Pattern of Haemoglobin Disorders

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Abstract

Background: To study the pattern of different haemoglobin disorders in our set up.

Methods: In this descriptive study, patients referred for haemoglobin electrophoresis were included. Detailed clinical history was taken. Physical findings were noted. Peripheral blood film and haemoglobin electrophoresis was performed.

Results: Out of total 666 referred cases, 176 (26.4%) were detected to have abnormal haemoglobin. The commonest disorder was beta thalassaemia trait (13.2%). Beta thalassaemia major was found in 9.9% cases. Other haemoglobin disorders like HbD, HbE, and HbS alone or in combination with beta thalassaemia were detected in 2.95% cases. One case of beta thalassaemia minor revealed normal pattern on haemoglobin electrophoresis and was diagnosed on molecular studies.

Conclusion: The increased trend of haemoglobin electrophoresis employment predicts a decline in homozygous states of autosomal recessive haemoglobin disorders.

Key Words: Haemoglobin disorders. Haemoglobin electrophoresis. Thalassaemia

Introduction

Hereditary disorders of haemoglobin synthesis e.g., thalassaemias, and of haemoglobin structure e.g., Hb-S, Hb-C and Hb-D have a worldwide distribution. Thalassaemias are probably present in every racial and ethnic group, whereas structural haemoglobin defects are distributed within certain geographic and racial limitations. Hb-S and Hb-C is primarily an African and Negro characteristic whereas Hb-E is present mainly in the Far East and South East Asian populations. There are substantial number of patients having anaemia due to haemoglobin disorders. Among haemoglobin disorders, beta - thalassaemia is probably the most common single gene disorder and its prevalence, as trait is 5-7% in Pakistan. Other haemoglobin disorders like HbD, HbE and HbS, alone or in combination with beta thalassaemia also exist in our population.1,2

For the diagnosis of haemoglobin disorders blood counts, peripheral film examination and red cell indices are the first line investigations. Then on the basis of haemoglobin electrophoresis a definitive diagnosis can be established.

Patients and Methods

The study was carried out on the patients who were sent for haemoglobin electrophoresis, from January 2000 to March 2004. Detailed clinical history regarding blood transfusion, family history and physical findings like splenomegaly were noted.

Three ml of blood sample was collected in EDTA. Complete blood counts were measured on automated haematology analyzer. Peripheral blood films were examined for red blood cells morphology and any other relevant finding.

Haemolysate was prepared by adding carbon tetra chloride and distilled water. Electrophoresis was performed on cellulose acetate strips and densitometric evaluation was performed. The results were reported after the review of clinical notes, peripheral blood findings, examination of cellulose acetate strips and densitometric graphs.

Results

A total of 666 requests for haemoglobin electrophoresis were received from January 2000 to March 2004. Out of these 176 (26.4%) were found to have abnormal haemoglobins. Beta thalassaemia minor (13.2%) was the commonest. Beta thalassaemia major was found in 9.9% cases. Other haemoglobin disorders like HbD, HbE and HbS alone or in combination with beta thalassaemia trait were detected in 2.95% patients (Table-1).

In patients detected as beta thalassaemia trait HbA2 was increased (more than 3.5%). Blood counts revealed RBC count of more than 5X10^6 /l and MCV < 60fl (Table 2;Fig 1&2) Peripheral blood film in these
cases revealed microcytic and hypochromic blood picture with target shaped red blood cells. One case revealed normal red cell morphology but electrophoresis showed increased HbA2. In another case, haemoglobin electrophoresis was normal and beta thalassaemia was diagnosed on polymerase chain reaction (PCR) test.

All the patients of beta thalassaemia major had severe anaemia, hepatosplenomegaly and a history of blood transfusion before the age of two years. Two patients were diagnosed at an age of more than 2 years and they were not transfusion dependent. Both these were labelled as beta thalassaemia intermedia.

The peripheral blood films of beta thalassaemia major cases revealed marked aniso-poikilocytosis with red cell fragmentation and nucleated red blood cells. Haemoglobin electrophoresis in all these cases revealed high fetal haemoglobin (Fig 1&3).

Most of the cases with Hb-D trait were asymptomatic, without any positive finding on clinical examination. None of these had any history of blood transfusion. Haemoglobin electrophoresis in these cases revealed a distinct band in HbS/D region. Sickling test was negative in these cases. The blood picture in HbE trait showed mild hypochromia and microcytosis. Haemoglobin was within normal limits. One patient who was compound heterozygous (E/beta thalassaemia) had mother having HbE trait and father with beta thalassaemia trait. Clinical presentation of this patient was just like beta thalassaemia major (Fig 4).

**Table 1: Haemoglobin disorders: Distribution (n=666)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Number (%)</th>
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<tbody>
<tr>
<td>Heterozygous beta thalassaemia</td>
<td>88 (13.2%)</td>
</tr>
<tr>
<td>(Beta thalassaemia minor/trait)</td>
<td></td>
</tr>
<tr>
<td>Homozygous beta thalassaemia</td>
<td>66 (9.9%)</td>
</tr>
<tr>
<td>(Beta thalassaemia major)</td>
<td></td>
</tr>
<tr>
<td>Beta thalassaemia intermedia</td>
<td>2 (0.3%)</td>
</tr>
<tr>
<td>Hb-D heterozygous</td>
<td>11 (1.6%)</td>
</tr>
<tr>
<td>Hb-S heterozygous</td>
<td>2 (0.3%)</td>
</tr>
<tr>
<td>Hb-E heterozygous</td>
<td>2 (0.3%)</td>
</tr>
<tr>
<td>Hb-E/Beta thalassaemia</td>
<td>1 (0.15%)</td>
</tr>
<tr>
<td>Hb-S/Beta thalassaemia</td>
<td>1 (0.15%)</td>
</tr>
<tr>
<td>Hb-D/Beta thalassaemia</td>
<td>1 (0.15%)</td>
</tr>
</tbody>
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The cases of HbS trait had mild anaemia and their sickling test was positive. Out of two cases of sickle cell anaemia, one presented with recurrent attacks of bony pains and the other one with recurrent infections. Peripheral film examination of both these cases revealed sickle cells and none had splenomegaly. Patient with Sickle/beta thalassaemia had splenomegaly. The peripheral film examination of this case revealed microcytic, hypochromic blood picture with target cells and sickle cells. The diagnosis was established on sickling test and haemoglobin electrophoresis (Fig 5).

**Table 2: Blood parameters in beta thalassaemia trait**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>90% Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>4.2 – 13</td>
<td>Less than 12</td>
</tr>
<tr>
<td>RBC Count (X109 /l)</td>
<td>3.88 – 8.70</td>
<td>Above 5.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>54.6 – 73.6</td>
<td>Less than 60</td>
</tr>
</tbody>
</table>

**Fig 1:** Beta Thalassaemia Major: Pattern of Electrophoresis

**Fig 2:** Densitometric Pattern of Beta Thalassaemia Minor

**Fig 3:** Densitometric Pattern of Beta Thalassaemia Major
Discussion

In Pakistan there is a strong tendency to marry within own ethnic group, ‘biradri’, ‘tribe’ or family. Cousin, especially first cousin marriages with a background of family history of a haemoglobinopathy, further strengthens this trend. This peculiar situation results in an unusually high frequency of autosomal recessive disorders, like haemoglobinopathies. The most common haemoglobinopathy in our set up is thalassaemia. In Pakistan it is seen in all parts of the country and over 5000 homozygotes are born each year. Carrier frequency varies from 4.0% to 5.0% in different groups in various parts of country. In a family having a patient of beta thalassaemia major (extended family) the prevalence of carrier is more than 30% Incidence of other haemoglobinopathies ranges from 0.7 % to 1.2% .

The requests for haemoglobin studies are usually made during antenatal screening. A proper screening before conception can further circumvent the chances of the birth of a homozygous child. If husband and wife both are carriers for an autosomal recessive disorder, then in every pregnancy there are 25% chances of beta thalassaemia major, 50% chances of beta thalassaemia minor and 25% chances of a normal offspring . The chorionic villous sampling and mutation analysis by PCR make an early detection of disease possible and prove to be an effective method in reducing the disease prevalence .

During routine work up of these cases it is important to differentiate beta thalassaemia minor from iron deficiency. Advances in carrier diagnosis using haematological indices as a useful tool can make an early detection of carrier possible. Microcytic hypochromic morphology of red blood cells in proportion to degree of anaemia, anisocytosis and poikilocytosis with presence of pencil shape red cells on peripheral blood, red blood cells count usually less than 5.0 millions/cmm and decreased MCV favours the diagnosis of iron deficiency anaemia. Whereas, uniformly microcytic hypochromic blood picture more pronounced as compared to the level of haemoglobin, minimal or absence of anisocytosis, presence of target shaped red blood cells, red blood cells count more than 5.0 millions/cmm and decreased MCV favours the diagnosis of beta thalassaemia trait. 3-11

The present study was not population based. The cases underwent haemoglobin electrophoresis as a part of antenatal screening or during the evaluation for anaemia. As compared to previous studies, performed more than ten years back, this study revealed a less prevalence of beta thalassaemia major. In recent years preventive strategies have been introduced . Many philanthropist societies are working to screen the carriers and then to give them genetic counselling. The population at large and the medical personnel are now more acquainted with the intricacies of the haemoglobin disorders. This can describe a declining prevalence of homozygous states of haemoglobin disorders. 1

Haemoglobin-D is the most common haemoglobin disorder other than beta thalassaemia . There is approximately 3% occurrence in the population in northern India. As HbD has normal solubility so most of the patients are asymptomatic. In HbD haematological parameters are usually normal in heterozygotes and homozygotes. However in combination with β thalassaemia it causes moderate hypochromic microcytic anaemia. In Pakistan Sickle cell disease cases usually belong to a tribe of Dera Ismail Khan(Bhatani tribe) and Balochistan. HbE is moderately unstable. Although traits are usually asymptomatic having only mild hypochromic microcytic blood picture but when in combination with beta thalassaemia they can cause moderate to severe anaemia with hepatosplenomegaly. On cellulose acetate electrophoresis, it is slow moving and migrates in the same position as HbA2 and HbC , but on quantification it is usually more than 7%. The heterozygous state of HbE is associated with microcytosis and target erythrocytes. There is no anaemia or reticulocytosis. Homozygosity for HbE is characterized by mild microcytic anaemia with many target cells . HbE/β thalassaemia resembles thalassaemia major clinically and haematologically. Alpha thalassaemia is a haemoglobin defect prevalent in Southern China and South East Asia. The diagnosis of alpha thalassaemia can not be made on peripheral blood or cellulose acetate findings. The HPLC and polymerase chain reaction are helpful modalities to diagnose α - thalassaemia. 12-15

In present study a case remained undiagnosed on
haemoglobin electrophoresis, but on a high suspicion the polymerase chain reaction studies were performed and the patient turned out as a case of beta thalassaemia minor. Cases of this nature can be ascribed to different silent mutations (Cap+1 in this case). 16 Cap +1 mutation phenotypically presents with silent beta thalassaemia trait and normal haematogical indices. Hence, it may create a tight spot in the screening programmes.17

**Conclusion**

An equivocal and an unexplainable haemoglobin electrophoresis result, in a given situation, warrants molecular studies

**References**

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