patients with Hb < 11 g/dL were screened. Out of 153 (26.74%) patients, 137 (23.95%) patients and their family members agreed tothe family study. Among 137 patients, 72 were females and 65 were males. Therefore M:F ratio was 0.90:1. Pallor was present in 121 (88.32%) cases and splenomegaly was seen in 49 cases (35.76%). HPLC along with family studies is a quick and minimally invasive method to screen high to medium-risk large communities, which in return helps in controlling the spread of clinically dreadful homozygous state of hemoglobin disorders.

Keywords: Family study, hemoglobinopathies, HPLC, screening

INTRODUCTION

The inherited hemoglobin disorders are the most common monogenic disorders present worldwide. As per WHO, the most common hemoglobin disorder is sickle cell disease. But in 11 Southeast Asian countries, the thalassemia subtypes likeβ thalassemia, α thalassemia, and hemoglobin-E are highly prevalent. Their prevalence rates range from 2.5%-11%.[1]

According to the World Health Organization (WHO),in India,the average prevalence of β thalassemia is between 3-4% of the population. In India, there are many multi-ethnic and linguistic groups, therefore, the distribution of hemoglobin variants varies from one province to another province. Gujrat, Sindh, Punjab, Tamil Nadu, and Maharashtra have the highest prevalence of β-thalassemia, whereas sickle cell disease is found mainly in central and southern India.[1]

Hence, active screening should be done in a high-risk population. It will help in subduing the morbidity and mortality related to homozygous hemoglobinopathies.

Currently, the gold standard for hemoglobin variants is automated cation-exchange High-Performance Chromatography (HPLC). Installation, and maintenance of HPLC machines along with training of associated staff is expensive proposition especially in lower- and middle-income countries. Ironically, these countries have maximum prevalence of hemoglobinopathies.[2]

The downside is, in certain patients, HPLC does not give confirmatory results. For example, it might be difficult to differentiate between double heterozygous of β-thalassemia and HbE from the HbE homozygous state with HPLC values of individual patients alone. Similarly, delta beta thalassemia and Hereditary Persist of Fetal Hemoglobin (HPFH) might yield similar values on HPLC. Overall prognosis is very differentin these different entities. Therefore, family studies will play a vital role in differentiating these entities and confirming the diagnosis. Subsequent counseling of these affectedpatients will help in curbing the spread of these hereditary disorders.

This research aimed to studythe relevance of family study in differentiating various hemoglobinopathies with overlapping valueson HPLC.

METHODS

A retrospective study of 48 months from January 2019 to January 2024, 137 patients along with their family members were included. Patients attending Outpatient Department (OPD) and admitted in Inpatient Department (IPD) with anemia and abnormal values of different hemoglobin (HbA, HbA2, HbF, etc.) along with their family members were part of this study. Patients with less than 3 months of history of blood transfusion orpatient with less than 6 months of age were excluded from the study.

Four (4) mL of intravenous blood was collected in EDTA vial and sent for complete blood count using an automatic hematology analyzer (Sysmex 800). EDTA sample was analyzed using Bio Rad D-10 that separated different hemoglobin through chromatography using ion-exchange high performance liquid chromatography.

The institutional Ethical committee granted leewayand permission for conducting the retrospective study. The consent forms were already explained and filled by patients before HPLC testing.

RESULTS AND DISCUSSIONS

The number of patients screened on HPLC was 572. They had < 11g/dL of hemoglobin. Out of which 153 patients (26.74%) showed hemoglobinopathy or a variant of physiological hemoglobin. Only, 137(23.95%) patients agreed for family study. Among 137 patients, 72 were females and 65 were males.Therefore M:F ratio was 0.90:1

Pallor was seen in 121 (88.32%) cases and splenomegaly was present in 49 cases (35.76%).Hemolytic face was present in one patient with homozygous β-thalassemia.

Microcytic picture was present in 107 (78.10%) and hypochromasia was seen in 71 patients (51.82%). Microcytosis and hypochromasia were seen in both homozygous as well as heterozygous hemoglobinopathies but normocytic and normochromic blood picture was present in physiological variants of hemoglobin i.e., HbD_{Punjab} and HbD_{Iran}.Table 1 denotes the number of patients in each subtype of hemoglobin disorder.

In this present study, 137 patients family studies were performed. The most common hemoglobinopathy present was β-thalassemia trait consisting of 73 (53.28%) cases, it is more than the percentage of cases found by Garje *et al.*, (38.73%), Dhara *et al.*, (36.66%) and Phalak *et al.*, (17%). Present study was conducted in North India where as the

Table 1. Distribution of 137patients in present study

Diagnosis	No. of Patients
Homozygous β-thalassemia	6 (4.37%)
Heterozygous β-thalassemia	73 (53.28%)
Homozygous Sickle cell disease	2 (1.45%)
Heterozygous Sickle cell disease	7 (5.1%)
Double heterozygous β-thalassemia and HbE	11 (8.02%)
Homozygous HbD_{Punjab}	4 (2.91%)
Heterozygous HbD_{Punjab}	15 (10.94%)
Heterozygous HbD_{Iran}	3 (2.18%)
Homozygous HbE	1 (0.72%)
Heterozygous HbE	8 (5.83%)
Heterozygous delta β-thalassemia	3 (2.18%)
Hereditary Persist of Fetal Hemoglobin (HPFH)	4 (2.91%)
Total patients	**137**

study population of Garje *et al.*, and Phalak *et al.*, was South-western India and Dhara *et al.*,was performed in Western India.[3-5] Kavitha *et al.* studying South Indian population recorded maximum number of cases of β-thalassemia trait of 69.13%.[6] Yadav *et al.* stated in its study about prevalence of β-thalassemia trait in various parts of India, Central India had 1.4-3.4% whereas South India had 8.50%-37.90% and North and Western India too had higher prevalence between 0-30.8%.[7]

The present study had 8 (5.83%) patients of heterozygous HbE and 1 (0.72%) patient of homozygous HbE. Garje *et al.* (6.3%) had maximum number of heterozygous HbE cases. Dhara *et al.* did not have a single case of HbE, whereas Phalak *et al.* had only 1% of cases. Kavitha *et al.* had 4.1% of cases of HbE, which is closer to percentage of cases in this study.[3-6] It (HbE) is generally found in population of North Eastern India and Eastern India along with South East Asia.[1]

Patel *et al.* had reported, in some parts of India, endogamy and consanguineous marriages being very common, thus leading to many double heterozygous cases.[7] It led to the first use of family study in this study. In this present study, consanguineous marriages were present in 17 (12.78%) families out of 137 families studied. Consanguineous marriages were mainly found in tribal population. In Bangladesh, consanguineous marriages has been linked to an increase of carrier state as well as double heterozygous state.[1]

In cases where individual patient HPLC values were not sufficient to confirm the diagnosis, the blood samples from mother, father and siblings were

taken. Following examples, explain the relevance of family study in this study. The patients hadsimilar values of various hemoglobinon HPLC testing, but they had different hemoglobinopathies. Thus, family study helped in diagnosing different entities, which were not able to be diagnosed via individual patient HPLC.

In example number one, family studies were done for differentiating HbE homozygous and double heterozygote of HbE and β-thalassemia. Both had raised HbA2, that is >50 percent of HbE of total hemoglobin. In homozygous HbE patient, both parents had values indicating towards heterozygous HbE. Homozygous HbE has a relatively benign course but double heterozygote HbE and β-thalassemia might have a similar clinical course as thalassemia major. Confirmation of diagnosis using family study and differentiation between these two entities is very crucial. In double heterozygote of HbE and β-thalassemia, the father had HPLC values of 20-30% HbA2 indicating heterozygous HbE and the mother had HbA2 value between 4%-7% indicating heterozygous state of thalassemia.[8]

In this present study, there were 4 (2.91%) cases of HPFH, which was very high compared to other studies.[3-6] Cases of HPFH as well as delta β-thalassemia show approximately 10-90% of HbF on HPLC.[9] Patients with HPFH have relatively normal hematological parameters but delta β-thalassemia patients have reduced hemoglobin and microcytosis.

Family study in this second example helped in confirming the diagnosis through parental screening. If the value of HbF was more than 5% after the age of 1 years old, along with blood smears showing microcytic and hypochromic RBCs, delta β-thalassemia can be suspected. The father of a delta β-thalassemia patient had similar values. Helping the confirmation of diagnosing delta β-thalassemia. Prognosis of HPFH is benign and prognosis of delta β-thalassemia is quite variable. In normal individuals, HbF values are less than 2% after 1 year of age.[9,10]

Three cases (2.18%) of delta β-thalassemia were reported in local population from upper caste community. The delta β-thalassemia is generally found in Turks, Greeks, Italians, Indians and Chinese.[11]

Third instance, where family studies helped in differentiating was HbD_{Punjab} and HbS. Average range of retention time of HbD Punjab (3.50-4.00s), and HbS (4.02-4.30s) are close to each other.[12,13] Thus HbD_{Punjab} could elute in HbS window, especially if the working conditions of HPLC machine were not ideal. One patient in this present study, had normal hematological parameters with HPLC values indicating towards sickle cell disease. Family studies helped in ruling out sickle cell disease because samples from both parents had HbD_{Punjab} and yielded results in the HbD window. It was clear that patient had HbD_{Punjab} instead of sickle cell disease.

It prevented wrong presumption of sickle cell disease in initial stage. Furthermore, confirmation could be done through sickling test. HbD_{Punjab} hemoglobin was present in local population belonging to Punjab. HbD_{Punjab} heterozygous is a phenotypically normal hemoglobin with mutation at B121 (GH4) Glu-Gln (GAA-CAA) of the β chain. Its prevalence is 3% in the North Indian population.[12,13] Present study reported 4 (3.00%) and 15 (11.27%) cases of homozygous and heterozygous HbD_{Punjab}, respectively.

Ethnically, this study showed HbSwas present in tribal and Muslim population, who migrated from Central India to North Western Provinces in search of job opportunities. Garje *et al*. found sickle cell disease in the Buddhist followed by Muslim community in Aurangabad district of Maharashtra. Dhara *et al*. recorded that sickle cell disease affected mostly Buddhists, followed by Banjara (nomadic people) and Muslims.[3,4]

Similarly, two heterozygous cases of HbD_{Iran} was found in a patient from the adjacent province of Punjab. Presence of delta β-thalassemia and HbD_{Iran} suggests a connection between the population of India and countries of the Middle East since ancient times, which eventually led to marriages and inter mixing of genetic material. Nasiri *et al.* studied Iranian population and HbD_{Iran} was the second most variant of hemoglobin after beta thalassemia.[14]

HbD_{Iran} has a normal phenotype with chain mutation of $alpha_2beta_2$ 22 Glu-Gln (GAA-CAA). It was first described in 1973, and was found in Iranian, Pakistanis and Indians. It was present in North-Western Region of Punjab. In the heterozygote state, it is phenotypically normal but in the homozygous state mild hemolysis is present but with an overall benign course.[15,16]

CONCLUSIONS AND SUGGESTIONS

In countries of low- or lower middle-income, where hemoglobinopathy could not be diagnosed by HPLC due to scarcity of resources, family case study should be the approach to take instead of DNA analysis. Though DNA analysis is the gold standard for diagnosis but family studies improve HPLC sensitivity and reduces turnaround time as well as cost.

More individual-level and family studies should be done through HPLC at provincial government as well as institutional level in different ethnicities and geographical locations. It will allow us in the future, more accurate mapping of the prevalence of hemoglobinopathies in under-resourced countries.

It will also help us to find not only pathological but also physiological variants of hemoglobin like HbD_{Punjab} and HbD_{Iran}.

REFERENCES

1. World Health Organization. Regional desk review of haemoglobinopathies with an emphasis on thalassemia and accessibility and availability of safe blood and blood products as per these patients' requirement in South-East Asia under universal health coverage. New Delhi, 2021; 1-10.
2. Ran An, Muhammad Noman Hasan, Yuncheng Man, Umut A. Gurkan. Integrated anemia detection and hemoglobin variant identification using point-of-care microchip electrophoresis. Blood, 2019; 134: 37.
3. Garje M, Sonwane BR, Gupta S, Narkhede P. Importance of family studies with High Performance Liquid Chromatography (HPLC) in hemoglobin disorders: An analysis of 37 Families. Int J Pathol Clin Res, 2020; 6:110.
4. DharaP.Trivedi, Vijay C. Popat, Arpita Chaudhari. Importance of family studies with High Performance Liquid Chromatography (HPLC) in hemoglobin disorders: An analysis of 34 families.IntlJSR, 2023; 12:23-25.
5. Phalak P, Pratap A. Haemoglobin variant study by HPLC method at tertiary care centre. J. Med. Chem. Sci, 2023; 6(11): 2841-2848.
6. Kavitha A, Kavita V, Mukilarasi. Incidence of hemoglobinopathies in South Indian population-4 year study. Annals of Pathology and Laboratory Medicine, 2023; 10(5): 47-52.
7. Yadav SS, Panchal P, Menon KC. Prevalence and management of β-thalassemia in India. Hemoglobin, 2022; 46(1):27-32.
8. Barbara J. Bain, Imelda Bates, Michael A. Laffan. Practical Haematology.Twelfth Ed., London, Dacie and Lewis, 2017;294-303.
9. Sharma DC, Singhal S, Woike P, Rai S, Yadav M, Gaur R. Hereditary persistence of fetal hemoglobin. Asian J Transfus Sci, 2020; 14(2): 185-186.
10. Pedro A. de Alarcón, Eric J. Werner, Robert D. Christensen. Neonatal hematology: Pathogenesis, diagnosis, and management.2nd Ed., New York, Cambridge University Press, 2021; 3: 93-100.
11. Dipika Mohanty.Variant hemoglobin system. Bio Rad Pvt Ltd, 2010; 30-40.
12. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC & modulating effects of nutritional deficiency anaemias from North India. Indian J Med Res, 2010; 132(5): 513-9.
13. Ankur N. Sarvaiya, Sanjaykumar C. Chauhan.Variant hemoglobin spectrum by cation exchange high performance liquid chromatography: A study of 2035 subjects. Annals of Pathology and Laboratory Medicine, 2017; 4(3): 297-303.
14. Nasiri A, Rahimi Z, Vaisi-Raygani A. Hemoglobinopathies in Iran: An updated review. Int J Hematol Oncol Stem Cell Res, 2020; 14(2): 140-150.
15. Rohe RA, Sharma V, Ranney HM. Hemoglobin D Iran alpha A2 beta 2 22-Glu leads to Gln in association with thalassemia. Blood, 1973; 42: 455-462.
16. Bhat VS, Mandal AK, Mathew B. Co-inheritance of HbD_{Iran}/beta thalassemia IVS1-5 (G > C) trait in a Punjabi lady with diabetes. Ind J Clin Biochem, 2012; 27: 202-206.