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Hematology

SPECTRUM OF HEMOGLOBINOPATHIES AND THALASSEMIAS DIAGNOSED ON HPLC - A TERTIARY CARE HOSPITAL BASED STUDY

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ABSTRACT **Background -** The most common single gene disorders in the world are hemoglobinopathies and thalassaemias. The prevalence of thalassaemias and haemoglobinopathies varies by region. However, some communities have a very high incidence due to consanguinity, caste, and area endogamy, making this group of diseases a major public health problem in our country. High performance liquid chromatography (HPLC) is an automated instrument that is highly sensitive and specific, has a high resolution, and aids in the quantification of various haemoglobins. It is a viable alternative to electrophoresis. **Materials and method -** the study comprises of total 220 patients presented with anaemia and were examined in the department of Medicine. Thereafter, blood smaples were taken and the hemoglobin typing were done in the department of Pathology by Cation exchange high performance liquid chromatography. **Results**- Among the 220 cases majority were females with 64.1% of cases and 35.9% (79) of cases are male. Out of total cases, 119 (54.1%) cases had normal hemoglobin and 101 (45.9%) cases had variant hemoglobin. Out 101 cases of variant hemoglobin, 38 (17.3%) cases were diagnosed with Hb E trait, 27 (12.3%) cases were detected with Hb E disease, 18 (8.2%) cases were diagnosed with Hb E-β Thalassemia, 17 (7.7%) cases were diagnosed as β-Thalassemia trait and 1 (0.5%) case was detected as β-Thalassemia major.

KEYWORDS : HPLC, Hb, Thalassemia

INTRODUCTION

The most common single gene disorders in the world are hemoglobinopathies and thalassaemias. The prevalence of thalassaemias and haemoglobinopathies varies by region. According to World Health Organization estimates, 5% of the world's population carries a potentially pathological haemoglobin gene. In India, the prevalence of thalassemia trait and sickle cell haemoglobinopathy ranges from 3-17% and 1- 44%, respectively. However, some communities have a very high incidence due to consanguinity, caste, and area endogamy, making this group of diseases a major public health problem in our country. (1) According to the World Health Organization's most recent Bulletin, inheritance of thalassemic mutations is a significant public health problem in the majority of nations (71% of 229 countries). It is estimated that up to 1.5% of the global population, or approximately 270 million people, may carry a genetic mutation affecting Hb production, and that approximately 1% of couples worldwide are at risk of having children with an Hb disorder. (2)

There are an estimated 65,000-67,000 beta-thalassemia patients in India, with an additional 9,000-10,000 cases added each year. The beta-thalassemia gene carrier rate ranges from 1 to 3% in Southern India to 3 to 15% in Northern India. Thalassemia can result in life-threatening situations as well as chronic illness. They impose a financial and psychological burden on the individual, his family, and society as a whole. As a result, a comprehensive study is required, as well as steps to improve the quality of life of thalassemia patients and to prevent thalassemia through population screening and genetic counselling. (3)

HbS is very common in Orissa, whereas HbE Disease is the most common in West Bengal. In Punjab, Sikhs (2%) have the highest prevalence of Hb D punjab. According to the ICMR study, HbE was most prevalent in Assam (23.9%) and Kolkata (3.92%). The HbE gene has been found in all ethnic groups in Assam, including the Ahom, Koch, Chutia, Muttock, Deori, Sonowal, and Mishing groups of North Eastern India. The Bodo Kacharis, an ethnic group speaking Tibetan and Burmese languages, have had the highest incidence, despite sharing a common ancestor. It is well established that the incidence of the HbE gene is highest in North Eastern India. HbE prevalence varies by state in the North Eastern region, ranging from 16.2% to 47.3%. Hb S is also prevalent in a large migrant tea garden population. (4)

The high frequency of consanguineous marriages, which tend to increase the frequency of any Mendelian recessive disorder, is the first critical factor for the high frequency of haemoglobin disorder. The second factor is the epidemiological transition, in which, as neonatal and childhood mortality rates fall as a result of improved social conditions, babies who would have died with serious haemoglobin disorders before being diagnosed are now alive to present for management. Founder effects and gene drift are two other factors that contribute to the high gene frequency.(5)

A detailed clinical history is followed by haematologic evaluation (including haemoglobin level, complete blood count (CBC), reticulocyte count, and red blood cell morphology), protein-based analytic methods (alkaline and acid Hb-electrophoresis), high performance liquid chromatography (HPLC), and nucleic acid-based methods such as polymerase chain reaction (PCR)(6) ,High performance liquid chromatography (HPLC) is an automated instrument that is highly sensitive and specific, has a high resolution, and aids in the quantification of various haemoglobins. It is a viable alternative to electrophoresis. (1)

AIMS AND OBJECTIVES

1. To evaluate the different patterns of Hemoglobinopathies and thalassemias by using Cation exchange high performance liquid chromatography (CE-HPLC)
2. To study various demographic factors of Hemoglobinopathies and Thalassemias.

MATERIALS AND METHODS

The study is a hospital based cross- sectional study conducted from September 2021 to August 2022, in the Department of Pathology, Fakhruddin Ali Ahmed Medical College and Hospital,Barpeta,Assam. A total of 220 blood samples were received in the department of Pathology from patients presenting with clinical features of anaemia without any known cause and from patients with family history of Hemoglobinopathies or Thalassemias. However patient presenting with anaemia of known cause , patients less than 6 months of age and those with history of blood transfusion in last 3 months are excluded from the study. Thereafter, complete haemogram and then hemoglobin typing were done in the department of Pathology at BIO –RAD D10 machine by cation exchange high performance liquid chromatography.

RESULTS

A total of 220 cases were included in this study based on inclusion criteria and exclusion criteria. Among the 220 cases majority were females with 64.1% of cases and 35.9% (79) of cases are male. Also, 17.7% (39) of cases are below 10 years, 38.2% (84) of cases are between 11 to 30 years, 24.1% (53) of cases are between 31-45 years, 15.0% (33) of cases are between 46-65 years and 5.0% (11) of cases are between 66-95 years. In the study, Out of total cases, 119 (54.1%) cases had normal hemoglobin and 101 (45.9%) cases had variant

hemoglobin. Out of 101 cases of variant hemoglobin, 38 (17.3%) cases were diagnosed with Hb E trait, 27 (12.3%) cases were detected with Hb E disease, 18 (8.2%) cases were diagnosed with Hb E-β Thalassemia, 17 (7.7%) cases were diagnosed as β-Thalassemia trait and 1 (0.5%) case was detected as β-Thalassemia major. Among the 101 cases of variant hemoglobin, 88 cases had no history of consanguinal marriage of parents, and 13 cases had history of consanguinal marriage of parents. Mean RDW level of cases with normal hemoglobin was 15.85% and those with variant hemoglobin was 11.82%. All the cases included had microcytic hypochromic anemia with mean MCV level of cases with normal hemoglobin was 75.16fl and those with variant hemoglobin was 74.11fl.

Table: 1 - Age distribution of Variant Hemoglobin and normal Hemoglobin.

Age groups	Normal hemoglobin	Variant hemoglobin	Total cases
6 months - 10 years	14	25	39
11- 30 years	52	32	84
31- 45 years	31	22	53
46- 65	15	18	33
66-95 years	7	4	11
Total cases	119	101	220

Table: 2- Frequency and Percentage of different types of Variant haemoglobin

	Number of cases	Percentage (%) of cases
Normal Hemoglobin	119	54.1%
Hb E trait	38	17.3%
Hb E Disease	27	12.3%
Hb E-β Thalassemia	18	8.2%
β Thalassemia trait	17	7.7%
β Thalassemia major	1	0.5%
TOTAL CASES	220	100%

Table: 3 - Distribution of Normal Hemoglobin and Variant Hemoglobin cases according to sex

Sex	Normal Hemoglobin		Variant Hemoglobin		Total number of cases
	Number of cases	%	Number of cases	%	
Female	80	36.3%	61	27.7%	141
Male	39	17.7%	40	18.1%	79
Total	119	54.1%	101	45.9%	220

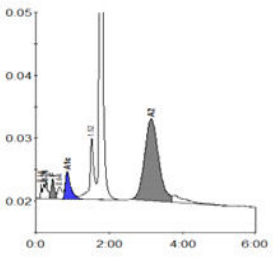

Figure 1- Graph of Hb E trait with A2 level of 31.5% and Hb F 1.4%.

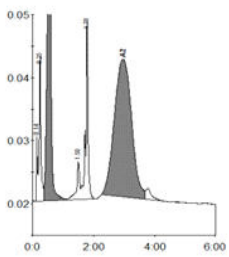

Figure 2- Graph of Hb E Disease with A2 level of 98.7% and Hb F 7.9%.

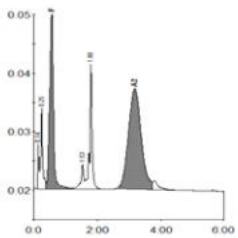

Figure 3- Graph of Hb E beta Thalassemia with A2 level of 66.3% and Hb F 29.1%.

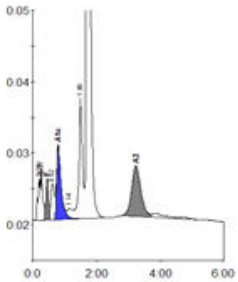

Figure 4- Graph of Beta thalassemia trait with A2 level of 5.6% and Hb F 1.1%.

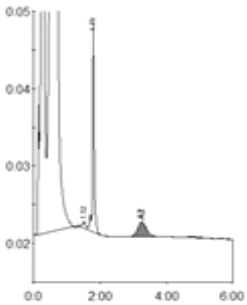

Figure 5- Graph of Beta thalassemia major with A2 level of 1.0% and Hb F 76.8%.

DISCUSSION

Thalassaemias and haemoglobinopathies are inherited blood disorders that primarily affect the haemoglobin globin moiety. These disorders, which were previously confined to specific areas, religions, castes, and tribes, particularly with endogamous marriage norms, are now widespread throughout the world. (6) Because of the migration of various races over the ages and thus being home to an assortment of socio-cultural, linguistic, and ethnically diverse people, the North Eastern region of India, particularly Assam, is a rich reservoir of hemoglobinopathies and thalassemias. Previous research has shown that a high gene frequency for HbE is prevalent in Assamese autochthonous inhabitants with cultural and linguistic ties to the population of South East Asian countries, whereas HbS is restricted to tea garden labour communities, a group of population brought to Assam by British colonial tea planters from central, eastern, and southern India during the mid-nineteenth century. (6)

HPLC is sensitive and accurate technique alternative to electrophoresis for identification and quantification of normal and abnormal hemoglobin fractions.

Total 220 cases of both the genders included in the study. All the cases had microcytic hypochromic anaemia. Out of these, 119 (54.1%) cases had normal hemoglobin and 101 (45.9%) cases had variant hemoglobin. Of these 220 cases tested 141 (64.1%) cases were Female and 79 (35.9%) cases were Male. The age range of patients was from 6 months to 95 years with a mean age of 30.3 years.

In the present study, out of 220 cases , 101 cases were diagnosed with variant hemoglobin. The most commonly detected Hemoglobinopathies was Hb E trait with 38 (17.3%) number of cases followed by Hb E disease seen in 27 (12.3%) cases . Apart from Hb E , thalassemia was also detected along with double heterozygous state. β

thalassemia trait was detected in 17 (7.7%) cases and one (0.5%) case of β thalassemia major was also detected.18(8.2%) cases of double heterozygous variant i.e. Hb E-β Thalassemia was also seen.

Pathak MS eta al conducted a study in Department of Paediatrics at Gauhati Medical College and Hospital within a period of 25 months from June 2011 till July 2013 also got Hb E trait as most common Hemoglobinopathies (23.5%), followed by β thalassaemia trait (18.12%) and Mrinal Kumar Baruah et al also found most common hemoglobinopathies to be Hb E trait (47.9%) followed by Hb E disease which is 25.4% of cases. In the present study , we also found Hb E trait with 17.3% of cases to be most prevalent Hemoglobinopathies in Barpeta district and two adjacent districts i.e. Baksa and Bongaigaon of Assam. This result is consistent with the above studies.(6) (7)

In the present study 65.3% of cases with general cast had variant hemoglobin followed by 14.5% cases seen in SC community. Out of this 66 (65.3%) cases of variant hemoglobin 41(62.1%) cases are from Muslim community. 60 (59.4%) cases of variant hemoglobin seen in Hindu community. In this study hindu community are most commonly affected. This result is consisitent with Dr. Mauchumi Saikia Pathak et al however inconsistent with Bidyut Krishna Goswami et al , Mrinal Kumar Baruahet al and Nilanjana Ghoshet al in which they all got Muslim population as most commonly affected community.(6)(7)(8)(9)

In SK Behera et al, SK Mondal et al and S Raman et al study of Hemoglobinopathies few cases of Hemoglobinopathies had history on consanguineous marriage in their family.(10)(11)(1) This result is comparable with the present study in which 87.1% of cases with variant hemoglobin did not had history of consanguineous marriage of their parents and 12.8% cases had history of consensual marriage.

In the present study all the cases are anaemic with mean hemoglobin level of 8.76gm/dl in cases with variant hemoglobin. The haematocrit level and red cell distribution width (RDW) are also reduced with mean value of 30.85% and 11.82% respectively in cases with abnormal hemoglobin.

CONCLUSIONS

Anaemia caused by abnormal haemoglobin should be evaluated, as morbidity and mortality in homozygous haemoglobinopathies are significant. Haemoglobin anomalies are mostly restricted to specific regions, castes, and tribes and by understanding the prevalence, we can raise public awareness about the diseases and their consequences, as well as take action to treat and prevent them. Cation exchange HPLC is gradually becoming the primary diagnostic test for haemoglobinopathies. It is a more precise, simple, and effective method for detecting various haemoglobin abnormalities, which aids in patient care and has prognostic value.

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