ORIGINAL RESEARCH PAPER

Pathology

DETECTION OF HAEMOGLOBIN VARIANTS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN A TERTIARY CARE HOSPITAL OF NORTHEAST INDIA.

KEY WORDS: hemoglobin variants, HPLC, BIORAD D10

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ABSTRACT

Background: The most prevalent single gene disorders in India are hereditary haemoglobin (Hb) abnormalities. Because North East India lacks information on the pattern of hemoglobinopathies and thalassemias, it was thought beneficial to research these conditions using a series of patients sent to a clinical diagnostic laboratory. **Aim-** To study the spectrum of different haemoglobin variants in patients with Anaemia **Methods:** The study is carried out for a period of one year from July 2021 to June 2022. A total number of 90 cases of anemia were studied during this period. HPLC was done using BIORAD D-10 machine and the data were analysed. **Results:** Of the 90 cases, 23 was diagnosed as HbE Trait, 5 as HbE Disease, 8 as β- Thalassaemia Trait, 2 as Sickle Cell Trait, 1 each as HbE- β- Thalassaemia Trait and HbD Punjab, 33 as Iron Deficiency Anaemia and 17 as Anaemia of chronic disease. There is a female preponderance of the haemoglobin variants.

INTRODUCTION:

The prevalence of anaemia in India among six groups as per the National Family Health Survey 5 (2019-21), is 25.0 percent in men (15-49 years), 57.0 percent in women (15-49 years) and 67.1percent in children (6-59 months). The scenario in Assam is also similar with prevalence of 68.4% in 6-59 months age group, 54.2% in women of reproductive age group and 67% in adolescent girls[1].

The most prevalent genetically inherited illnesses are haemoglobinopathies. According to World Health Organization (WHO) statistics, 5% of the global population is thought to be a carrier of genetic haemoglobin (Hb) abnormalities. Haemoglobinopathies have a cumulative gene frequency of 4.2% in India. Global population movement and the comparatively greater frequency of consanguineous marriages in several of the high frequency nations have both contributed to the rising haemoglobinopathies burden[2]. With an average allele frequency of 10.9%, haemoglobin E (Hb E) is mostly limited to the North-eastern Indian states of West Bengal, Assam, Andhra Pradesh, Nagaland, Manipur, Tripura, and Meghalaya among the clinically significant haemoglobinopathies[3]. The prevalence of beta-thalassaemia trait and sickle cell in various regions of India is around 3%–17% and 1%–44%, respectively. Assam in particular, is home to a variety of socioculturally, linguistically, and ethnically varied populations, making it a rich reservoir of haemoglobinopathies and thalassaemias due to historical migration of different races[4].

High performance liquid chromatography (HPLC) being an automated instrument is highly sensitive and specific, has high resolution and helps in quantification of various haemoglobins. It acts as a good alternative to electrophoresis[5]. With the help of basic haematological parameters like Hb% and RBC indices along with HPLC, a laboratory can identify around 75% of the common variants encountered, without the need for confirmatory studies such as alkaline and acid electrophoresis.

MATERIALS AND METHODS:

Study Design: The study is a hospital based prospective cross- sectional study.

Study Center: The study is carried out in the Department of Pathology, Jorhat Medical College & Hospital, Jorhat.

Duration Of Study: The study is for a period of one year from July 2021 to June 2022.

Sample Size: A total of 90 cases were included in the study based on pevious hospital records.

Inclusion Criteria : All cases of microcytic hypochromic anaemia (MCV < 80 fl, MCH < 27 pg) not responding to conventional treatment, clinically suspected cases of haemoglobinopathy, antenatal, and other cases with or without symptoms and patient with nutritional deficiency anaemia, where a co-existent haemoglobinopathy is suspected.

Exclusion Criteria: Patients requiring blood transfusion sampling was deferred for at least 4 weeks after or just before next transfusion and non cooperative patients.

Scheme Of Study: The whole procedure will consist of the following: 1) Informed consent 2) Ethical clearance 3) History and clinical examination (general, systemic examination). 4) Five millilitres (5 mL) of whole blood was collected in a vacuum collection tube containing EDTA which can be stored at 2–8 degrees C for maximum 7 days if processing is delayed. No preparation was required unless the sample was in a tube other than the recommended tube 5) Samples were run on SYSMEX XN-550 AUTOMATED ANALYZER before performing HPLC to obtain the Hb values and red blood cell (RBC) indices.

The tests were performed on an instrument manufactured by BIO-RAD laboratories, USA. The instrument, known as BIO-RAD 'VARIANT II' (beta thalassemia short program) utilizes the principle of HPLC.Haemolysate is prepared using lysis buffer (10µl blood in 1 ml lysis buffer). Each sample takes 6.5 min for the result. The instrument is calibrated for HbA2/HbF using area percentage and retention time of HbA2 for which HbA2/F calibrator is provided which has been assigned values (in units of area percent of total haemoglobin) for both HbA2 and HbF and it is analysed at the beginning of each run. The value more than 3.5% of A2 fraction of haemoglobin wastaken as cut off point for determining the βthalassemia trait and more than 10% was assumed to be haemoglobin E.

Ethical Clearance: Provided by the Institute ethical committee, Jorhat medical college.

RESULTS:

Table - 1.1: Showing Age Distribution Of Cases Selected For Study.

AGE	NUMBER(n)	PERCENTAGE
0-10	11	12.22
11-20	17	18.9
21-30	20	22.22
31-40	18	20
41-50	11	12.22
51-60	9	10
61-70	4	4.44

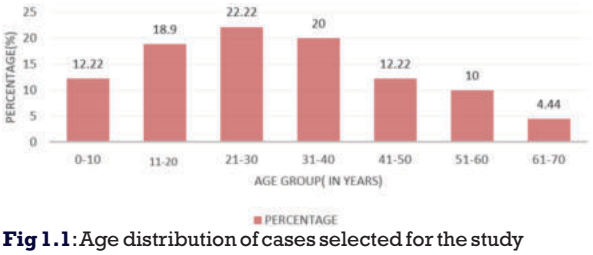

Fig 1.1: Age distribution of cases selected for the study

Observations: Of the total 90 cases selected for the study, the highest number was in the age group of 21-30 which was 20 cases(22.22%) followed by 31-40 with 18 cases(20%); 11-20 years 17 cases(18.9%), 0-1 and 41-50 each with 11 cases(12.22%), 51-60 9 cases(10%) and 61-70 with 4 cases(4.44%).

Table 1.2: Distribution of cases according to sex

Gender	Number	Percentage (%)
Male	34	38
Female	56	62

Observations: Out of 90 cases 34(38%) were males and 56(62%) were females.

Table 1.3: Haemoglobin typing findings of the cases.

Sl. No	HPLC Finding	No. of Cases	Percentage (%)
1	Normal Adult heamoglobin	50	55.55
2	HbE Heterozygous	23	25.55
3	HbE Homozygous	5	5.55
4	β-Thalassaemia Trait	8	8.9
5	HbS Heterozygous	2	2.22
6	HbD Punjab Heterozygous	1	1.11
7	HbE-β Thalassaemia	1	1.11

Observations: Among the 90 cases selected 50(55.55%) had normal adult haemoglobin, 23 (25.55%) had HbE heterozygous, 5(5.55%) were HbE homozygous, 8(8.9%) had β-Thalassaemia Trait, 2(2.22) had HbS heterozygous and 1(1.11%) each had HbD heterozygousand HbE- β-Thalassaemia compound heterozygous.

Table 1.4: Distribution Of Cases According To Final Diagnosis

Final Diagnosis	Number(n)	Percentage
HbE Trait	23	25.55
HbE Disease	5	5.55
β- Thalassaemia Trait	8	8.9
Sickle Cell Trait	2	2.22
HbE-β- Thalassaemia compound heterozygous	1	1.11
HbD Punjab Trait	1	1.11
Iron Deficiency Anaemia	33	36.67
Anaemia of chronic disease	17	18.89

Observation: Of the 90 cases, 23(25.55%) was diagnosed as HbE Trait, 5(5.55%) as HbE Disease, 8(8.9%) as β-Thalassaemia Trait, 2(2.22%) as Sickle Cell Trait, 1(1.11%) each as HbE- β- Thalassaemia Trait and HbD Punjab, 33(36.67%) as Iron Deficiency Anaemia and 17(18.89%) as Anaemia of chronic disease.

Table 1.5: Age Distribution According To Final Diagnosis

AGE	HbE Trait	HbE Disease	β-Thalassaemia Trait	Sickle Cell Trait	HbD Punjab trait	HbE-β Thalassaemia	Iron Deficiency Anaemia	Anaemia of chronic disease
0-10	3	1	3	0	0	0	3	1
11-20	3	3	0	1	0	1	5	4
21-30	6	0	0	0	1	0	11	2
31-40	6	0	2	1	0	0	6	3
41-50	3	0	2	0	0	0	4	2
51-60	1	1	1	0	0	0	4	2
61-70	1	0	0	0	0	0	0	3

Observations: Among the cases diagnosed as HbE Trait, the age groups 21-30 and 31-40 had the highest frequency. 11-20 age group showed most cases with HbE disease, 0-10 age group showed β-Thalassaemia Trait as the most common diagnosis, sickle cell trait was seen in 11-20 and 31-40 age group (1 each). HbE-β Thalassaemia trait was seen in 11-20 age group, HbD Punjab Trait was seen in 21-30 group. Iron Deficiency Anaemia was seen highest is the 21 -30 age group while Anaemia of Chronic Disease was seen mostly in 11-20 age group.

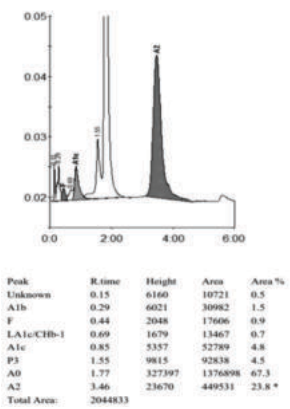

Fig 1.2: Chromatogram of HbE trait

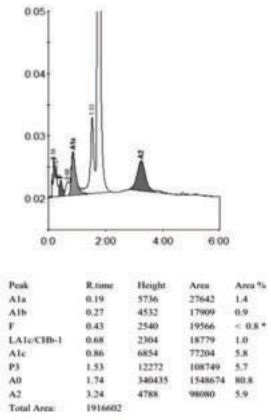

Fig 1.3: Chromatogram of β-Thalassemia trait

DISCUSSION:

Of the total 90 cases selected for the study, the highest number was in the age group of 21-30 which was 20 cases (22.22%) followed by 31-40 with 18 cases (20%); 11-20 years 17 cases (18.9%), 0-1 and 41-50 each with 11 cases (12.22%), 51-60 9 cases (10%) and 61-70 with 4 cases (4.44%). Assam is among the worst performing states, with a huge spike in anaemic cases. The figure for children has increased to 68.4 per cent (NFHS 5)[1] from 35.7 per cent (NFHS-4). The second spike was in the age group of 20-29 (NFHS 5). Our study was however a hospital-based study while the study that NFHS conducts is population based. Out of 90 cases with anaemia in our study, 34(38%) were males and 56(62%) were females. The figure for all women increased to 65.9 % (NFHS-5) from 46 % (NFHS-4). The figure for anaemia in men is slightly better at 36 per cent (NFHS-5)[1], up from 25.4 per cent (NFHS-4). These results correspond with the present study.

Of the 90 cases in this study 23(25.55%) was diagnosed as HbE Trait, 5(5.55%) as HbE Disease, 8(8.9%) as β-Thalassaemia Trait, 2(2.22%) as Sickle Cell Trait, 1(1.11%) each as HbE- β- Thalassaemia Trait and HbD Punjab, 33(36.67%) as Iron Deficiency Anaemia and 17(18.89%) as Anaemia of chronic disease. These findings correlate with the studies conducted by R. Deka *et al*.[6] and Madhusnata De *et al*.[7] where it was seen that HbE trait was the most common variant haemoglobin in the people of Assam. Iron Deficiency Anaemia still remains the most common cause of anaemia which is also reflected in the study conducted by Rita Panyang *et al*.[8] in 2018.

In this present study, among the cases diagnosed as HbE Trait, the age groups 21-30 and 31-40 had the highest frequency. 11-20 age group showed most cases with HbE disease, 0-10 age group showed β-Thalassaemia Trait as the most common diagnosis, sickle cell trait was seen in 11-20 and 31-40 age group (1 each). HbE-β Thalassaemia trait was seen in 11-20 age group, HbD Punjab Trait was seen in 21-30 group. Iron Deficiency Anaemia was seen highest is the 21 -30 age group while Anaemia of Chronic Disease was seen mostly in 11-20 age group. Sushanta Chakma *et al*.[9] in their study conducted from September 2019 to October 2020 yielded similar results. Bidyut Krishna Goswami *et al*.[10] in their study also found similar age distributions of the cases of haemoglobin variants.

CONCLUSION:

The present study was undertaken from June 2021 to May 2021 in the Department of Pathology in collaboration with the Department of Biochemistry, Jorhat Medical College and Hospital. This study showed the high prevalence of anaemia in females attending Jorhat Medical College. The young adult age group had the greatest number of cases. Most were asymptomatic while generalized weakness was the commonest symptoms in those where present. Most cases had moderate degree of anaemia. HPLC using BIORAD D10 system was done revealing HbE Trait as the most common variant. Additional variants detected were HbE Disease, □-Thalassaemia Trait, HbS heterozygous, HbD Punjab heterozygous, Hb Lepore and HbE- β-Thalassaemia compound heterozygous. This implicates the prevalence of a variety of haemoglobin variants in Assam thus proving the necessity of haemoglobin typing and proper screening for these variants. Proper haematological investigations and biochemical profile help ruling out Iron Deficiency Anaemia and Anaemia of Chronic Disease which is prevalent all over India. However, this being a hospital-based study conducted over only a period one year, it would not be able to completely represent the existing scenario. Extensive population-based study over a longer period of time is warranted to throw more light on this topic.

Limitations Of The Study:

This being a hospital-based study conducted over only a period one year, it would not be able to completely represent the existing scenario. Extensive population-based study over a longer period of time is warranted to throw more light on this topic.

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