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PATTERN AND QUANTIFICATION OF HEMOGLOBINOPATHIES IN ANAEMIC PATIENTS BY HPLC

Pathology

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ABSTRACT

Haemoglobinopathies are a group of genetic disorders of haemoglobin divided into structurally abnormal haemoglobin variants and those in which one or more of the normal polypeptide chains of haemoglobin are synthesized at a reduced rate. In this study, we screen for haemoglobinopathies in anaemic patients using HPLC. HPLC is a reliable, accurate, fast and reproducible diagnostic tool for early and direct identification of haemoglobin variants with a high degree of precision in quantification of normal and abnormal haemoglobin variants.

KEYWORDS

Haemoglobinopathies, HPLC, Anaemia.

INTRODUCTION

Haemoglobinopathies are a group of genetic disorders of haemoglobin.[1]Inherited abnormalities of haemoglobin synthesis are divided into: those characterized by structurally abnormal haemoglobin variants and those in which one or more of the normal polypeptide chains of haemoglobin are synthesized at a reduced rate.

The term haemoglobinopathy is used to designate the structural disorders and the term thalassemia is associated with the quantitative disorder. The thalassemias are a heterogeneous group of disorders caused by inherited mutations which result in reduced synthesis of either α globin or β globin chains that compose adult haemoglobin HbA ($\alpha_2\beta_2$) [2] They are among the most common genetic disorders worldwide, occurring more frequently in the Mediterranean region, the Indian subcontinent, Southeast Asia, and West Africa. [3]

Thalassemias and other structural haemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. The general incidence of thalassemia trait and sickle cell anaemia in India varies between 3-17% and 1-44% respectively but, because of consanguinity, caste and area endogamy some communities show a very high incidence, making the disease a major public health problem in our country.[4,5]

The use of cation - exchange high performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal haemoglobin (Hb) fractions has been increasing.[6] It is a highly sensitive, specific, quick but more expensive method for diagnosis.[7] The purpose of this study is to screen for haemoglobinopathies in anaemic patients using HPLC.

MATERIALS AND METHODS

This study was carried at a tertiary care hospital in Navi Mumbai and patients who were in patients or out patients. This study included patients who presented to the hospital with mild to severe anemia including pregnant females. This was a prospective observational study with a sample size of 230 patients over a period of 24 months. We included patients of all ages with mild to severe anemia. We excluded patients with Hb>11 gm/dl, anaemic patients with history of trauma, GI bleed or chronic kidney disease, patients with previous history of blood transfusion and patients who did not consent to be a part of this study.

On receiving information from the concerned clinical department, patients were examined and evaluated. A relevant clinical history of these patients along with their particulars like name, age, sex, religion, native place was recorded. The chief complaints and family history also was a part of our proforma for evaluation. All the patients were screened by High Performance Liquid Chromatography (HPLC).

OBSERVATIONS AND RESULTS

Table 1: Age wise and gender wise distribution of cases

Age Group	Females	Males	Total	Percentage (%)
<1 year	6	17	23	10
>1 year – 10 years	13	27	40	17.39
>11 years – 20 years	21	9	30	13.04
>21 years – 30 years	53	14	67	29.13
>31 years – 40 years	29	13	42	18.26
>41 years – 50 years	7	4	11	4.78
>51 years – 60 years	5	2	7	3.04
>61 years	7	3	10	4.34
Total	141	89	230	100

We found that the most common age group to be affected by haemoglobinopathies was 21 years to 30 years followed by age group of 31 years to 40 years.

The mean age group of total 230 cases was 23.61 ± 17.25 years and median age was 25 years. In our study, 89 out of 230 (38.7 percent) of our patients were males and 141 patients (61.3 percent) were females.

Table 2: Types of Haemoglobinopathies by HPLC

Diagnosis by HPLC	Number of cases	Percentage (%)
Beta Thalassemia Major	5	2.2
Beta Thalassemia Trait	37	16.1
Beta Thalassemia trait + Sickle cell homozygous	14	6.1
Delta Beta Thalassemia trait	2	0.9
Hb S trait	5	2.2
Sickle cell homozygous	15	6.5
Hb E trait	5	2.2
Hb E homozygous	3	1.3
Total hemoglobinopathies	86	37.4
Non haemoglobinopathy group	144	62.6
Total	230	100

We observed total haemoglobibopathies to be in 86 cases (37.4%) out of which beta thalassemia trait was the most frequent haemoglobinopathy followed by sickle cell homozygous disease and least number of cases with Delta beta thalassemia trait while non haemoglobinopathies were diagnosed in 144 patients (62.6%).

Table 3: Haemoglobin components by HPLC in respective haemoglobinopathies

Diagnosis	Analysis of HbA2 (%) Mean ± SD	Analysis of Hb F (%) Mean ± SD	Analysis of Hb S (%) Mean ± SD	Analysis of HbA2 (%) Mean ± SD	Analysis of Hb E (%) Mean ± SD
Beta Thalassemia Major	3.14 ± 0.71	79.2 ± 5.48			
Beta Thalassemia Trait	5.68 ± 1.33	----			
Hb S Trait		----	25.58 ± 2.15	3.44 ± 0.36	
Sickle Cell Homozygous		15.69 ± 6.12	76.32 ± 6.07	2.88 ± 1.00	
Sickle Cell Homozygous with beta thalassemia trait		17.40 ± 9.20	64.55 ± 10.55	5.35 ± 1.02	

Hb E trait		----			36.46± 5.04
Hb E Homozygous		4.1±1.70			84.03± 4.23

DISCUSSION

Disorders of globin chain synthesis are common in India and constitute a major public health problem. HPLC helps in identifying many abnormal haemoglobin variants which are useful in neonatal screening of hemoglobinopathies as well as in the prenatal diagnosis of thalassemia.[8]

AGE AND GENDER

In our study, around 29 percent of our patients who presented with anemia were of 21- 30 years followed by patients under 10 years. 141 out of 230 patients (61.3 percent) were females. In a study of 69,440 patients in rural India done by Gerardo Alvarez Uria et al, they found that the highest prevalence of anaemia was seen in children <10 years followed by women and older adults.[9]

DISTRIBUTION OF DIAGNOSES BY HPLC

Out of the 230 cases of anaemia studied, 86 cases showed abnormal hemoglobin fractions on HPLC. Of these, 37 cases of beta thalassemia trait was the predominant abnormality (16.1%). The other cases diagnosed as sickle cell homozygous were 15 (6.5%) followed by sickle cell homozygous with beta thalassemia trait amounting to 14 cases (6.1%). The cases with normal hemoglobin fractions were 144 (62.6%).

In study by Mondal et al beta thalassemia trait was the most common abnormality found, followed by Hb E trait and then E-beta thalassemia followed by beta thalassemia major. Other variants detected included sickle cell trait, Hb E disease, sickle cell disease, sickle β thalassemia, Hb D-Punjab trait, double heterozygous state of Hb S and Hb E, double heterozygous state of Hb S and Hb D, HbLepore, Hb J-Meerut and HbH.[10]

In study done by Patel U et al in population of Gujarat, beta Thalassemia trait was most common haemoglobinopathy, followed by beta thalassemia major, sickle cell anemia and sickle cell trait.[11]

FEASIBILITY OF HPLC FOR TESTING

In a tertiary care hospital/ public health care centre, where a test costs Rs 800/- it can be possible to generate funds by hospital authorities and NGOs for it, as its usefulness in reaching the diagnosis outweighs its cost. With such a small amount of blood volume required for this test, it is possible to run the same EDTA sample which was collected for running CBC. As the results of HPLC are machine generated, there is no inter-observer variation which exists for sickling test and reticulocyte count. And hence, HPLC is a very accurate, reliable and a reproducible test.

CONCLUSION

HPLC is a reliable, accurate, fast and reproducible diagnostic tool for early and direct identification of haemoglobin variants. It has a high degree of precision in quantification of normal and abnormal haemoglobin variants. In India, anaemia is most commonly caused by nutritional deficiency, but the differential diagnosis of abnormal haemoglobin variants/ haemoglobinopathies should also be kept in mind. Thus, HPLC should be used for early detection and proper management of haemoglobinopathies which is important in view of increased incidence of β thalassemia trait nowadays.

In India, various strategies should be implemented to tackle these haemoglobinopathies which are:

1) Educate the people at the grass root level about the harsh outcomes of the abnormal haemoglobin variants.
2) Mass screening of the patients with genetic counselling.
3) Introducing more antenatal screening programs.
4) Infrastructure for the management and therapy of the affected patients.
5) Retrospective and prospective genetic counselling for prevention and management of haemoglobinopathies should be implemented.
6) Premarital screening

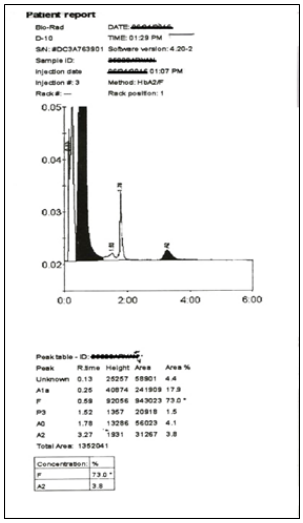

Fig 1. Chromatogram of Beta Thalassemia major

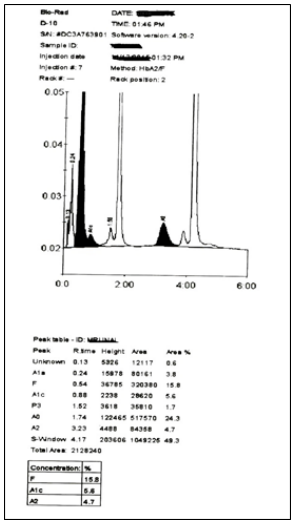

Fig 2. Chromatogram of Sickle cell homozygous

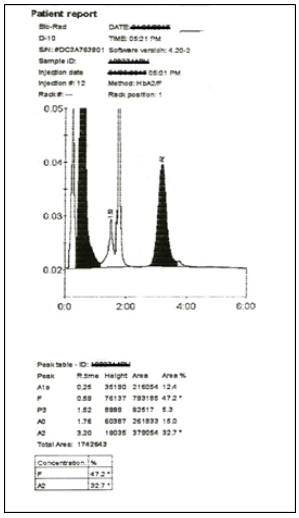

Fig 3. Chromatogram of Hb E trait

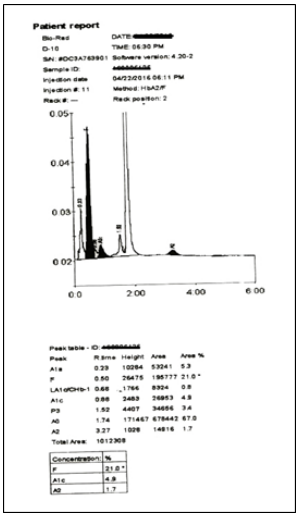

Fig 4. Chromatogram of Delta Beta thalassemia trait

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