ORIGINAL ARTICLE

Screening Significant Haemoglobin Disorders HPLC Vs Electrophoresis

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ABSTRACT

Background: Haemoglobin , protein responsible of carriage of gases consists of hetrotetramers of two alpha and two beta globin chains with one haem molecule in the center of each chain .Mutations of the globin genes can result in either a quantitative decrease output from that gene or modify the amino acid sequences of the protein produced. Quantitative defects produce thalassemia syndrome. The qualitative alterations are referred to as Hb variants. Inherited disorders of haemoglobin are the commonest single gene disorder. Screening and accurate identification of hemoglobin (Hb) variants have become increasingly important in antenatal diagnosis and prevention of Hb disorders. [5] Two most widely used screening methods are haemoglobin electrophoresis and high performance liquid chromatography (HPLC). Electrophoresis is one of the most common methods for haemoglobinopathy screening. Although cost-effective, the technique is relatively laborious, requiring manual sample preparation .High-performance liquid chromatography (HPLC), is a method that allows the detection of abnormal Haemoglobins quickly and precisely, using a small sample amount. I

Aim: To compare the results of HPLC and Haemoglobin Electrophoresis technique for screening of clinically significant haemoglobin disorders.

Methodology: This cross sectional comparative study was conducted at Chughtais Lahore Lab from August 2015 to October 2015. 100 consecutive patients of suspected haemoglobinopathy were included. Peripheral blood was collected in EDTA anticoagulant. Haemoglobin electrophoresis was done using cellulose acetate membrane at pH 8.4-8.6 on Interlab Genu electrophoresis system and Elfolab software version 7.03 was utilized for the calculation of results. The cases with co migration on alkaline electrophoresis were confirmed on acid electrophoresis. All the samples were run in parallel on Biorad D10 HPLC analyzer using HbA2 and Hb F programme.

Results: A total of hundred cases were enrolled in study. Testing the samples yielded that 50(50%) had normal haemoglobin electrophoretic pattern. 30(30%) had haemoglobin A_2 levels more than 3.5% (Thalasaemia trait) Thalasaemia major was detected in 13% of patients. Both the screening methods produce comparative results in detecting haemoglobinopathies.

Conclusion: Our study denotesthat the results obtained from the two techniques are comparable in screening significant haemoglobin disorders. There is considerable difference in cost, making screening by electrophoresis more affordable for economically burdened affected families

Keywords: Hb disorder, HPLC, electrophoresis

INTRODUCTION

Haemoglobin is the protein present in red blood cells responsible for transport of gases to and from the body tissues[1]. In normal adult the various haemoglobins are HbA, (94%), Hb A2 (upto 3.5%), and Hb F (<1.0%). Adult Hamoglobin (Hb A) consists of hetrotetramers of two alpha and two beta globin chains with one haem molecule in the center of each chain[2].Mutations of the globin genes can result in either a quantitative reduction of output from that gene or modify the amino acid sequences of the protein produced. Quantitative defects produce thalassemia syndrome. The qualitative alterations are referred to as Hb variants. They result in a wide spectrum of diseases including sickle cell disease, unstable Hb, decreased or increased oxygen affinity haemoglobins and methemoglobinemia[3]. Inherited disorders of haemoglobin are the commonest single gene disorder. WHO figures quote that 5% of the world population is carrier for Hb disorders [4].Most of the disorders are not clinically apparent .Screening and accurate identification of hemoglobin (Hb) variants have become increasingly important in antenatal diagnosis and prevention of Hb disorders[5]. Two most widely used screening methods are haemoglobin electrophoresis and high performance liquid chromatography (HPLC). Electrophoresis is one of the most common methods for haemoglobinopathy screening. Although cost-effective, the technique is relatively laborious, requiring manual sample preparation. It allows for the separation of the major hemoglobins and a number of less common Hb variants. High-performance liquid chromatography (HPLC) is a method that allows the detection of abnormal Haemoglobins quickly and precisely, using a small sample amount. It allows the quantification of Hb A2, Hb F, Hb A, HbS and Hb C and screening for Hb variants[5].Carrier detection and genetic counseling are helpful in reducing the incidence.

The objective of study was to compare the results of HPLC and Haemoglobin Electrophoresis technique for screening of clinically significant haemoglobin disorders.

METHODOLOGY

This cross sectional comparative study was conducted at Chughtais Lahore Lab from August 2015 to October 2015. 100 consecutive patients of both genders referred for

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haemoglobinopathy screening were included in the study.Peripheral venous blood was collected in EDTA anticoagulant after ensuring aseptic technique. Haemoglobin electrophoresis was done using cellulose acetate membrane at pH 8.4-8.6 on Interlab Genu electrophoresis system and Elfolab software version 7.03 was utilized for the calculation of results. The cases with co migration on alkaline electrophoresis were confirmed on acid electrophoresis. All the samples were run in parallel on Borad D10 HPLC analyzer using HbA2 and HbF programme.

Data collection and data analysis: Data was collected in a specially designed proforma. Data derived using both electrophoresis and HPLC was analyzed on SPSS version 18. T test was applied and p value ≤ 0.05 was considered significant

RESULTS

A total of hundred cases were enrolled in study. Testing the samples yielded that 50(50%) had normal haemoglobin electrophoretic pattern. 30(30%) had haemoglobin A_2 levels more than 3.5% (Thalasaemia trait) Thalasaemia major was detected in 13% of patients (Fig. 1).

Fig. 1: Distribution of disorders

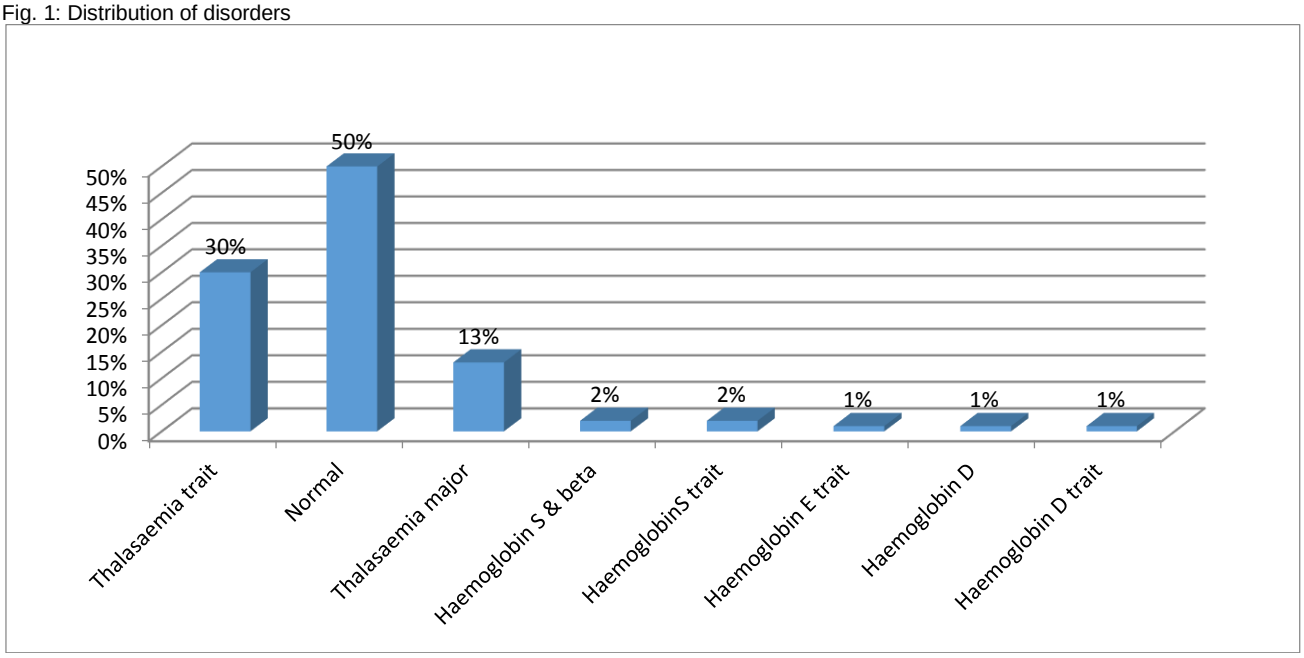

Fig. 2 :Comparison haemoglobin A and A2 levels on alkaline electrophoresis and HPLC

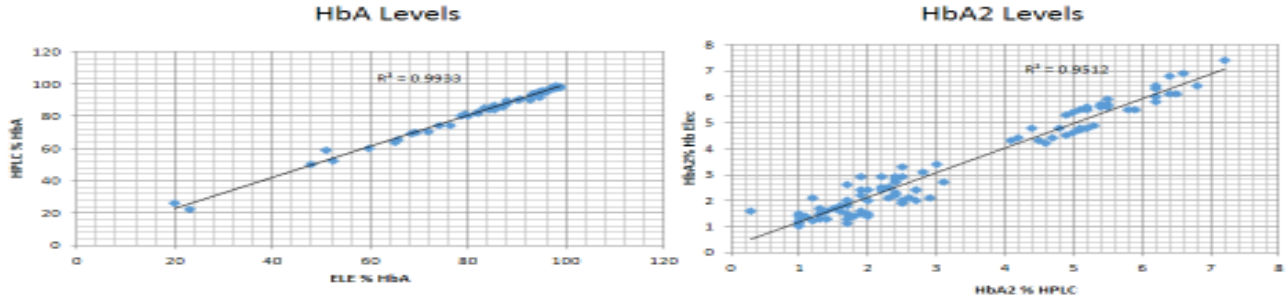

Table 1: Comparison of different haemoglobins on electrophoresis and HPLC

	Haemoglobin Electrophoresis	HPLC	P value (≤ 0.05)
Haemoglobin A (%)	96.2 ±1.3	96.1 ±1.4	>1.1
H aemoglobinA 2 (%)	4.1± 1.3	4.2 ±1.2±	> 0.99
Haemoglobin F (%)	33.9± 1.8	33.0 ±1.6	> 0.82
Haemoglobin S (%)	49.1 ± 1.3	48.5 ± 1.4	> 0.23
Haemoglobin D (%)	61.4± 2.2	61.6 ± 2.6	> 0.18

DISCUSSION

Overall frequency of haemoglobinopathy was 50% in the study. A retrospective analysis of 2731 patients by Shaista et al showed a frequency of 34.2%.· As haemoglobinpathies in our country have ethinic patterns, larger samplesize reflects better burdern of disease Waheed U et al[7] in a study found beta thalasamia 25.6% with haemoglobin S or D 1.4%. In our study beta thalasamia carriers were 30% while haemoglobin S and D were 2% and 1% respectively.

All the samples were analysed for normal and variant haemoglobins on both cellulose acetate haemoglobin electrophoresis and HPLC. Both the techniques yielded similar results. When the results were statistically analyzed there was no statistical difference in detecting the vairianthaemoglobins (p ≤0.05). In a study conducted by Shafi et al·[8] no statistical difference was observed between the two techniques in determination of haemoglobin A 2 levels. However they found that in co morbid condition i.e., coexisting iron deficiency anaemia both the techniques fail to determine low levels of haemoglobin A 2 levels reliably.

In another study conducted by Shane Rauf et al[9], hundred confirmed cases of bata thalassemia trait by Polymerase chain reaction were analyzed on both cellulose acetate and HPLC. They also stated that there is no significant difference in detection the haemoglobin A 2 levels between the two techniques. In our study the levels of haemoglobin A 2 determined by both the techniques were not statistically significant.

High performance liquid chromatography is a swift and reliable method for detection of haemoglobinopathies, It is easy to perform and saves time .in contrast to cellulose acetate electrophoresis which is laborious and time consuming. On the contrary it is an expensive technique not freely available in public sector setups. In economically burdened coutries where health care facilities and infra structure are not sufficient to serve the purpose, the screening of haemoglobinopathies by cellulose acetate provides an inexpensive and reliable method.

CONCLUSION

Our study denotes that the results obtained from the two techniques are comparable in screening significant haemoglobin disorders. There is considerable difference in cost, making screening by electrophoresis more affordable for economically burdened affected families

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