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Original Article

Retrospective Study of Screening of Haemoglobin Disorders by Hb: Electrophoresis in Tribal Part of Maharashtra

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Abstract

Haemoglobin disorders are a major public health problem in India. We have studied haemoglobin patterns of various HB disorder's at Gokunda sub district hospital in Nanded district of Maharashtra during period of June 2011 to June 2014. We tested 959 samples for electrophoresis. Out of them 526 (54.8%) were sickle cell carrier and 49 (5.1%) were sickle cell disease. Electrophoresis is simple, cost effective method for screening sickle cell disorders. It is cheap, reliable and effective tool for screening of haemoglobinopathies.

Keywords: Electrophoresis; Sickle cells disease.

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Introduction

Inherited disorders of haemoglobin are the commonest genetic disorders in the world. Haemoglobin disorders are grouped into three main categories the haemoglobin disorders are grouped into three main categories 1. Those due to structural variants e.g. sickle cell disorder 2. Those due to failure to synthesize normal haemoglobin e.g. Thalassemia's 3. HPFH (hereditary persistence of fetal haemoglobin.

The thalassemia's are a heterogeneous group of inherited disorders of haemoglobin characterized by reduced or absent production of one (or rarely more) of the globin chains. They are the commonest single gene disorders in the world. Sickle cell disease results due to point mutation in beta chain where substitution of valine for glutamic acid at sixth position takes place.

Materials and Methods

Blood samples from patients in EDTA bulb at Primary Health Centre. Two peripheral smears were prepared at same time. First smear done 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 Methylene Blue dye.

The samples which were detected as positive on Solubility tests carried at Primary Health Centre under Kinwat, Himayatnagar and Mahur talukas and also at Gokunda itself were further tested on cellulose acetate agarose gel electrophoresis at Gokunda electrophoresis center. Further confirmation of all positive samples done

by High Performance Liquid chromatography. Hematological findings taken into account were haemoglobin percent, peripheral smear examination with special on presence of normoblasts and polychromatic RBCs, anisopoikilocytosis, presence of sickle like cells and Reticulocyte count. History, clinical and laboratory correlation done in every step for reaching up to accurate diag-nosis.

Electrophoresis

Sample Preparation: The sample was washed three times with normal saline. Hemolysate was prepared using Saponin. The concentration of hemolysate was uniformly adjusted at 1 gm % using Tris buffer as diluent.

Gel preparation: 1% Agarose gel was made from agarose powder and Tris buffet after heating. This liquid gel was poured on a slide to make it an even solid gel. After cooling of gel hemolysate 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 8.63.

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 and 25 empires for 30 minutes. The gel was stained using Ponceau's stain for clear and identifiable bands of hemoglobin fractions. Excess stain was removed using 5% acetic acid. The electrophoretic bands were observed and interpreted.

Results

Table 3.1: Electrophoresis pattern on agarose gel.

Predominant Hemoglobin Fraction	No of Cases	Diagnosis
Haemoglobin S	40	Sickle cell disease
HAemoglobin S and F	09	Sickle cell disease
Haemoglobin S and A	526	Sickle cell trait
Haemoglobin A and F	24	Thalassemia (Further evaluated by HPLC)
Haemoglobin F	20	Thalassemia (Further evaluated by HPLC)
Haemoglobin A	361	Further evaluated by HPLC
Total	959	

Maximum number of electrophoretic patterns found was AS (Sickle cell trait) HbS and HbSF pattern were found in 40 and 9 cases respectively which were suspected cases of sickle cell anaemia. HbAF and HbF patterns were found in 24 and 20 cases respectively, they were suspected of thalassemia. All the suspected of thalassemia. All the suspected cases of haemoglobin disorders were further confirmed by HPLC.

Table 3.2: Hematological features.

Diagnosis	Hb Fraction	Avg Hb	Avg Normo-blasts %	Average Reticu-locytes%
Sickle cell disease	Haemoglobin S	6.5	10	3.8
Sickle cell disease	Haemoglobin S and F	7.2	8	3.6
Sickle cell trait	Haemoglobin S and A	9.3	2	2.1
Thalassemia major	Haemoglobin F	4.3	30	2.5
Thalassemia major	Haemoglobin F and A	4.7	18	2.5

Average haemoglobin of Sickle cell anaemia patients showing HbS pattern was 6.5 gm% while those showing HBSF had an average of 7.2 gm%. Average haemoglobin of thalassemia patients HbF pattern was 4.3 gm % while those showing HbAF had an average of 4.7 gm%.

Table 3.3: History of consanguinity.

	Total	Percentage
H/o consanguineous marriages	105	11.0%

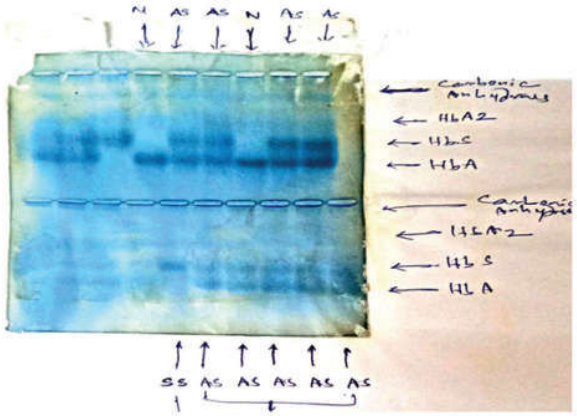

Fig. 3.1: Cellulose acetate alkaline electrophoresis showing bands of respective disorders.

AS: Sickle cell trait, SS: Sickle Disease, N: Normal.

Table 3.4: Sex wise distribution.

Disorder	Male	Female
Sickle cell disease	24	25
Sickle cell trait	264	262
Total	268	267

Discussion

Considering the high prevalence of sickle cell disease and beta thalassemia in Nanded region of Maharashtra. Haemoglobin electrophoresis remains a cost-effective method for diagnosis of haemoglobinopathies. Both beta thalassemia and sickle cell disease have considerable morbidity and mortality so early diagnosis is very advantageous.

Sickle cell disease is an autosomal recessive disease, haemoglobin electrophoresis along with sickle solubility test helps to diagnose both homozygous form (sickle cell anaemia) and heterozygous form (sickle cell trait), thus can be employed for mass screening programmed. In homozygous sickle cell anaemia, patients having haemoglobin S and haemogolobin F on electrophoresis presents with milder clinical and hematological features compared to patients having haemoglobin S only thus haemoglobin F have a somewhat protective role. In homozygous thalassemia (thalassemia major) patients having haemoglobin F and haemoglobin A on electrophoresis (beta 0/beta+ or beta+/beta+) presents with clinical and hematological features compared to patients having haemoglobin F only (beta 0/ beta 0) thus production of haemoglobin A has somewhat protective role.

In the present study total 959 cases of clinically and haematologically suspected haemoglobinopathies, 49 cases of sickle cell anaemia (5.1 %) and 526 cases of sickle cell trait (54.8%) were diagnosed. Cases shown f band and combined F and A were of thalassemia major. Out of cases shown only Hiba band on electrophoresis (361) maximum were of Thalassemia Trait (324). Alkaline electrophoresis failed to pick this iceberg.

Conclusion

As carrier state of thalassemia (thalassemia minor) cannot be accurately diagnosed by haemoglobin electrophoresis, mass screening can be done using haemoglobin electrophoresis along with NESTROFT Test and Mentzer's criteria (MCV/RBC count). NESTROFT is sensitive cost effective rapid and reliable screening test for thalassemia trait.

In the present study, total 959 cases of clinically and haematologically suspected haemoglobinopathies were subjected to agarose gel electrophoresis. Out of these 526 cases of sickle cell trait (54.8%) and 49 cases of sickle cell anaemia (5.1%) were diagnosed, hence electrophoresis remains a cost-effective method for confirmatory diagnosis of Sickle cell disorders. however, for screening thalassemia battery of screening tests such as NESTROFT / Mentzer and Electrophoresis.

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