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Detection of Haemoglobinopathies and Thalessemias by Capillary Electrophoresis

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Abstract

Introduction: Haemoglobinopathies and thalessemias are inherited disorders of haemoglobin synthesis.It is estimated that at least 5.2% of the world population and more than 7% of pregnant women are carriers of abnormal haemoglobins.The aim of the present study was to evaluate capillary electrophoresis method for the detection of thalessemias and haemoglobinopathies in patients visiting our haemotology department for Hb electrophoresis. *Materials and Methods:* The present retrospective study was carried out in our haematology department between September 2015 to August 2016 . A total of 500 patients in both the sexes registered for Hb electrophoresis were included in the study. Blood samples were collected intravenously in K3 EDTA tubes using standard protocols.A complete blood count was performed on each sample on LH 750 (Beckman coulter) fully automated haematology analyser within 12 hours of blood collection. All the blood samples were later run on capillary electrophoresis system (Sebia)as per standard protocols. *Results:* There were 38.6% males and 61.4% females .The male to female ratio was 0.6:1. 311 patients(62.2%)had normal Hb electrophoretic pattern .Sicle cell trait was detected in 68(13.6%) patients.Thalessemia minor was detected in 51(10.2%) patients, Sickle cell disease in 47(9.4%)patients,.17 patients (3.4%) were detected to have Thalassemia major.Two cases (0.4%) had HbD. One each(0.2%) case was observed for Hb Barts, HbE, HbH and hereditary persistence of foetal haemoglobin (HPFH). *Conclusion:* Detection of haemoglobinopathies and Thalessemias have become easier due to rapid technical advances in the field of haematology. Capillary electrophoresis has an edge over other methods currently in use because it is rapid, precise and has high resolution and is extremely useful in screening population for haemoglobinopathies and thalessemias and can be used in conjunction with HPLC method for detection of new and rare Hb variants.

Keywords: Capillary Electrophoresis; Haemoglobinopathies; Thalessemia; HPLC.

Introduction

Haemoglobinopathies and thalessemias are inherited disorders of haemoglobin synthesis and are common globally because of increased incidence of migration,which was originally known to be concentrated to the tropics. It is estimated that at least 5.2% of the world population and more than 7% of pregnant women are carriers of abnormal haemoglobins. These often go unnoticed or undetected due to lack of proper health care diagnostic facilities or due to lack of awareness among the people. It is estimated that approximately 1.1% of the married couples are at risk of giving birth to children with a haemoglobinopathy and approximately 2.7per 1000 pregnancies are affected by this [1].

Haemoglobin molecule is a polypeptide tetramer made up of two pairs of globin chains, alpha, beta, delta and gamma, and four oxygen binding heme groups. Healthy adults have two alpha and two beta globin chains which constitutes about 97% of the total adult haemoglobin. HbA2 has two alpha and two delta globin genes and is below 3.5% in normal adults. Foetal haemoglobin, HbF, has two alpha and two gamma globin genes and may be present in small

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quantities in healthy adults.

Haemoglobinopathies and thalessemias account for two distinct genetic haemoglobin abnormalities. Thalessemias are characterized by reduced quantities of normal globin chains either due to mutations or gene deletions. Clinically, thalessemias may present as mild anaemia with microcytosis as in beta thalessemia trait or as a fatal condition Hb Barts in which all the four alpha genes are deleted. Haemoglobinopathies, on the other hand, are characterized by substitution of amino acids in either globin chain.

It is estimated that around 1179 total haemoglobin variants have been characterized so far [2]. Out of these abnormal variants HbS (sickle cell)is the most common, others being HbC, HbE, HbD Punjab.There are numerous rare variants which may be detected during routine screening procedures [3].

Several methods are now in use for screening and detection of abnormal haemoglobins such as High performance chromatography (HPLC) and capillary electrophoresis(CE) [4-6]. Capillary electrophoresis carries the advantage of high resolution and automation with online detection and quantification of numerous abnormal haemoglobins and both alpha and beta thalessemias [7-11].

The aim of the present study was to evaluate capillary electrophoresis method for the detection of thalessemias and haemoglobinopathies in patients visiting our haemotology department for Hb electrophoresis.

Material and Methods

The present retrospective study was carried out in our haematology department between September 2015 to August 2016 .A total of 500 patients in both the sexes registered for Hb electrophoresis were included in the study.The patients were divided into different age groups, 0-20, 21-40, 41-60, 61-80 and more than 80 years age group. (Table 1). All patients with anaemias, generalized weakness, fever, splenomegaly, evidence of haemolysis in peripheral smear examination or family history of any haemoglobinopathy or thalessemia were included in the study. Patients receiving blood transfusion within three months of sampling were excluded from the study. Blood samples were collected intravenously in K3 EDTA tubes using standard protocols. A complete blood count was performed on each sample on LH 750 (Beckman coulter) fully automated haematology analyser within 12 hours of blood collection. A peripheral blood smear was prepared by finger prick and examined under the microscope for RBC morphology. A sickling test was performed on blood samples showing drepanocytes and evidence of haemolysis. All the blood samples were later run on capillary electrophoresis system (Sebia)as per standard protocols.Location of HbF, HbS was confirmed by pooling the samples with blood from normal individuals. Diagnosis of haemoglobinopathies and thalassemias was made on the basis of haematological parameters,laboratory tests and clinical findings [12-14].

Results

500 patients in both the sexes and all age groups with low haemoglobin and family history or RBC morphology suggestive of haemoglobinopathy or thalessemia were included in the study. There were 38.6% males and 61.4% females .The male to female ratio was 0.6:1(table1). 311 patients (62.2%)had normal Hb electrophoretic pattern out of which 93 were males and 218 were females. Sickle cell trait was detected in 68(13.6%) patients out of which 30 were males and 38 were females. Thalessemia minor was detected in 51(10.2%) patients with 28 females and 23 males.Sickle cell disease was observed in 47(9.4%)patients out of which 31 were males and 16 were females. 17 patients (3.4%) were detected to have Thalessemia major with 13 being males and 4 were females. Two cases (0.4%) had HbD. One each (0.2%) case was observed for Hb Barts, HbE, HbH and hereditary persistence of foetal haemoglobin (HPFH). 50.6%(253/500) patients of haemoglobinopathies were in 0-20 years age group, followed by 45%(225/500) in 21- 40 years of age group, 3.6%(18/500)in 41-60 years, 0.8%(4/500)in 61-80 years age group. Sickle cell disease and sickle cell trait, followed by thalessemia major were detercted in 0-20 years age group while in 21-40 years age group Thalessemia minorand sickle cell trait detection was most common followed by sickle cell disease and Thalessemia

Table 1: Showing demographic data of patients

	Male	Female	Total
T.Major	13	4	17
T.Minor	23	28	51
Sicklecell Disease	31	16	47
Sickle cell trait	30	38	68
Normal	93	218	311
HbBarts	1		1
HPFH	1		1
HbH	1		1
HbD		2	2
HbE		1	1
TOTAL	193	307	500
percent	38.6	61.4	100

Table 2: Showing incidence of different haemoglobinopathies

Age In Year	T. Major	T. minor	SC disease	SC trait	Normal	HbBarts	HPFH	HbH	HbD	HbE
0 to 20	14	19	34	42	141	1	1	1		
21 to 40	2	29	13	22	156				2	1
41 to 60	1	1		4	12					
61 to 80		2			2					
>80										
TOTAL	17	51	47	68	311	1	1	1	2	1
percent	3.4	10.2	9.4	13.6	62.2	0.2	0.2	0.2	0.4	0.2

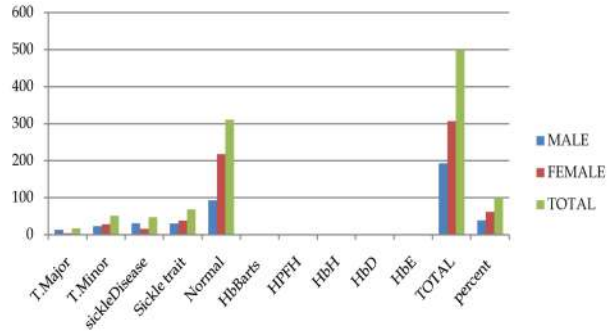

Fig. 1:

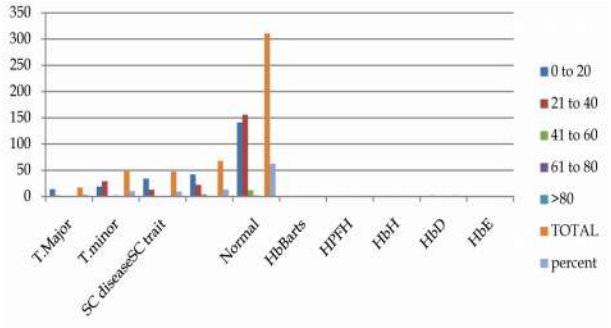

Fig. 2:

major.Two cases of Thaessemia minor were detected in 61-80 years age group.

Discussion

Haemoglobin electrophoresis is a method of detecting abnormal haemoglobins and is based on the principle of migration of electrically charged molecules under an applied electric field. Pauling et al in 1949 discovered the first abnormal haemoglobin molecule Hb S(sickle cell) [15]. Several methods of electrophoresis like gel electrophoresis, HPLC and capillary electrophoresis etc are in use. Capillary electrophoresis is based on the electroosmotic flow and electrophoretic mobility of haemoglobin variants in alkaline buffer (Ph9.4). Samples are separated by high resolution in silica glass capillaries and is a fast method of electrophoresis, taking about eight minutes for analyzing the sample. The wavelength used is UV at 415 nm. It is similar to HPLC in many respects and is considered to be a hybrid between gel electrophoresis and HPLC. The graph generated consists of 300 readings divided into different zones which are displayed as peaks. These peaks are automatically identified and labeled by the system thus minimizing subjective errors.

In our study sickle cell trait was the commonest finding(13.6%), followed by Thalessemia minor (10.2%), sickle cell disease (9.4%) and Thalessemia major (3.4%). Shivashankara A.R. et al in 2008 in their study observed T. minor as the major finding followed by T.major. Sickle cell trait was observed in only 2 patients in their study [16]. The prevalence of sickle cell gene in India is around 5%. Highest incidence of sickle cell gene is found in Orissa (9%), followed by Assam(8.3%), Madhya Pradesh(7.4%)and lowest in Gujarat(6.4%) [17,18,19]. Beta thalessemia gene is unevenly distributed in the Indian subcontinent. Gujarat leads in the incidence of Beta thalessemia trait(10 to15%), followed by Sindhis(10%), Punjab(6.5%),Tamil Nadu(8.4%) and Maharashtra [12,17,20]. In our study, we found a prevalence rate of 10.2% for beta thalessemia trait (T. minor).

Other abnormal haemoglobins like HbD are also frequent in Sindhi populationwith a prevalence rate of 0.5% [21]. Hb D patients are diagnosed most often incidentally because they have normal haemoglobin usually with no significant clinical symptoms.HbD Punjab is another Hb variant in which beta 121(GH4) is affected where Glu is replaced by Gln.HbD Punjab cannot be differentiated using conventional agar gel electrophoresis. Capillar electrophoresis carries the advantage of separating HbD Punjab as a distinct band. Similarly, HbBarts and HbH, which were detected in our study, was possibly because capillary electrophoresis detects both alpha and Beta thalessemias.

HbE is also common in South east Asia [22]. It results from replacement of Glutamic acid by a Lysine and cannot be distinguished from HbC by agar gel electrophoresis.It is separated as a distinct band in capillary electrophoresis.

Conclusion

Detection of haemoglobinopathies and Thalessemias have become easier due to rapid technical advances in the field of haematology. Capillary electrophoresis has an edge over other methods currently in use because it is rapid,precise and has high resolution and is extremely useful in screening population for haemoglobinopathies and thalessemias.It has the advantage of accurate quantification and complete automation .These systems are also robust and may likey replace the cumbersome gel electrophoresis method and can be used in conjunction with HPLC method for detection of new and rare Hb variants.

Conflict of Interest

none

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