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STUDY OF PATTERN OF HEMOGLOBINOPATHIES AND THALASSEMIA IN THE BARAK VALLEY REGION OF NORTH EAST INDIA

Pathology

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ABSTRACT

Background: Identification of inherited abnormalities of hemoglobin synthesis is immensely important epidemiologically. Data pertaining to the pattern of hemoglobinopathies and thalassemias is scarce in this part of India, and hence this study.

Objectives: To analyze the different Hb variants in the population of Barak Valley and adjacent areas and study their clinicohematological profile.

Materials & Methods: The study was done by Sebia Capillary electrophoresis machine. Cases were referred either after getting abnormal hemogram or for premarital counseling.

***Results*:** Out of 1074 cases 63.78% (685 cases) had normal and remaining 36.2% (389 cases) had abnormal Hb pattern on electrophoresis. The male female ratio was 1.3. HbE trait (11.3%) was the commonest abnormality encountered followed by beta thalassemia trait (9.3%) among the hemoglobinpathies.

Conclusion: Abnormal hemoglobins such as HbE and beta thalassemia are common among the populations of Barak Valley and its adjacent areas.

KEYWORDS

Hemoglobin electrophoresis, Hemoglobinopathy, Thalassemia.

INTRODUCTION

Hemoglobin disorders, including thalassemias and hemoglo binopathies, are among the commonest genetic diseases, and the clinical laboratory is essential for the diagnosis of patients with these abnormalities.[1] The clinical spectrum ranges in severity from asymptomatic laboratory abnormalities to death in utero.[2]

WH0 estimates 5% of the world population are carriers of a potentially pathological hemoglobin gene.[3] In India, hemoglobinopathies and thalassemia constitute major bulk of non-communicable genetic disease and the carrier frequency varies from 3 to 17 percent.[4] The common hemoglobin variant found in the North Eastern states of India are HbE and beta thalassemia.

Detection of carriers through population screening and genetic counseling is a must in prevention of these disorders.

AIMS & OBJECTIVES:

To analyze the different Hb variants in the population of Barak Valley and adjacent areas and study their clinicohematological profile.

MATERIALS AND METHODS

The Study was done in the Department of Pathology, Silchar Medical College & Hospital from Jan 2009 to Dec 2013. Samples were taken both from the patients referred by the clinicians as well as those who came to the hematology section for complete blood count and had abnormal hemograms.

Hematological parameters were done with automated hematology analyzers and hemoglobin electrophoresis using Sebia Capillary Electrophoresis machine.

Solubility test was done to differentiate between HbS & HbA aided the electrophoresis report. Peripheral blood smear was studied using Leishman Stain.

Complete family history could not be obtained in good number of cases.

Exclusion criteria:

1. Patients below 1 year of age- as significance of HbF (Fetal Hemoglobin) levels are hard to interpret.
2. Patients having recent blood transfusions.

Inclusion criteria:

1. Patients with abnormal hemograms suggestive of hemolytic anemia. (i.e,low Hb, low MCV, abnormal RDW, high RBC count.)

RESULTS AND OBSERVATIONS:

In this retrospective study, a total of 1074 cases were studied for a 5 year period. Out of these patients, 685 (63.78%) had normal hemoglobin pattern and remaining 389 (36.2%) cases had abnormalities. The male female ratio was 1.3. HbE trait (11.3%) was the commonest abnormality encountered followed by beta thalassemia trait (9.3%).

HbE trait was found in 121 cases (11.3%). Most of these patients were asymptomatic and few presented with generalized weakness. These patients presented with mean hemoglobin of 10.1 ±2.4 g/dl, RBC count 4.1 ± 1 millions/cu mm, MCV 77.4 ± 8.3fl and RDW-SD of 16.2 ± 1.8 %. Peripheral blood smear showed microcytic hypochromic RBC's and target cells. On hemoglobin electrophoresis mean HbA was 75.3±7.8% and HbE was 24.6 ± 8.4%. Few had mild increase in HbF which ranges from 0%-3%.

HbE disease was found in 34 cases (3.2%). Patients with HbE disease presented with greater degree of anemia, generalized weakness, palpitation, exertional dyspnea etc. The mean Hb was 7.7 ± 2.3g/dl, RBC Count 4.1 ± 1.8 millions/cumm, MCV 58.2 ± 10.7fl and RDW-SD 15.3 ± 2.9%. Peripheral blood smear greater amount of microcytosis and plenty target cells. On hemoglobin electrophoresis mean HbA was 3.9±2.5% and HbE was 88.7 ± 5.9%. Few cases had an increase in HbA_2 which ranges from 3.5%-6%.

Beta thalassemia trait was the second most common abnormality, comprising of 100 cases (9.3%). Peripheral blood smear showed microcytosis with mean hemoglobin of 9.2 ± 2.9 g/dl, MCV 69.5 ± 10.1 fl, RBC Count 3.8 ± 1.2 millions/cu mm and RDW-SD 16.4 ± 1.2%. On hemoglobin electrophoresis mean HbA was 92.5 ± 0.9% and HbA_2 was 5.9 ± 0.5%. There were 9 cases of beta thalassemia major, comprising of 0.8%. These patients presented in early childhood with severe anemia (Mean Hb of 4.7 ± 2.4 g/dl), MCV was 68.7 ± 2.1 fl, RBC Count was 2.4 ± 1.6 millions/ cu mm and RDW-SD was 24.5 ± 4.2%. Peripheral blood smear showed severe anisopikilocytosis with microcytic hypochromic RBC's, pencil cells, target cells, polychromatic RBC's. HbF was the major hemoglobin in these patients with a mean of 77.9 ± 5.2% and mean Hb A was 9.5 ±3.1 %.

Table 1: Abnormal Hemoglobin Variants

Hemoglobinopathies	Number of cases	Percentage
HbE Trait	121	11.3
Beta thal trait	100	9.3

HbE beta Thal	45	4.2
HbS trait	38	3.5
HbE disease	34	3.2
Hb S disease	24	2.2
Hb S β thalassemia	16	1.5
Beta thal major	9	0.8
HbD trait	2	0.2

Hb Eβ thalassemia was another hemoglobinopathy requiring multiple transfusions. There were 45 cases (4.2%) of HbE β thalassemia. Patients presented in childhood or early adult life with moderate to severe anemia (Mean Hb value of 6.5±2.5 g/dl). The RBC count was reduced with mean RBC count of 2.8±1.6 million/µl. The mean HCT was 20.2±9.4 %. MCV was reduced ranging from 51.9- 79.8fl and a mean of 65.2±8.6 fl. MCH and MCHC were both decreased with a mean MCH of 21.9±2.6pg and mean MCHC of 30.6±1.9gm/dl. The red cell distribution width was greatly increased. Peripheral blood smear showed moderate to severe anisopoikilocytosis with microcytic hypochromic RBC's, target cells, pencil cells, polychromatic RBC's, nucleated RBC's etc. On hemoglobin electrophoresis mean HbA was 9.2±4.6%, HbE was 59.5±8.7%, HbA_2 was 5.9±1.8% and HbF was 34.6±6.8%.

Sickle cell disease and sickle cell trait were also common in this region. There were 24 cases (2.2%) of Sickle cell disease, 38 cases (3.5%) of Sickle cell trait and 16 cases (1.5%) of Sickle cell β thalassemia. Patients with HbS trait had mild anemia (mean Hb of 9.8 ± 2.8 gm/dl) mild microcytosis (mean MCV 75.5 ± 12.9fl), mean HbS concentration of 31.8±4.2% and mean HbA concentration of 61.2±5.0%.

Table 2: Hemograms in different types of hemoglobin variants (Mean ± SD)

Hemoglobinopathies	Hb%	MCV	MCH	MCHC	TRBC	HCT	RDWCV
HbE Trait	10.1 ± 2.4	77.4 ± 8.3	26.2 ± 3.8	31 ± 4	4.1 ± 1	29.2 ± 12.9	16.2 ± 1.8
Beta thal minor	9.2 ± 2.9	69.5 ± 10.1	23.1 ± 3.9	30.9 ± 2.8	3.8 ± 1.2	26.8 ± 11.4	16.4 ± 1.2
HbE beta Thal	6.5 ± 2.5	65.2 ± 8.6	21.9 ± 2.6	30.6 ± 1.9	2.8 ± 1.6	20.2 ± 9.4	24.5 ± 5.8
HbS trait	9.8 ± 2.8	75.5 ± 12.9	26.2 ± 4.3	31.1 ± 2	2.4 ± 2.8	18.1 ± 8.7	20.1 ± 3.6
HbE disease	7.7 ± 2.3	58.2 ± 10.7	21.4 ± 3.6	30.1 ± 1.9	4.1 ± 1.8	26.4 ± 9.1	15.3 ± 2.9
Hb S disease	6.8 ± 2.6	80.2 ± 10.6	24.0 ± 3.8	30.5 ± 2.4	2.4 ± 2.1	20.5 ± 6.5	21.4 ± 5.1
Hb S β	8.1 ± 1.9	70.2 ± 12.8	23.7 ± 2.5	31.5 ± 2.1	3.2 ± 2.2	26.5 ± 7.1	18.5 ± 2.2
Beta thal major	4.7 ± 2.4	68.7 ± 2.1	21.1 ± 2.5	31.1 ± 1.2	2.4 ± 1.6	14.5 ± 5.9	25.6 ± 4.2
HbD trait	11.3	81	26.2	31.9	4.5	35.4	20.4

Patients who were homozygous for HbS (i.e HbSS), had greater anemia (mean Hb of 6.8 ± 2.6 gm/dl), similar microcytosis (average MCV 80.2 ± 10.6fl) and an average HbS conc of 83.01± 6.9%. These patients also had a modest elevation of HbF with a mean of 15.57±5.9%.

showing increased levels of both HbS and HbF.

HbSβ thalassemia patients had an average Hb concentration of 8.1 ± 1.9 gm/dl, mean MCV of 70.2 ± 12.8fl, mean HbS concentration of 74.8 ± 4.7% and mean HbF concentration of 17.2 ± 6.4 %. Majority of the cases of sickle cell trait, sickle cell disease and HbSβ thalassemia belonged to the tea tribes from the various parts of Barak Valley.

There were 2 cases of HbD trait. Both were father and daughter who were Punjabis and now settled in Barak Valley. They presented with hemoglobin of 12.1 gm/dl and 10.4gm/dl and MCV of 81.6 fl and 80.4 fl. On hemoglobin electrphoresis the concentration of HbD-Punjab was 37.2% and 25.6%.

DISCUSSION:

This study was conducted including patients of Barak Valley and its adjacent areas. In this study HbE trait (11.3%) was the commonest abnormality encountered followed by beta thalassemia trait (9.3%), HbEβ thalassemia (4.2%), Sickle cell trait (3.5%) and HbE disease (3.2%). HbE was found to be the commonest hemoglobinopathy prevalent in the north eastern part of India in various studies such as Baruah et al[5], Pathak et **al[6], Hazarika D et al**[7]. In a study done by Sengupta et al. HbE was found to be of highest frequency in the tribal population of Tripura.[8] HbE was also found to be of highest incidence in four population groups of another North eastern state, Manipur. Hb E is the most prevalent variant hemoglobin in ethnic groups affiliated to Tibeto-Burman linguistic family.

The frequency of beta thalassemia trait has been reported from 3.48%[5] to 18.12%[6] among the north eastern population of India. Jain BB et al has reported beta thalassemia trait to be the commonest hemoglobinopathy among the population of West Bengal.[9] The next common hemoglobinopathy in the present study was HbEβ thalassemia which is similar to the studies of Pathak et al. and Jain BB et al.[6,9] It is the commonest severe hemoglobinopathy requiring multiple transfusion. The frequency of Eβ thal is reported from 0.7%[10] to 4.6%[11] in different studies. The average frequency of HbE gene in Indian population is about 10.9%.[11] In the present study the frequency of Hb E disease was found to be 3.2%. However various studies in this part of the country had reported a higher frequency of HbE disease. [5,12]

HbS is mainly prevalent in central India, national average being about 4.3%.[11] In Orissa, HbS is very common making it the commonest hemoglobinopathy in the state.[10] Sickle cell disease is prevalent among the tea garden population of Assam. In 1952 Dunlop and Mazumder reported the presence of sickle hemoglobin in the tea garden workers of Upper Assam who were migrant labourers from tribal groups in Bihar and Odisha.[13] In the present study 3.5% cases were Sickle cell trait, 2.2% cases were Sickle cell disease and 1.5% cases were sickle cell beta thalassemia. The patients were primarily from the tea tribe population.

β thalassemia major constituted 0.8% of the total cases which is similar to Baruah et al, Pathak et **al , Hazarika D et al.**[5,6,7] Sachdev R in his study among the patients of northern India showed the prevalence of thal major to be 0.67%.[14]

HbD is mainly prevalent in Punjab & Uttarpradesh with a frequency of 0.5% to 3.1%[15] and is relatively rare in this part of the country. In the present study HbD was found to be 0.2% (2 cases). Both cases were father and daughter, who were Punjabis and had settled in Barak Valley. Baruah et al has reported a frequency of 0.07% (i.e 6cases) of HbD trait in Upper Assam.[5]

Hemogram was done in all the cases and the lowest mean hemoglobin (4.7gm/dl) was seen in β thalassemia major cases. Anisopoikilocytosis was also greatest having the mean RDW-CV of 25.6%. These cases presented in early childhood with signs and symptoms of anemia, splenomegaly etc. and are transfusion dependent which is similar to various studies.[5,6,16]

Among the homozygous hemoglobinopathies, HbSS had the lowest average Hb level of 6.8 gm/dl. Amongst the heterozygous hemoglobinopathies, HbS trait had the lowest average Hb level of 9.8 gm/dl. Among the double heterozygotes, Eβ thal had lower Hb concentration than S β thal (6.5 gm/dl vs 8.1 gm/dl), hence requiring more frequent transfusions than Sβ thal. The findings are similar to Mondal et al. [16] but Pathak el al.[6] has found the average hemoglobin concentration to be 3.4gm/dl in patients with Sβ thalassemia. The degree of microcytosis was more in Hb Eβ thalassemia as compared to Sβ thalassemia. HbF concentration was more in Eβ thalassemia (34.6%) as compared to Sβ thalassemia (17.2%)

Thus to conclude that HbE & its variants are the major abnormal hemoglobins in the north eastern part of India and hemogram analysis with hemoglobin electrophoresis is a quick and accurate method to detect various Hb variants in the community.[17] As the incidence of thalassemia in our country is still unacceptably high, such studies as ours (particularly pre-marital testing) will prevent or reduce the number of more serious hemoglobinopathies like thalassemia major in future generations.

As this study is a hospital based study and comprises of limited number of cases the pattern observed may not be a true reflection on a

community basis. A larger population based sudy is necessary for detailed assessment and conclusion.

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