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Original Research Paper

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PATTERN OF HEMOGLOBINOPATHIES AND THALASSEMIAS.

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ABSTRACT

Introduction: Thalassemia and other structural haemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. Aims: To study the pattern of different haemoglobin disorders .Methods: In this retrospective study, we included all the cases referred to our centre, for High performance liquid chromatography(HPLC) using BIORAD D 10 system. Results: Out of total 500 referred cases , 225 (45%) were detected to have abnormal haemoglobin. The commonest disorder was Hb E trait(145 cases),followed by Hb E disease(39 cases),B Thalassemia trait(20 cases),Compound heterozygous for Hb E and beta thalassaemia (15 cases), Beta thalassaemia major(03 cases),Hb S trait(03 cases) . Conclusion: This study provides a comprehensive database on the spectrum of haemoglobinopathies . HPLC was found to be a simple, rapid and reliable method for the detection of hemoglobin variants

KEYWORDS : High performance liquid chromatography, Thalassaemia, haemoglobinopathy ,pattern.

INTRODUCTION:

Thalassemia and other structural haemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. Inherited disorders of haemoglobin synthesis are an important cause of morbidity and mortality worldwide. They place a large burden on the patients, their families and even the community. They can be managed by expensive bone marrow transplantation, which is always not possible in a developing country like ours. Population screening, genetic counselling and prenatal diagnosis can prevent these genetic disorders; as it has been a success in countries like Greece, Cyprus and Italy.1 Haemoglobinopathies are the most prevalent genetic defect worldwide, with an estimated 269 million carriers.2 Globally, the populations of certain regions are at higher risk of having a haemoglobinopathy ,2,3while approximately 5% of the world's population carries a gene for sickle-cell anaemia or thalassaemia, the percentage of carriers can reach 25% in some regions.4A majority of the haemoglobinopathies are not clinically apparent but some produce serious life-threatening diseases and constitute a significant health care burden.5 These are quantitative (thalassaemia syndromes) or qualitative (variant Hb).5,6,7,8, Thalassaemia syndromes are sub-classified based on the gene involved, i.e. α and β. These α- and β- thalassaemias are further sub-divided into α+, β+ or αo, βo depending on whether some (+) or no(o) globin protein is produced as a result of the causative mutation.9,10 Data pertaining to the pattern of hemoglobinopathies and thalassemias is scarce in this part of the country, and hence it was considered worthwhile to study these disorders. Aims: To study the pattern of different haemoglobin disorders. Materials and methods: In this retrospective study, we included all the cases referred to our centre, for HPLC. Details of patient's age and sex were recorded. For each patient a 2 mL intravenous blood sample was collected in EDTA-containing vacutainer blood collection tubes. The samples were subjected to testing within 2 hours of sampling using Bio Rad D 10 system. The Bio Rad D 10, an automated cation exchange HPLC instrument has been used to quantify Hb A2, Hb F, Hb A along with screening haemoglobin variants like Hb S, Hb D,E,C in a single highly reproducible system. Hb A2/F calibrator was analyzed at the beginning of each run. The D-10 operates on the principle of HPLC and the column comprises of a small cation exchange cartridge, with a requirement of only 2 ml of blood sample, and each sample takes six and a half minutes for analysis. The samples are injected into the analysis stream and seperatad by the cation exchange cartridge using a phosphate ion gradient generatad by mixing two buffers of different ionic strengths to elute the different haemoglobins. A dual wavelength filter photometer monitors the eluent from the cartridge as it passes through the photometer cell. Changes in optical density at 415 nm are measured. A secondary filter at 690 nm corrects the effects caused by mixing buffers of different ionic strengths. The data is processed and the report giving the chromatogram where the different peaks are identified in defined windows with relevant information like retention time,relative percentage and area.

Acceptance criteria for a chromatogram:

1. The total area count of the chromatogram should be in the range of 1 million to 4 million.

2. The peak shape should be sharp and symmetrical, there should be no dergradation peaks at the end of the run.

HPLC reveals:

A1c: Minor component of Adult haemoglobin.
A1b: Minor component of Adult haemoglobin.
Hb F: Fetal haemoglobin.
LA1c/CHb1: Labile component of glycosylated Adult haemoglobin.
LA1c/CHb1: Carbamylated Adult haemoglobin.
A1c: Glycosylated Adult haemoglobin
A0: Non Glycosylated Adult haemoglobin
P3: Peak on HPLC
A2: Haemoglobin A2.

The fetal haemoglobin level in a normal adult is found to be less than 1%. The HbA2 for a normal adult has been reported to be less than 3.5%. The value reported between 3.5 to 4% should be reported with caution keeping in mind the red cell indices and silent beta thalasssemia. It is to be noted that iron deficiency tend to reduce the Hb A2 value. Megaloblastic anemia could fasely elevate Hb A2 value.

In B Thalassemia trait, HbA2 lies between 4-9% .Hb A2 levels of >7% are generally seen in beta globinn deletional mutations like the 619 bp deletion.

In B Thalassemia major, on the D-10 haemoglobin testing system, a marked increase in Hb F is seen (>85%) with a concomitant marked reduction in Hb A, constituting usually <3% of total haemoglobin in B0 thalassemia and variable in B+ Thalassemia. If the sample contains greater than 16.5% Hb F, the Hb F may elute in the LA1c/CHb window or A1c window and no Hb F will be reported. HbA2 levels may be reduced, normal or elevated.

Hb E trait is the most common haemoglobin variant in South east

Asia and second most prevelant haemoglobin variant worldwide. Hb E heterozygous have about 30% Hb E. Hb E elutes in the Hb A2 window. Hb F levels are normal.

In HbE homozygous-HbE accounts for 85-95% of haemoglobin. On the D10 haemoglobin testing system, Hb E elutes in the HbA2 window. Hb F may be mildly increased or normal.

In compound heterozygous for Hb E and B Thalassemia trait there is increase Hb F(15-50%) and Hb E(50-80%) are present in untransfused cases, however the % of HbF decreases in post transfused patients.

Results and observations: Out of 500 cases, 275 (55%) were normal and 225 (45%) cases had abnormal haemoglobin pattern. Of the 225 abnormal cases, 118 (52.4%) were males and 107 (47.5%) were females. The commonest disorder was Hb E trait(145 cases),followed by Hb E disease(39 cases),B Thalassemia trait(20 cases),Compound heterozygous for Hb E and beta thalassaemia (15 cases), Beta thalassaemia major(03 cases),Hb S trait(03 cases) as shown in Table 1 .

TABLE – 1SPECTRUM OF HAEMOGLOBINOPATHY

	TOTAL	%
HB E Trait	145	64.4%
Hb E Disease	39	17.3%
B Thalassemia trait	20	8.8%
Compound heterozygous for Hb E and B Thalassemia	15	6.6%
B Thalassemia major	03	1.3%
Hb S trait	03	1.3%
TOTAL	225	100%

DISCUSSION:

A large number of haemoglobin variants prevalent in the population indicate that haemoglobinopathies are not uncommon amongst our population. The inherited disorders of haemoglobin synthesis are one of the important public health problems in India.1 Since data on the prevalence of hemoglobinopathies and thalassemias is scarce in India, the Indian Council of Medical Research (ICMR) conducted a multicenter study in six cities of six states, and have found that the overall incidence of beta thalassemia trait (BTT) was 2.78%. 10 Other studies from different parts of India have shown an incidence of beta thalassemia to be 3-15%. 11,12 The incidence of hemoglobinopathies also differs in different parts of India. In Orissa13 HbS is very common whereas in West Bengal the commonest hemoglobinopathy seen is HbE. 14,15 The ICMR study showed that HbE was mainly seen in Assam (23.9%) and Kolkata in West Bengal (3.92%). 10 It is well established that the incidence of HbE gene in the North Eastern region of India is one of the highest in the world. Different states of the North Eastern region show a variable incidence of HbE varying from 16.2% to 47.3%. 16 Though the HbE gene has been detected across all ethnic groups in Assam like the Ahom, Koch, Chutia, Muttock, Deori, Sonowal and Mishing groups, the highest incidence has been detected in the Bodo-Kacharis an ethnic group speaking Tibeto-Burman languages, although all have a common ancestry.10

45% cases were detected to have abnormal haemoglobin in our study .Maximum number of patients had Hb E Trait comprising 64.4% of cases which was in accordance to the study done by MK Baruah et al where Hb E trait was the predominant variant followed by Hb E disease.[16]

Conclusion: This study provides a comprehensive database on the spectrum of haemoglobinopathies. HPLC was found to be a simple, rapid and reliable method for the detection of hemoglobin variants. An accurate diagnosis can be provided in majority of cases by use of retention time, proportion of total haemoglobin, and peak characteristics of HPLC

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