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Original Research Article

The Role of Capillary Zone Electrophoresis in Hemoglobinopathy Diagnosis

Dilip Kumar Das[1], Madhusmita Mishra[2], Swagatika Agarwal[3]

[1]Assistant Professor, Department of Pathology, Hi Tech Medical College and Hospital. Bhubaneswar, Odisha

[2]Assistant Professor, Department of Pathology, Hi Tech Medical College and Hospital. Bhubaneswar, Odisha

[3]Professor, Department of Pathology, IMS and SUM 2 Hospital, Phulnakhara, Bhubaneswar, Odisha

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Corresponding Author: Dr Swagatika Agarwal

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Abstract:

Hemoglobinopathies are inherited blood disorders that affect the hemoglobin molecule, the protein in red blood cells that carries oxygen. These disorders can be caused by mutations in the genes that code for the globin chains that make up hemoglobin. They can result in abnormal hemoglobin structure, reduced hemoglobin production, or both. The purpose of the present study was to evaluate the prevalence of hemoglobninopathies detected by capillary electrophoresis method in individuals by capillary electrophoresis method.

Methods: This study was carried out on 395 individuals referred medical college. Blood samples were collected in EDTA vacutainer tubes, then CBC including blood indexes (MCV, MCH), level of Hemoglobin A, Hb F, Hb A2 and other hemoglobin's were evaluated and also genetic tests applied for them to confirm results that were acquired by capillary electrophoresis method.

Results: 77 (19.7%) subjects had HbA2 ≥3.5%, thus were classified as beta thalassemia carrier and 3.3%, 2.5%, 1.5% and 0.5% of the individuals were heterozygote for Hb S, Hb D, Hb C and Hb Bart, respectively. Results of the genetic analysis showed the mutations in these subjects; cd36-37(-T) was the most frequent mutation in beta thalassemia carriers in this geographic region.

Conclusion: This study showed high frequency of beta thalassemia mutations in the (19.7), and 7.85% of the individuals had hemoglobin variants including Hb S, Hb D and Hb C detected by capillary electrophoresis. Capillary electrophoresis could be used for detection of hemoglobinopathies.

Keywords: HPLC, Hemoglobin disorders, Capillary Zone Electrophoresis.

Introduction

Hemoglobinopathies are inherited hemoglobin disorders caused by mutations of the globin genes. Globin chain which contained four polypeptide chains; these chains are of four types: α, β, δ, and g. Each hemoglobin chain consists of two pairs of different form of globin chains. The hemoglobin disorders fall into two main groups: the structural hemoglobin variants and the thalassemia syndromes. Single nucleotide substitutions can lead to hemoglobin variants or hemoglobinopathies. 96-98% of the Hb is Hb A (α2β2) in normal adults, with small amounts (23.5%) of HbA2 (α2δ2) and about 1.5 % of Hb F (α2g2).[1] about 500,000 infants are born with a severe hemoglobin disorder annually and approximately 7% of the total population carries an inherited Hb disorder gene according to recent statistics.[2] At present up to 1000 hemoglobin variants have been registered.[3] The most common worldwide hemoglobin variants are such as Hb S, Hb C, Hb E and Hb D-Punjab.[4] Likewise, other hemoglobin variants such as D, S, C, Lepore, CS, Q, J and other hemoglobin's have been reported in many countries.[5]

B-thalassemia is commonly observed in individuals of Mediterranean, African, and Southeast Asian ancestry. The gene frequency of ß-thalassemia mutations is high in Iran and varies from area to area. In southern Iran, the gene frequency is also high and is about 8-10%.[6] As a result, diagnosis of hemoglobin variants and thalassemia has become increasingly important in clinical laboratories. The guidelines of the British Committee for Standards in Hematology was accepted with numerous techniques for the screening and detecting hemoglobinopathies have been developed such as cellulose acetate electrophoresis (CAE), isoelectric focusing (IEF), low-pressure liquid chromatography (LPLC), high-performance liquid chromatography (HPLC), capillary zone electrophoresis (CZE) and finally genetic

analysis.[7] Evaluation of hemoglobinopathies Cellulose acetate method is a common method; however, differentiation of Hb variants with low concentrations, especially unstable hemoglobin can be difficult.

HLPC is a useful method for screening and diagnosis of hemoglobinopathies, but interference of glycated Hb S and Hb E with HbA2 quantitation may result in incorrect diagnosis of beta thalassemia in the presence of glycated Hb S and Hb E.[8,9] In 2007, Food and Drug Administration (FDA) approved the Capillary zone electrophoresis system for the evaluation of hemoglobinopathies.[10]

Reliable evidence has indicated that CZE may be an accurate tool for the screening and diagnosis of hemoglobinopathies. You-Qiong and colleagues analyzed adult and cord blood sample of patients heterozygous for Hb New York by using both CZE and HPLC. Interestingly, all cases could be diagnosed with CZE, whereas none of them could be detected by HPLC.[11]

Material and Methods

This study was carried out on 395 individuals (55%men and 45% women) that were from clinical departments. EDTA vacutainer tubes were used for collection of blood samples, then CBC including blood indexes (MCV, MCH) were performed and hemoglobin A, Hb F, Hb A2 and other hemoglobin variants were evaluated by capillary electrophoresis (CE). The instrument is equipped to re-suspend, lyse, separate, and analyze EDTA whole blood for hemoglobin variants. Samples were tracked using a built-in bar code reader and electropherograms were produced automatically. The lysed red cells are electrophoresed in alkaline buffer (pH 9.4) allowing separation to be directed by pH and endosmosis. Using the change in absorbance 415 nm hemoglobin species was eluded. An electropherogram is divided into 15 zones that each zone is entitled as Z.[12] Then genetic analysis including, ARMS-PCR, RFLP- PCR and sanger sequencing applied for confirming the results of CE method.

Results

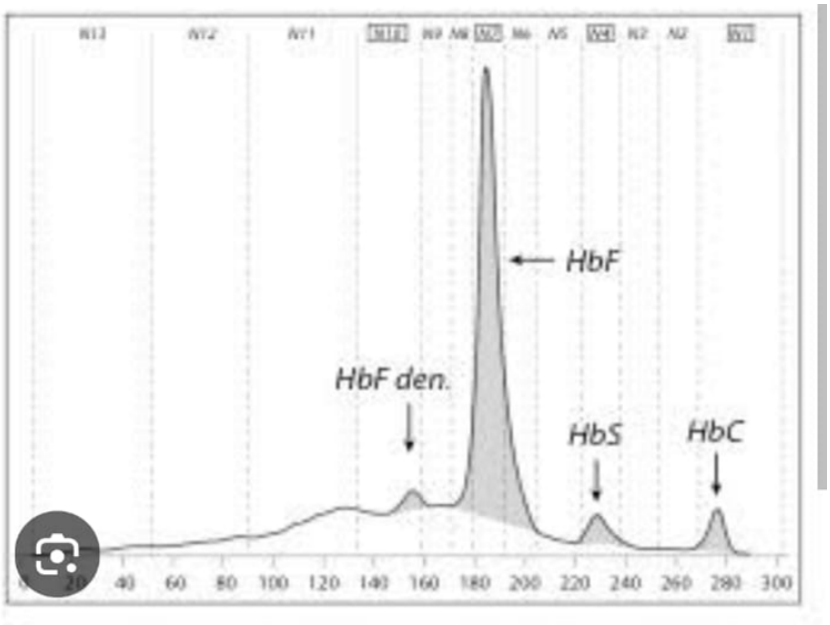
77 of 395 samples (19.7%) showed Hb A2 >3.7 %, thus were classified as beta thalassemia carrier state. 74(18.9%) of them had MCV<80.0 fL, MCH<27.0 pg, but 3(0.78%) had normal blood indexes (MCV, MCH). The genetic analysis revealed various mutations in minor beta thalassemia; the most frequent was cd 36-37(-T) (table 1). There were individuals who were heterozygote for Hb S, Hb D, Hb C and Hb Bart while their blood indexes were in normal range (table 2). The genetic analysis for these variants showed mutations in the β-globin gene, HBB: c.20A>T), HBB: c.67G> C and HBB:c.19G>A in Hb S, Hb D and Hb C, respectively. The subjects with Hb A2 < 3.7% could be suspected of having a thalassemia or iron deficiency or association of β thalassemia with iron deficiency

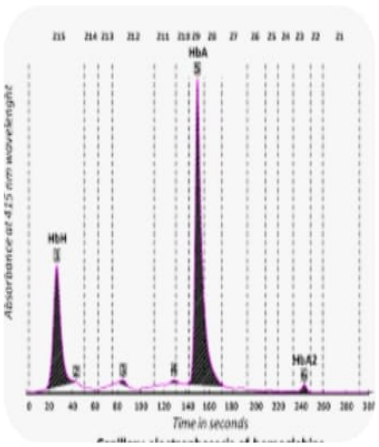
Table 1: Blood Indexes and mean and standard deviation of hemoglobin variants

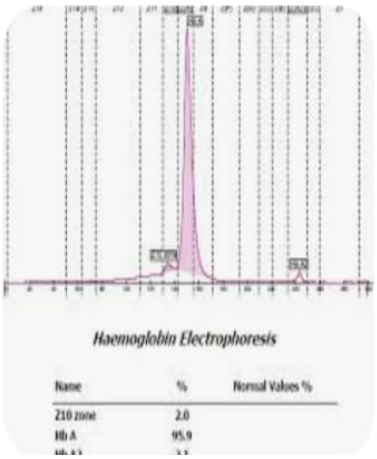
Indexes of blood	Hemoglobin variants	Mean percent ±SD	Frequency No (%)
MCV≥ 80,MCH≥ 27	Hb S	16.24±3.9	12 (3.6)
MCV≥ 80,MCH≥ 27	Hb D	35.67±13.8	10 (2.55)
MCV≥ 80,MCH≥ 27	Hb C	8.16±2.48	4 (1.57)
MCV<80,MCH< 27	Hb Bart	1.3±.53	2 (0.6)
Total			28 (7.87)

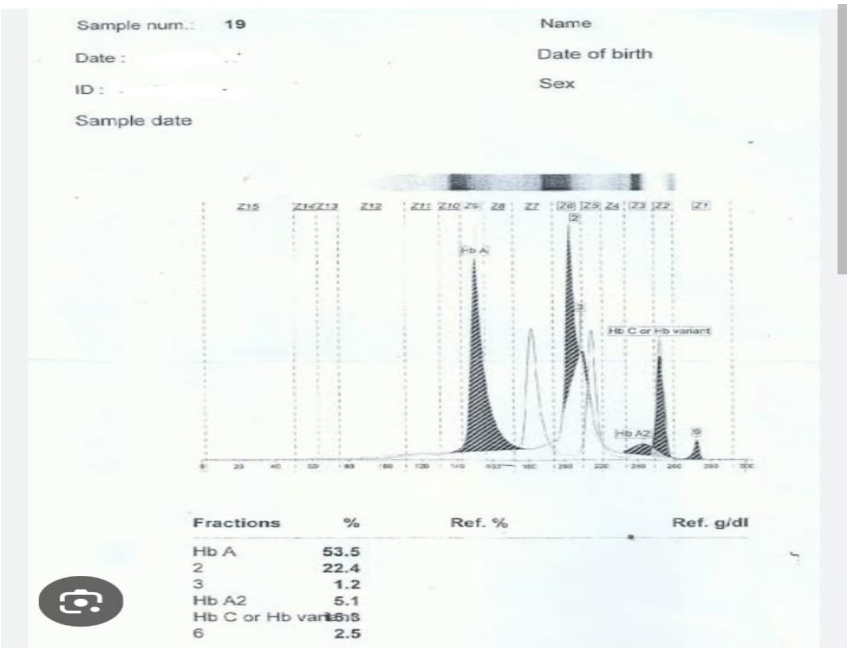
Table 2: Frequency of Mutations of Beta thalassemia

Type of mutations of beta thalassemia	Number	Frequency
cd 36-37(-T)	28	36.39%
IVSII-1 (G>A)	16	20.78%
IVSI-110(G>A)	11	14.30%
Cd 82-83(-G)	8	10.39%
5UTR+20(C>T)	6	7.80%
IVS II-745(C>G)	4	5.22%
cd82-83(-G)	3	3.93%
Fr 8.9	1	1.31
Total	77	100%


HbF
HbF den.
HbS
HbC
40 60 80 100 120 140 160 180 200 220 240 260 280 300


HbA
HbH
HbA2
Absorbance at 415 nm wavelenght
Time in seconds


Haemoglobin Electrophoresis
Name
%
Normal Values %
Z10 zone
2.0
Hb A
95.9


Sample num.: 19
Name
Date :
Date of birth
ID :
Sex
Sample date
Hb A
Hb C or Hb variant
Hb A2
Fractions
%
Ref. %
Ref. g/dl
Hb A 53.5
2 22.4
3 1.2
Hb A2 5.1
Hb C or Hb variant
6 2.5

Discussion

Hb D could be seen in combination with sickle hemoglobin and beta thalassemia. Co-inheritance of beta-thalassemia and Hb D together can result in the slightly lower hemoglobin levels[13-14].

Hb S results from a point mutation in beta globin chain gene. Sickle cell disease is very frequent in southern of Iran, especially in Khuzestan province that sickle cell trait is usually asymptomatic with normal RBC indexes.[15] On the other hand, measurement of Hb A2 is challenging because its level could be low and also interference with other hemoglobin variants would change the quantity of Hb A2 and since molecular techniques are not routinely used in many medical laboratories, so that the most unknown hemoglobin variants may not be correctly diagnosed.[16]

The aim of this study was screening of hemoglobin abnormalities by using of capillary zone electrophoresis. In this study 19.5% of the individuals were classified as beta thalassemia carriers. 3.3%, 2.5%, 1.5% and 0.5% of the subjects were heterozygote for Hb S, Hb D, Hb C and Hb batrs, respectively. The numbers approximately were similar to the study performed by Joshaghani and colleagues in North of Iran. In that study, Hb electrophoresis was carried out by capillary electrophoresis and 0.27%, 4.68%, 55%,0.27% and 0.41% were recorded for Hb E, Hb D, Hb S, Hb H and Hb Bart, respectively. In that study, Hb D had a higher frequency than our study.[17] We did not have any case of Hb E in our samples.

Zandian et al. studied frequency of alpha and beta thalassemia mutations and hemoglobin C, D, and S in Ahvaz. Hemoglobin S was the most frequent hemoglobinopathy that was similar to our study.[13]

In another study which was performed in Ahwaz, the frequency of alpha and beta thalassemia and other hemoglobinopathies was investigated. The Results of their study showed the frequency of Hb S, D, C, and α–globin gene mutations to be 16.2%, 3.2%, 1%, and 9.7%, respectively which again was similar to the present study.[18]

In the present study, we used capillary zone electrophoresis for detection of hemoglobinopathies. Recent studies have illustrated that capillary electrophoresis separates HbA2 well from Hb E, Hb C, and Hb S and is suitable for screening. Cellular acetate electrophoresis is routinely used in clinical laboratory; however, is not much accurate. On the other hand, HPLC method is costly and not routinely available. It can achieve simultaneous analysis, fast separation, good resolution high accuracy, and full automation. Furthermore, capillary electrophoresis also is capable to separate Hb A2 from Hb Lepore than the HPLC method.[19]

kim et al. compared the capillary electrophoresis method with cellulose acetate method for screening of hemoglobinopathies. The study was performed in two groups, one group with normal CBC and the other group were subjects with hypochromia and microcytosis. No statistically significant difference was found for Hb quantification (P>0.05). The study indicated that capillary electrophoresis was more sensitive than cellulose acetate for detecting Hb fractions.[20]

Higgins and coworkers analyzed evaluation of Hb A2 in patients with and without beta-thalassemia, and assessed heterozygous patients for Hb E, Hb S, Hb C and Hb D Punjab by using of capillary system. The results of this study demonstrated that the capillary method is superior to the Variant II method for HbA2 quantified measurement.[21]

Conclusion

This study showed high frequency of beta thalassemia mutations in the (19.5), and 7.85% of the individuals had hemoglobin variants including Hb S, Hb D and Hb C detected by capillary electrophoresis. Capillary electrophoresis could be a considerable method for detection of hemoglobinopathies

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