



Estimation and Correlation of Different Hemoglobin Levels in HbE Hemoglobinopathies in Indian Population Using Capillary Electrophoresis Method

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ABSTRACT

Background: Capillary electrophoresis (CE) estimates Hemoglobin E (HbE) in HbE hemoglobinopathies, which was previously not possible with other method due to combined elution of HbA₂ with HbE. Associated hemoglobin abnormalities can be estimated with separation of HbA₂ from HbE.

Methods: The study is retrospective using CE to detect abnormal HbE and differentiate the HbE syndromes. Student t-test was used for statistical analysis.

Result: 119 cases of HbE syndromes were identified and divided into HbE heterozygotes (71), HbE heterozygotes with borderline HbA₂ (15), compound heterozygotes of HbE with Thalassemia (13 HbE with β -Thalassemia / 8 HbE with α -Thalassemia), compound heterozygotes of HbE with HbS (HbSE) (2) and HbE homozygotes (10). Mean HbA₂ shows increasing pattern with increasing severity of HbE syndrome. However, compound heterozygote of HbE and β -Thalassemia (HbE- β -Thal) shows maximum mean level of HbA₂ (5.46%). HbA₂ of HbE heterozygote and HbE heterozygote with borderline HbA₂ was not found to be significantly different, statistically. Fetal haemoglobin (HbF) of HbE homozygotes is found significantly higher than that of HbE heterozygotes, but significantly lower than that of HbE-Thalassemia. The HbE values of HbE with α -Thalassemia (HbE- α -Thal) and HbE- β -Thal were found to be below -3SD value (14.77%) and beyond +3SD value (37.77%) of mean of HbE of HbE heterozygote (26.27%), respectively.

Conclusion: The study identifies range of different hemoglobin levels in HbE syndromes, with special reference to HbA₂. Concurrent iron deficiency anaemia also needs to be kept in mind in dealing with a population where iron deficiency is very common.

Keywords: Capillary Electrophoresis, Hemoglobin E heterozygote, Hemoglobin E

Introduction

Hemoglobin E (HbE) was the fourth abnormal hemoglobin described ⁽¹⁾. HbE is the second most prevalent hemoglobinopathy after Sickle cell hemoglobinopathy (HbS), showing the highest prevalence in South-East Asia ⁽²⁾. In India, it is prevalent in the north-eastern states ^(2,3,4,5). Heterozygosity and homozygosity of HbE are clinically mild, whereas compound heterozygosity for HbE and HbS (HbSE) and compound heterozygosity for HbE and β -Thalassemia (HbE- β -Thal) are clinically severe ^(3,6,7,8,9). The co-inheritance of HbE with a host of other globin mutants results in a wide spectrum of hemoglobinopathies with varying degrees of severity (HbE disorders or HbE syndromes) ^(1,10).

All the methods used previously were not able to distinguish between HbE and HbA₂. While reverse phase HPLC has been reported to provide an estimate of HbA₂ in the presence of HbE, this method is not routinely used in

clinical laboratories ⁽¹¹⁾.

Capillary electrophoresis (CE) has the ability to completely separate HbA₂ from HbE ^(12,13,14). This enables to quantitate exact levels of HbE and HbA₂, assisting in identification of co-inherited hemoglobinopathies.

The present study is conducted to identify levels of different hemoglobins in HbE syndromes and to find their correlation, if any. Most of the studies conducted previously on hemoglobinopathies in the Indian population have highlighted the increased prevalence of HbE and associated syndromes. This study attempts to define the range of abnormality in the HbE syndromes.

Materials and Methods :

The present study is a retrospective study conducted for a period of one year (June 2015 to May 2016). Capillary electrophoresis method was used for identification of abnormal hemoglobin variants. Patients with abnormal



HbE peaks and without any treatment history (particularly of blood transfusion) were included in the study, to have an accurate estimation of the hemoglobin levels. Cases with abnormal hemoglobin E peaks were correlated with the Complete blood count (CBC) and peripheral blood smear (stained with Leishman's stain) to conclude a final diagnosis. Cases were classified as HbE heterozygotes, HbE homozygotes and compound heterozygotes of HbE with other hemoglobinopathies (like sickle cell and Thalassemia). Another category, HbE with borderline HbA₂, was classified in the present study, to find out if any significance exists. The mean values of HbA₂ were compared statistically (using student t-test) amongst the HbE categories / syndromes, and also with control group of 30 normal individuals and 30 β -Thalassemia cases.

Result

A total of 119 cases of HbE syndromes were identified during the study period, out of which 71 were HbE heterozygotes, 15 were HbE heterozygotes with borderline HbA₂, 21 were compound heterozygotes of HbE with Thalassemia (13 with β -Thalassemia and 8 with α -Thalassemia), 2 were compound heterozygotes of HbE with HbS and 10 were HbE homozygotes. The distribution of the cases into the different categories, along with levels of HbA₂ are shown in Table 1.

On statistical analysis, there was no significant difference (p -value >0.05) between the mean value of HbA₂ in HbE heterozygotes, HbE heterozygotes with borderline HbA₂, and normal control group. The mean of HbA₂ in

compound heterozygotes of HbE with Thalassemia (both α -Thalassemia and β -Thalassemia combined) was found to be significantly higher (p -value <0.05) than that of HbE heterozygotes, HbE heterozygotes with borderline HbA₂ and HbE homozygous, but there is no significant difference (p -value >0.05) with that of β -Thalassemia control group. Compound heterozygotes of HbE with HbS was not considered for statistical analysis due to small size. There is an increasing trend in the mean value of HbA₂ from HbE heterozygote to HbE homozygous, as shown in Table 1. However, the mean of HbA₂ in HbE- β -Thal exceeds that of HbE homozygotes.

The mean value of HbE was found to be 26.27% in HbE heterozygotes, 31.08% in HbE heterozygotes with borderline HbA₂ and 83.67% in HbE homozygotes. The compound heterozygotes of HbE with Thalassemia have been divided into two groups - HbE- β -Thal (Mean HbE level 51.97%) and HbE- α -Thal (Mean HbE level 16.65%). Although the sample size for HbSE was too small for calculation, however, the mean HbE in HbSE was calculated to be 9.75%.

The fetal hemoglobin (HbF) level (Table 2) was found to be increased (than normal range in adults) in compound heterozygotes of HbE- α -Thal (Mean 19.71%), compound heterozygotes of HbE with β -Thalassemia (Mean 9.28%), compound heterozygotes of HbE with HbS (Mean 4.9%) and HbE homozygotes (Mean 5.41%). It was within normal range in HbE heterozygote (Mean 0.48%) and HbE heterozygote with borderline HbA₂ (0.36%).

Table 1: HbA₂ levels in different HbE syndromes.

SI no.	Type of HbE (with total number of cases)	HbA ₂ level(%) (Mean, $\pm 2SD$)
1	HbE heterozygote (71)	2.98, ± 0.8
2	HbE heterozygote with borderline HbA ₂ (15)	3.76, ± 0.31
3	Compound heterozygote of HbE with β -Thalassemia (13)	5.46, ± 2.48
4	Compound heterozygote of HbE with α -Thalassemia (08)	3.10, ± 1.64
5	Compound heterozygote of HbE with HbS (2)	3.1, ± 0.56
6	HbE homozygotes (10)	3.52, ± 2.0

Table 2: Fetal haemoglobin (HbF) level in HbE syndromes

SI no.	Type of HbE (with total number of cases)	HbF level(%) (Mean, $\pm 2SD$)
1	HbE heterozygote (71)	0.48, ± 1.68
2	HbE heterozygote with borderline HbA ₂ (15)	0.36, ± 1.28
3	Compound heterozygote of HbE with beta-Thalassemia (13)	19.71, ± 26.60
4	Compound heterozygote of HbE with alfa-Thalassemia (8)	9.28, ± 7.84
5	Compound heterozygous of HbE and HbS (2)	4.9, ± 6.50
6	HbE homozygotes (10)	5.41, ± 6.17

Discussion

The CE method allows detection and separation of most of the common hemoglobin variants ^(15,16). The complete separation of HbA₂ from HbE deserves note ⁽¹⁷⁾.

The data obtained from 117 cases shows an increasing trend of the level of HbA₂ in the HbE syndromes with increased severity of HbE. However, the level of HbA₂ in HbE-β-Thal is significantly higher than all other categories, including HbE homozygous. This trend is similar to the study conducted by Mais DD *et al* ⁽⁶⁾, but the significant increased level of HbA₂ seen in HbE heterozygotes compared with that of normal control group in the study of Mais DD *et al* ⁽⁶⁾, is not seen in the present study. Moreover, the category defined in this study “HbE with borderline HbA₂” also does not have significant difference of HbA₂ level from the normal control and HbE heterozygous categories. The study conducted by Keren DF *et al* ⁽¹⁷⁾ suggested that the levels of HbA₂ are over – estimated by capillary electrophoresis method but the study also argues that in HbE syndromes, relative increase in HbA₂ levels is expected, because HbE is a structural form of beta - Thalassemia. The range defined for HbA₂ for HbE heterozygotes in the present study (Table 1) is lower than that described by Mais DD *et al* ⁽⁶⁾ (Mean ± 2Sd, 3.4% ± 0.4%) and Winichagoon P *et al* ⁽¹⁸⁾ (Mean ± 2SD, 3.5% ± 0.4%). However, the range of HbA₂ defined for HbE heterozygous with borderline HbA₂ is found to be in comparison with the mentioned studies. This can be attributed to concurrent iron deficiency anaemia, particularly in the Indian population, which lowers the levels of HbA₂, as evidenced by studies of Rao S *et al* ⁽¹⁹⁾ and Denic S *et al* ⁽²⁰⁾.

The fetal hemoglobin (HbF) of HbE homozygotes is found to be significantly higher than that of HbE heterozygotes, but significantly lower than that of HbE - Thal. The level of HbF inversely correlate well with proportion of HbA in the haemoglobin variant. In HbE homozygous, however, the HbE levels lowers the HbF levels, inspite of low HbA level (Mean HbA 7.4%). HbF is affected significantly by alfa / Beta - globin chain imbalances, as evidenced by study of Lin WF *et al* ⁽²¹⁾.

The mean of HbE levels of HbE heterozygote in the present study was found to be 26.27% ± 7.6% (Mean ± 2SD) which is in comparison with the study of Charoenkwan P *et al* ⁽²²⁾ (23.3% ± 6.2%) and Hafiza A *et al* ⁽²³⁾ (24.28% ± 6.76%). The HbE values of HbE with α – Thalassemia were found to be below the 3SD value (14.77%) of the mean of HbE level of HbE heterozygote (26.27%), with few exceptions. However, in those cases where HbE values are within 3SD of mean of HbE, the low hemoglobin level and the

peripheral blood picture were suggestive of an associated Thalassemic abnormality. Again, the HbE levels of HbE-β-Thal were beyond the 3SD value (37.77%) of the mean of the HbE heterozygote. This is in correlation with the study of Mais DD *et al* ⁽⁶⁾.

Only two cases of HbSE were encountered during the study period, the mean of sickle peak being 23.55% and HbE 9.75%, with mean HbF peak being 4.9%. The peripheral blood picture showed elongated cells, suggestive of the sickle abnormality. However, blood transfusion history was elucidated in these patients, so exact quantification of the different hemoglobin levels were not possible. Inclusion of more number of cases is required for further statistical analysis.

Conclusion

The present study identifies the range of different hemoglobin levels in HbE syndromes, particularly with reference to HbA₂ level, which helps to detect associated abnormalities. The concurrent iron deficiency anaemia also needs to be kept in mind in dealing with a population where iron deficiency is very common. Genetic studies need to be performed, particularly in compound heterozygotes, to define the exact range of HbE levels with each gene deletion.

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