Hemoglobin E Syndromes: Emerging Diagnostic Challenge in North India

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Abstract Hemoglobin E (HbE) is one of the world's most common and important mutations. HbE disorders may be found in heterozygous (AE), homozygous (EE) and compound heterozygous state. It is important to distinguish HbE disorders diagnostically because of marked differences in clinical course among different genotypes. To find out whether RBC indices as obtained from automated cell counter can provide a clue to the diagnosis of HbE disease. This study was carried out in the Department of Clinical Pathology, PGIMER, Dr. Ram Manohar Lohia Hospital, New Delhi. It included antenatal pregnant females brought for routine check-up as well as referred patients suspected of having hemoglobinopathies. High Performance liquid chromatography was used as a confirmatory test for identification of hemoglobinopathy. Total 20 cases of subtype homozygous HbE (3), HbE trait (12) and E β -thalassemia (5) were identified. Statistical analysis was done to find out correlation between levels of HbA₂, HbF with RBC indices. (a) There was negative correlation between HbA₂/E peak values and RBC indices (Mean corpuscular volume (MCV) and Mean corpuscular hemoglobin) among all the three groups taken together. (b) There was positive correlation between HbA₂/E and Red cell distribution width (RDW). (c) There was positive correlation between HbF values with MCV. The finding of positive correlation between HbA₂/E and RDW may help in differentiating

β thal (RDW normal) from HbE/ β thal. In a patient with microcytic hypochromic blood picture and increased RDW, diagnosis of HbE/ β thal should also be considered along with the more common Iron deficiency anemia. Thus, new insights into the knowledge of these diseases are important because they impart diagnostic challenges to all the experts involved in the treatment of anemic patients.

Keywords Hemoglobinopathy · HPLC · RBC indices · Hemoglobin E · Thalassemia

Introduction

Thalassemia and other structural hemoglobinopathies are the major genetic disorders prevalent in certain parts of the world including India. Hemoglobin E (HbE) is one of the world's most common and important mutations. It is important to distinguish HbE disorders diagnostically because of marked differences in clinical course among different genotypes [1]. HbE disorders may be found in heterozygous (AE), homozygous (EE) and compound heterozygous state (e.g. HbE with other abnormal hemoglobins or thalassemias) with widely variable clinical phenotypes [2].

The importance of HbE lies in the fact that

- patients with HbE have microcytic hypochromic picture which may be confused with thalassemia or initial stages of iron deficiency.
- patients who inherit both genes for HbE and β -thalassemia have severe anemia as is seen in thalassemia major.

Population screening, genetic counseling and prenatal diagnosis can prevent these genetic disorders as it has been a success in various other developing nations [3].

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Materials and Methods

This study was carried out in Department of Clinical Pathology, PGIMER, Dr. Ram Manohar Lohia Hospital, New Delhi. It included antenatal pregnant females brought for routine check-up as well as referred patients suspected of having thalassemia or other hemoglobinopathies. A complete hemogram with RBC indices was performed on automated cell counter, Medonic CA620/530. High Performance liquid chromatography (HPLC) using VARIANT™ β -thalassemia short program (Bio Rad Laboratories, Hercules, CA, USA). HPLC was used as a confirmatory test for identification of hemoglobinopathy in the study. A total of 20 cases of HbE disease, HbE trait and E β -thalassemia were identified and a comparative study was performed between levels of HbA2, HbF and RBC indices including RBC counts, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Packed cell volume (PCV) and Red cell distribution width (RDW).

Each type of hemoglobin has a characteristic retention time. HPLC uses the principle of cation exchange, based on differential retention time [3]. HbE, an abnormal Hb is interpreted by analyzing the HbA2% as HbE has the same retention time as HbA2 on HPLC but it can be distinguished by its high concentration. If the HbA2/HbE peak is greater than 3.5% but less than 10%, the patient is a beta thalassemia carrier i.e. HbE is not involved. If the A2 peak is greater than 10% and HbA is present, the patient can be E-trait or E/beta positive. If the patient is E/beta positive, HbA% is less than HbE. If the HbA2/E peak is greater than 10% and HbA is not present, the patient is homozygous E(EE) or E/ β 0 [4]. In case this patient has a high HbF%, it will suggest E/ β 0. Homozygous E(EE) are healthy and exhibit no clinical symptoms.

Results

Twenty cases (i.e. 0.878% out of total 2,164 cases) showed abnormal HbA2/E peak in this retrospective study. It included antenatal pregnant females brought for routine check-up as well as referred patients suspected of having thalassemia or other hemoglobinopathies. Out of these 20 cases, 12 cases were of HbE trait, 3 cases of homozygous

HbE and 5 cases were of E- β thalassemia respectively. An attempt was made to see if any correlation exists between levels of HbA2/HbE, HbF and RBC indices including RBC counts, MCV, MCH, MCHC, PCV and RDW.

The levels of HbA2/HbE, HbF and RBC indices between three groups of patients are shown in Table 1.

On statistical analysis, there was negative correlation between HbA2/E peak values and RBC indices like MCV and MCH among all the three groups taken together (Figs. 1, 2). There was positive correlation between HbA2/E and RDW (Fig. 3). There was no statistical correlation between values of HbA2/E and Hb level, RBC counts and PCV.

Correlation was found between values of HbF with MCV in our study (Fig. 4), while no correlation was seen with values of MCH, Hb level, RBC count and RDW.

Discussion

HbE is the most common Hb variant in South East Asia and second most common hemoglobin variant worldwide [1]. In India, HbE is mostly restricted to North East India, with an average allele frequency of 10.2% [2]. However rare case report from other parts of India including North India is also reported [5]. Hemoglobinopathies which were mainly confined to certain areas, religions, castes and tribes are now prevalent all over the world as a result of endogamous marriages and also because of ever increasing migration of people from one place to another [2].

Blood counts, hemoglobin, RBC indices, HPLC and DNA analysis are the various diagnostic modalities which are used worldwide to assess the prevalence of thalassemia and other hemoglobinopathies. HPLC provides effective separation of hemoglobins which gives an accurate quantitation of HbA2 and HbF and detects large majority of Hb variants [5].

Thalassemia and hemoglobinopathies are autosomal recessive disorders, primarily affecting globin moiety of Hb molecule [2]. HbE is caused by a substitution of glutamic acid by lysine at codon 26 of the β globin gene. This mutation also activates a cryptic mRNA splice site; which results in reduced synthesis of the β E chain and lead to a thalassemic phenotype [1].

Table 1 The mean values of Hb, RBC count, MCV, MCH, MCHC, RDW, PCV and levels of HbA0, HbA2 and HbF are given in a tabular form

Mean values	HB	RBC	MCV	MCH	MCHC	RDW	HbA0	HbA2/E	HbF	PCV
E hetero	11	4.52	74.7	25.9	33.6	18.6	62.7	27.24	0.792	33.0
E/ β thal	7.28	3.8	60.66	19.28	31.76	35.76	10.58	54.24	31.92	23
E homo	8.97	4.43	58.9	20.3	34.3	22.3	5.23	83.17	5.2	26.13

Fig. 1 Correlation between HbE and MCH is -0.6517 . Significance test that slope is zero resulted in a t value of -3.4990 ; significance level of this t test is 0.001 (<0.05)

Fig. 2 Correlation between HbE and MCV is -0.6574 . Significance test that slope is zero resulted in a t value of -1.358 ; significance level of this t test is 0.002 (<0.05)

It is important to distinguish HbE disorders as they have marked differences in their clinical course. Heterozygotes and homozygotes for HbE are microcytic, minimally anemic and asymptomatic. The microcytosis is attributed to the β -thalassemic nature of the β E gene, whereas the in vitro instability of HbE does not contribute to the phenotype [5]. The compound heterozygote state HbE/ β -thalassemia results in a variable and often severe anemia with the

phenotype ranging from transfusion dependence to a complete lack of symptoms [6].

Only one study by Kishore et al. [5] is available showing the correlation between HbA2/E levels with RBC indices. It was conducted in the same region of Indian continent as the present study. Both the studies have shown a negative correlation of HbA2/E values with MCV and MCH among all the three groups taken together [5]. Thus indicating that

Fig. 3 Correlation between HbE and RDW is 0.332. Significance test that slope is zero resulted in a t value of 0.866; significance level of this t test is 0.0002 (<0.05)

Fig. 4 Correlation between HbF and MCH is -0.567 . Significance test that slope is zero resulted in a t value of -1.919 ; significance level of this t test is 0.001 (<0.05)

microcytosis and hypochromasia is commonly seen with elevated HbA2 levels.

The characteristic finding of positive correlation between RDW and HbA2/E levels in HbE/ β thal group in our study was striking. The mean RDW value was higher in HbE/ β thal 35.76% (± 7.2). This observation may help in differentiating β thal from HbE/ β thal. RDW is usually within normal range in thalassemia minor [7].

Thus, in a patient with microcytic hypochromic blood picture with increased RDW, diagnosis of HbE/ β thal should also be considered along with the more common Iron deficiency anemia. Iron deficiency also lowers the HbE percentage [1]. Though iron studies were not done in our patients as HPLC for hemoglobin variant analysis was done as a part of routine antenatal checkup or for thalassemia screening. All those patients with abnormal

HbA2/E peak on HPLC were specifically picked up and analysed. However, the authors believe that iron studies should be done in all such cases to rule out any coexisting iron deficiency anemia.

Diagnosis of concomitant alpha thalassemia requires DNA testing. The concomitant inheritance of alpha thalassemia often occurs and lowers the percentage of HbE [1]. In our set up, DNA testing was not possible.

A study by Eldibany et al. [8] has discriminated normal, α -thalassemic, β -thalassemic and iron deficiency patients based on a set of linear discriminant functions including MCH, RBC count, MCV and RDW. However, this study does not include HbE syndromes.

There was no statistical correlation found between values of HbA2/E with Hb level, RBC counts and PCV which is in accordance with Kishore et al. However, Nuchprayoon et al. have documented that high RBC count (>4.4) and very low MCV (<69 fl) is a sensitive and highly specific (100%) criteria for diagnosis of mild thalassemia diseases (HbH, HbH-CS and homozygous HbEE) [9]. The reason may be due to the selective study of homozygous HbEE by Nuchprayoon et al. [9].

In our study, negative correlation was found to exist between values of HbF with MCH with no statistical correlation with Hb level, RBC counts and MCV which is contrary to the finding of Kishore et al. [5]. This may be due to the wide variation in clinical presentation and blood findings which are seen in HbE disorders.

Thus, our study documents that RBC indices as obtained from automated cell counter can provide a clue to the diagnosis of hemoglobinopathy and the type of hemoglobinopathy can be confirmed by HPLC.

Ittarat et al. [10] in their study concluded that identification of HbE especially the heterozygous form by using parameters from an electronic cell counter is not easy. Discriminant functions and red cell indices might be used as an initial diagnosis. But confirmation is needed in all cases.

Conclusion

In summary HbE disorders are a heterogeneous group of diseases that are rapidly increasing worldwide. In India, due to increasing awareness, it may gradually become a health problem as its prevalence will increase in areas other than the north eastern region. Thus, new insights into the knowledge of these diseases are important because they impart diagnostic challenges to all the experts involved in the treatment of anemic patients.

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