Electrophoresis Pattern In Clinically And Hematologically Suspected Cases Of Haemoglobinopathies.

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Abstracts: Objective: Haemoglobinopathies are a group of disorders with structural or quantitative variation in normal hemoglobin structure. There are various identified haemoglobinopathies worldwide, among these sickle cell disease and beta thalassemia are prevalent in Gujarat. Both of them are associated with marked morbidity and mortality.Method: Hemoglobin electrophoresis is a low cost method helpful in early diagnosis of many of these haemoglobinopathies. In current retrospective study- 33 cases of clinically and hematological suspected cases of haemoglobinopathies were subjected for hemoglobin electrophoresis on agarose gel at pH 8.6. Result and conclusion: Out of 33 suspected cases 13 cases of thalassemia major, 10 cases of sickle cell anemia and 7 cases of sickle cell trait were diagnosed with varying degree of clinical and hematological findings. [Patel S et al NJIRM 2012; 3(3) : 24-27]

Key words: Electrophoresis, haemoglobinopathies, sickle cell disease, thalassaemia.

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Introduction: Normal adult haemoglobin contains two alpha and two beta globin chains. Any alteration in these alpha or beta globin chain leads to various types of haemoglobinopathies worldwide with different ethical and geographical distribution. Clinical presentation varies with different types of haemoglobinopathies. Clinical symptoms vary from asymptomatic carrier state to severe haemolysis incompatible with life. .i.e.Hydrops foetalis[1,2].

Haemoglobinopathies are grouped into three main categories. 1) Those due to structural variants. e.g. Sickle cell disease. 2) Those due to failure to synthesize normal haemoglobin. e.g. Thalassaemias. 3) Those due to failure to switch from fetal haemoglobin to adult haemoglobin. e.g. Hereditary persistent of fetal haemoglobin[3].

Sickle cell disease and beta thalassaemia are the two most common haemoglobinopathies of Gujarat state. Sickle cell disease results due to point mutation in beta chain where substitution of valine for glutamic acid at the sixth position takes place[1,2]. Clinically homozygous patient presents with moderate to severe haemolytic anemia with repeated vaso-occlusive episodes due to the sickle shaped RBCs, the heterozygous patient may present with mild symptoms. Beta thalassaemia results from various genetic abnormalities of beta chain ranging from point mutation to large deletions which leads to decreased production of beta chains or complete absence of beta chain production. Clinically homozygous patient presents with severe haemolytic anemia, splenomegaly, jaundice, stunted growth etc, and heterozygous patient are mostly asymptomatic and are carrier of the disease[1,2].

Haemoglobin electrophoresis is a simple and cost effective method for diagnosis of some of the common haemoglobinopathies. At alkaline pH haemoglobin is a negatively charged protein and in a electric field will move from cathode to anode. Based on their surface charge differences- variants of haemoglobin will be fractionized[1,3]. Various types of haemoglobin based on their structure gives separate bands at separate distance[3].

Material and Methods: Total 33 cases of pediatric patients at PDU Medical College, Rajkot were selected for these retrospective study based on their clinical and hematological findings during September 2005 to May 2006. Only the patients presenting for the first time without past history of any blood transfusion and with clinical and hematological features suspicious of haemoglobinopathy were selected for present study. Patients already diagnosed at our institute

or any other hospital/institute was not included in the present study. Clinical signs and symptoms taken into account were pallor, jaundice, splenomegaly, vaso-occlusive events.

Hematological findings taken into account were haemoglobin%, peripheral smear examination with special emphasis on presence of normoblasts and polychromatic RBCs, anisopoikilocytosis, presence of sickle like cells and Reticulocyte count.

Taking aseptic precaution a smooth venepuncture was made to collect 2ml of blood in an EDTA bulb. Routine complete blood count was performed on three part cell counter (KX-21) at department of pathology, PDU Medical College, Rajkot. Two peripheral smears were prepared, first smear from the collected EDTA sample and second smear was prepared taking aseptic precautions by finger prick after tying the finger at the base to create hypoxia. Both the smears were stained by Leishman stain and observed under microscope. Reticulocyte count was also done from the EDTA collected sample using New Methelene Blue dye.

After routine hematological investigations sample in the EDTA bulb was processed for haemoglobin electrophoresis in the department of Biochemistry, PDU Medical College, Rajkot.

Sample preparation: The sample was washed 3 times with normal saline. Haemolysate was prepared using Saponin. The concentration of haemolysate was uniformly adjusted at 1gm% using Tris buffer as diluent.

Gel preparation: 1% Agarose gel was made from agarose powder and Tris buffer after heating. This liquid gel was poured on a slide to make it an even solid gel. After cooling of gel haemolysate was applied using an applicator. Positive controls of HbS and HbF were used from known cases of sickle cell disease and neonates respectively with each batch of study samples.

Tris buffer was prepared using Tris powder, EDTA acid, boric acid and distilled water. The pH was adjusted at 8.6[3].

After gel preparation and application of samples at the cathode end the gel was kept in the electrophoretic tank filled with Tris buffer. The gel was connected with Tris buffer using a filter paper soaked in Tris buffer. After these the electric current was started at 100V and 25 empires for 30 minutes.

The gel was stained using Ponceau's stain for clear and identifiable bands of haemoglobin fractions. Excess stain was removed using 5% acetic acid. The electrophoretic bands were observed and interpreted.

Result: Total 33 suspected cases of haemoglobinopathies based on their clinical and hematological findings were subjected for agarose gel electrophoresis at pH 8.6.

Age range varies from 9 months to 11 years. Out of 33 cases 18 were male patients and 15 were female patients. The electrophoretic pattern of all 33 cases on agarose gel at pH 8.6 was as table no.1

Table 1: Electrophoresis pattern on agarose gel.

Predominant Haemoglobin fractions	No. of cases	Diagnosis
HaemoglobinF	09	Thalassaemia Major
HaemoglobinF and HaemoglobinA	04	Thalassaemia Major
HaemoglobinS	07	Sickle cell anemia
HaemoglobinS and HaemoglobinF	03	Sickle cell anemia
HaemoglobinS and HaemoglobinA	07	Sickle cell trait
HaemoglobinA	03	Further evaluation done

As mobility of haemoglobinS, haemoglobinD and haemoglobinQ are similar on agarose gel at pH 8.6; all patients with haemoglobinS were further confirmed by positive sickle solubility test. All 3 patients with haemoglobinA turned out to be G-6PD (Glucose-6-Phosphate Dehydrogenase) deficient. The sex distribution of various haemoglobinopathies was as per the graph. 1

The clinical features and hematological findings of various haemoglobinopathies were as per tableno.2 and table no.3.

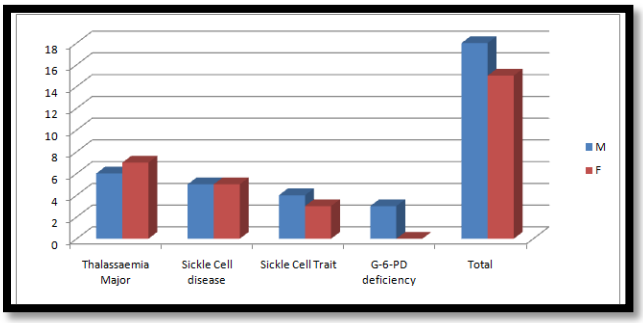
Table 2: Clinical Features

Diagnosis	Hemoglobin Fraction	Splenomegaly	Jaundice	Vaso-Occlusive events
Thalassemia Major	Hemoglobin-F	9(100%)	9(100%)	0 (0%)
	Hemoglobin-F and A	4 (100%)	3 (75%)	0 (0%)
Sickle Cell Anemia	Hemoglobin-S	4 (57%)	2 (28.6%)	7 (100%)
	Hemoglobin-S and F	1 (33.3%)	2 (66.6%)	3 (100%)
Sickle Cell Trait	Hemoglobin-S and A	0 (0%)	0 (0%)	2 (28.6%)

Table 3: Hematological features

Diagnosis	Hemoglobin Fraction	Average Hemoglobin%	Average Normoblast %	Average Reticulocyte %
Thalassemia Major	Hemoglobin-F	4.2 gm%	32 %	2.5%
	Hemoglobin-F and A	4.6 gm%	20%	2.3%
Sickle Cell Anemia	Hemoglobin-S	5.4 gm%	12%	4.2%
	Hemoglobin-S and F	6.2 gm%	8%	3.8%
Sickle Cell Trait	Hemoglobin-S and A	9.2 gm%	2%	2%
G-6PD Deficiency	Hemoglobin-A	7.8 gm%	3%	14%

Graph 1: Sex distribution of various haemoglobinopathies



The lowest haemoglobin was 2.8% in a case of thalassaemia major (HbF), while highest haemoglobin was 10.8% in a case of sickle cell trait (HbS and HbA). Highest normoblast count was 60% in a case of thalassaemia major (HbF). Highest reticulocyte count was 20% in a case of G-6-PD deficiency.

From the various above cited observations it is seen that thalassaemia major presents with most severe features of haemolysis, both clinically and hematologically[4]. In all thalassaemia major patients more severe clinical and hematological features were seen in patients having predominant HbF compared to patients having both HbF and HbA[4,5]. In all sickle cell anemia patients severe clinical and hematological features were seen in patients having HbS compared to patients having both HbS and HbF[6,7].

Discussion: Considering the high prevalence of sickle cell disease and beta thalassaemia in Gujarat state haemoglobin electrophoresis remains a cost effective method for diagnosis of haemoglobinopathies[1,4]. Both beta thalassaemia and sickle cell disease have considerable morbidity and mortality-so early diagnosis is very advantageous. Sickle cell disease is an autosomal dominant disease, haemoglobin electrophoresis along with sickle solubility test helps to diagnose both homozygous form (sickle cell anemia) and heterozygous form (sickle cell trait), thus can be employed for mass screening programme[4,6,8]. In homozygous sickle cell anemia, patients having haemoglobinS and HaemoglobinF on electrophoresis presents with milder clinical and hematological features compared to patients having haemoglobinS only- thus haemoglobinF have a somewhat protective role. In homozygous thalassaemia (thalassaemia major) patients having haemoglobinF and haemoglobinA on electrophoresis (ß0/ß+ or ß+/ß+) presents with milder clinical and hematological features compared to patients having haemoglobinF only (ß0/ß0)- thus production of haemoglobinA has

somewhat protective role[5,9]. Although carrier state of thalassaemia (thalassaemia minor) cannot be accurately diagnosed by haemoglobin electrophoresis, mass screening can be done using haemoglobin electrophoresis along with NESTROFT'S test and Mentzer's criteria(MCV/RBC count)[4,9]. Suspected cases of thalassaemia minor should be confirmed by HPLC (high performance liquid chromatography)[3]. As both beta thalassemia and sickle cell disease are genetical diseases mass screening and counseling can be employed to reduce the incidence of the disease and ultimately the burden on the society.

Conclusion: In the present study, total 33 cases of clinically and haematologically suspected haemoglobinopathies were subjected to agarose gel electrophoresis. Out of these 13 cases of thalassaemia major(39.4%), 10 cases of sickle cell anaemia(30%) and 7 cases of sickle cell trait(21%) were diagnosed. Hence electrophoresis remains a cost effective method for confirmatory diagnosis of various haemoglobinopathies

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