Research Article

A Clinico-Haematological study of hemoglobin E disease and trait

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Abstract

Introduction: HbE (Haemoglobin E) is one of the most important and common haemoglobinopathy. Its definitive diagnosis can be made on capillary electrophoresis. Our study aimed to analyze the clinico-haematological profile of patients having haemoglobin E disease and trait, including findings on capillary electrophoresis and iron profile. **Materials and Methods:** The samples were taken from patients who were referred to the haematology section of Department of Pathology, Silchar Medical College from January 2013 to December 2013. These patients were suspected of having haemoglobinopathy based on Peripheral Blood Film and Complete Blood Count. Study of complete haematological, eletrophoretic (by Capillary electrophoresis) and iron profile of the patients was done. **Result*:*** In our study, abnormal haemoglobins were detected in 61 out of the 100 cases examined, out of which HbE was detected in 45 cases. These patients presented with an asymptomatic to symptomatic phenotype, a decrease in mean corpuscular volume, microcytosis and target cells, a normal iron profile and increased HbE as well as HbA2 (Haemoglobin A2) levels on Capillary electrophoresis. ***Conclusion:*** Haemoglobin E constitutes an important haemoglobinopathy in lower Assam. An important finding was raised HbA2 (usually <6% on) capillary electrophoresis due to the β-thalassemic nature of HbE mutation. It needs to be differentiated from double heterozygous HbE-β thalassemia cases, as they also have elevated HbA_2 levels (usually >6%) along with raised HbF levels. Therefore a proper diagnosis is essential so that preventive measures could be undertaken to reduce the burden of this haemoglobinopathy.

Keywords: Electrophoresis, Haemoglobin A2, Haemoglobin E, Haemoglobinopathies, HbE-β thalassemia.

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Introduction

Haemoglobinopathies are caused by changes (mutations) in the coding, non-coding or regulatory sequences of the globin genes. When mutations abolish or reduce the expression of α or β globin genes, diseases are termed α and β thalassemia respectively. When mutations cause structural changes in the globin genes, they induce abnormal haemoglobins or Hb variants. Abnormal haemoglobins may be clinically silent or associated with severe disorder [1].

Haemoglobin E [HbE] is one of world's important and common mutations resulting from substitution of glutamine by lysine at codon 26 of β-globin gene ($\alpha_2\beta^{26Glu->Lys}$). It is one of the most common variant of normal haemoglobin, which can be detected on ***electrophoresis***. Since its first description by Chernoff and his colleagues in 1954 [2], HbE is increasingly reported from several parts of the world.

It is the most prevalent abnormal haemoglobin in South East Asia with its frequency approaching 60% in Northeast regions of Thailand, Laos and Cambodia.

Significant numbers were reported from other Asian countries such as Sri Lanka, North Eastern India [3], Bangladesh, Nepal, Vietnam and Malaysia. Additionally, population transmigration has led to its emergence in United States and Canada.

Assam has a mixed population consisting of original natives, Bengalis, immigrants from S-E Asia, Aryans, immigrant Oryahs comprising the Tea Garden Labourers and migrating population from other states.

Manuscript received 14th July 2016
Reviewed: 26th July 2016
Author Corrected: 10th August 2016
Accepted for Publication 25th August 2016

In Assam, HbE is reported to have high incidence in Ahoms, Kacharis, Totos, and also in Khasis of neighbouring Meghalaya. The heterogenous population in Assam has made this study of abnormal haemoglobin more complicated and interesting for workers from different fields, mainly clinicians, geneticians and anthropologists [4].

HbE variants usually manifest as homozygous (HbE disease), heterozygous (HbE trait), and double heterozygous forms as ***HbE-β thalassaemia*** & HbE-sickle cell.

This study which was conducted at Silchar Medical College and Hospital (Assam) in 2013 was the first of its kind, as such a study had never been done in this region before.

In summary, HbE disorders are a heterogeneous group of diseases that are rapidly increasing worldwide. The etiology for the marked variation in phenotype remains largely unknown.

Correct laboratory diagnosis is essential to separate asymptomatic genotypes from severe mutations. Comprehensive care, including genetic counseling, psychological support and access to new therapy, are important for all patients.

Materials and Methods

The study was performed in the Department of Pathology, Silchar Medical College and Hospital from January 2013 to December 2013.

The samples were taken from patients referred to the haematology section, who were suspected of having haemoglobinopathy based on Peripheral Blood Film (PBF) and Complete Blood Count (CBC) report (done on Sysmex 5 part automated haematology analyzer). Samples were collected in both EDTA vials (for CBC, PBF & Electrophoresis) and clotted vials (for Iron studies).

Inclusion criteria

1. Patients showing various stigma of haemo-globinopathy in CBC and PBF like microcytosis, target cells and unexplained anaemia.

2. Both male and female patients of all age groups, religions, and ethnicity were included.

Exclusion criteria: Patients found to have haemoglobinopathy other than HbE trait and HbE disease on Capillary electrophoresis excluded from the study.

Study of complete haematological, eletrophoretic and iron profile of the patients was done. The complete blood count was done in a 5 part automated analyser, which gives 22 parameters. It works on the principle of impedance and optical methods. PBF was examined in all the cases.

Haemoglobin electrophoresis was done by fully automated Sebia Capillary Electrophoresis machine in cases showing stigma of haemoglobinopathy on CBC and PBF. The sebia capillary electrophoresis system uses the MINICAP system.

The MINICAP system uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific P^H.

Separation also occurs according to the electrolyte P^H and electro-osmotic flow. This machine can differentiate between 24 different haemoglobin variants.

The Serum Ferritin, Serum Iron, TIBC and Transferring Saturation levels were measured by Beckman Coulter fully automated analyzer.

Result

A total of 100 cases suspected to have a haemoglobinopathy (on the basis of CBC and PBF), were taken up for capillary electrophoresis.

39 of these cases were found to have other causes of anemia such as Iron deficiency anaemia and were excluded. Of the 61 abnormal haemoglobins so obtained, 35 cases of HbE trait and 10 cases of HbE disease were found.

Rest of the 16 cases found to have other haemoglobinopathies (like sickle cell anemia, β-thalassemia) were excluded from study. HbE was observed to be the most common abnormal haemoglobin in this population. (Table1)

Table-1: Distribution of various abnormal haemoglobins in the study group.

Funding	Cases (n=61)	Percentage (%)
HbE trait	35	57.37
HbE disease	10	16.39
Thalassemia minor	8	13.11
HbE thalassemia	3	4.91
Thalassemia major	2	3.27
HbS trait	2	3.27
HbS disease	1	1.63
Total	**61**	**100**

We defined our results under the following sections:

Presenting signs and symptoms: Generalized weakness with easy fatiguability was the most common symptom of patients with HbE trait as well as disease. Exertional dyspnoea was the second most common symptom. Palpitation, giddiness, headache and yellowing of skin were seen in relatively less number of patients of both trait and disease.

Pallor was the most common sign in patients with HbE trait, and was observed in all the patients of HbE disease. Icterus was the second most common sign observed. Splenomegaly was seen in 20% cases of HbE disease (Table 2).

Table-2: Distribution of cases according to presenting signs and symptoms.

Sign/Symptom	HbE Trait cases (%)	HbE Disease cases (%)
Generalized weakness	71.4	100
Easy fatigability	71.4	100
Exertional dyspnoea	14.2	40
Pallor	71.4	100
Icterus	8.5	40
Splenomegaly	0	20

Hematological Parameters: In patients with ***HbE trait***, mean haemglobin was found to be 12.2 ± 1.0 g/dl. The values ranged from 10.4 to 14.6 g/dL. The mean RBC value was 5.25 ±0.6 million/mm^3. The mean HCT was 35.0±3.9 %, slightly lower than normal. MCV was decreased in majority of patients, with the mean value being 71.3±5.7 fl. MCH was also decreased with mean value being 23.7 ±2.5 pg. MCHC values were in the normal range in most of the patients. The WBC and platelet count were also within normal range in most patients.

In patients with ***HbE disease***, mean RBC count was found to be 5.74 ±0.5 million/mm^3 which is slightly higher than normal. The Hb values were decreased ranging from 10.1 g/dl to 12.6g/dl with mean of 11.2 ± 0.7g/dl. The mean HCT was 35.2±2.2%. The MCV was greatly decreased in all patients of HbE disease, ranging from 51.0 to 65.5 fL with a mean of 57.6±4.7 fl. MCH values were also decreased ranging from 19.7 to 23.8 pg with mean of 22.0±1.1 pg. MCHC was normal in majority of the cases. WBC and Platelet counts were within normal range. (Table 3)

Table 3: Haematological parameters

Haematological Parameters[Mean values]	HbE Trait	HbE Disease
Haemoglobin	12.2 ± 1.0 g/dl	11.2 ± 0.7g/dl.
RBC count	5.25 ±0.6 million/mm^3	5.74 ±0.5 million/mm^3
Haematocrit	35.0±3.9 %	35.2±2.2%.
Mean Corpuscular Volume	71.3±5.7 fl	57.6±4.7 fL
Mean Corpuscular Haemoglobin	23.7 ±2.5 pg	22.0±1.1 pg
Mean Corpuscular Haemoglobin Concentration	In normal range	In normal range

RBC Morphology: Most of the patients (43 out of 45) showed microcytosis. Anisopoikilocytosis was seen in over 50% cases of HbE trait and majority of patients with HbE disease. Target cells (Figure 1) were seen in almost all the cases.

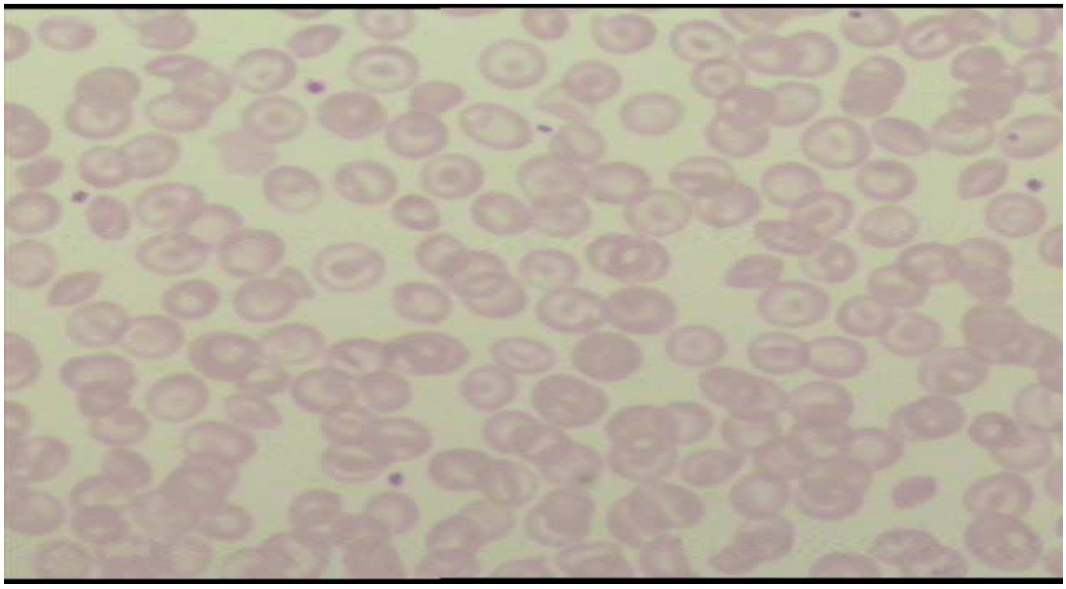

Figure-1: Target cells in HbE disease

Iron Profile: The Serum Ferritin levels were within normal limits for all the patients. Likewise the TIBC, Serum Iron and percentage saturation levels were also within normal limits for patients of HbE diease and trait.

Range of HbE (%) in HbE trait and disease as determined by capillary electrophoresis: HbE values in HbE trait ranged from 10.4%-30.2% with a mean of 22.7±3.9%; while in HbE disease it ranged from 80.5%-94.6% with a mean of 89.4±4.2% on capillary electrophoresis.

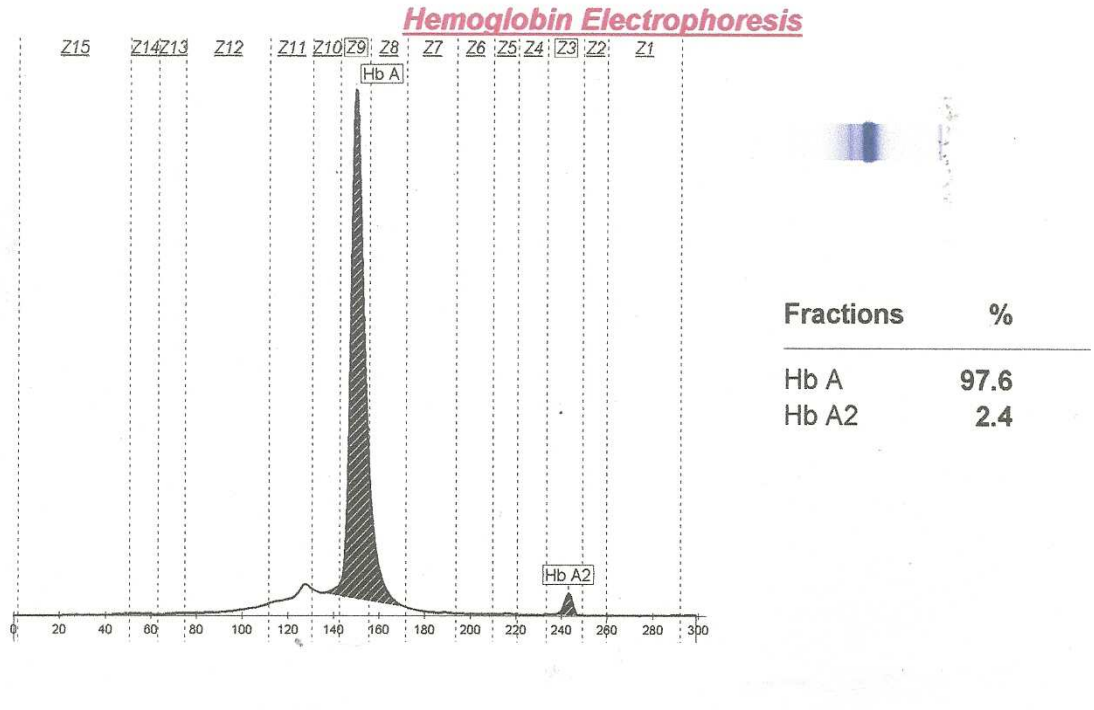

Figure-2: Normal pattern on capillary electrophoresis

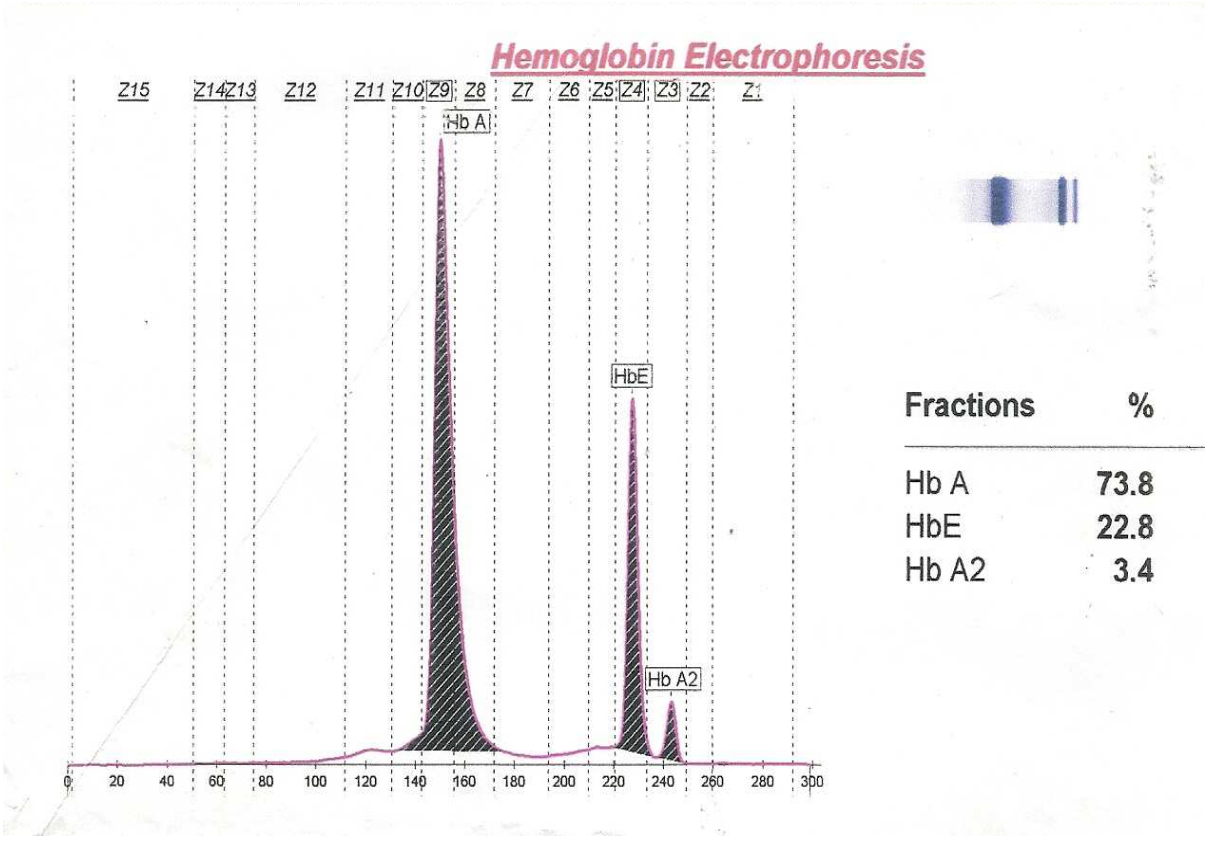

Figure-3: HbE trait on capillary electrophoresis

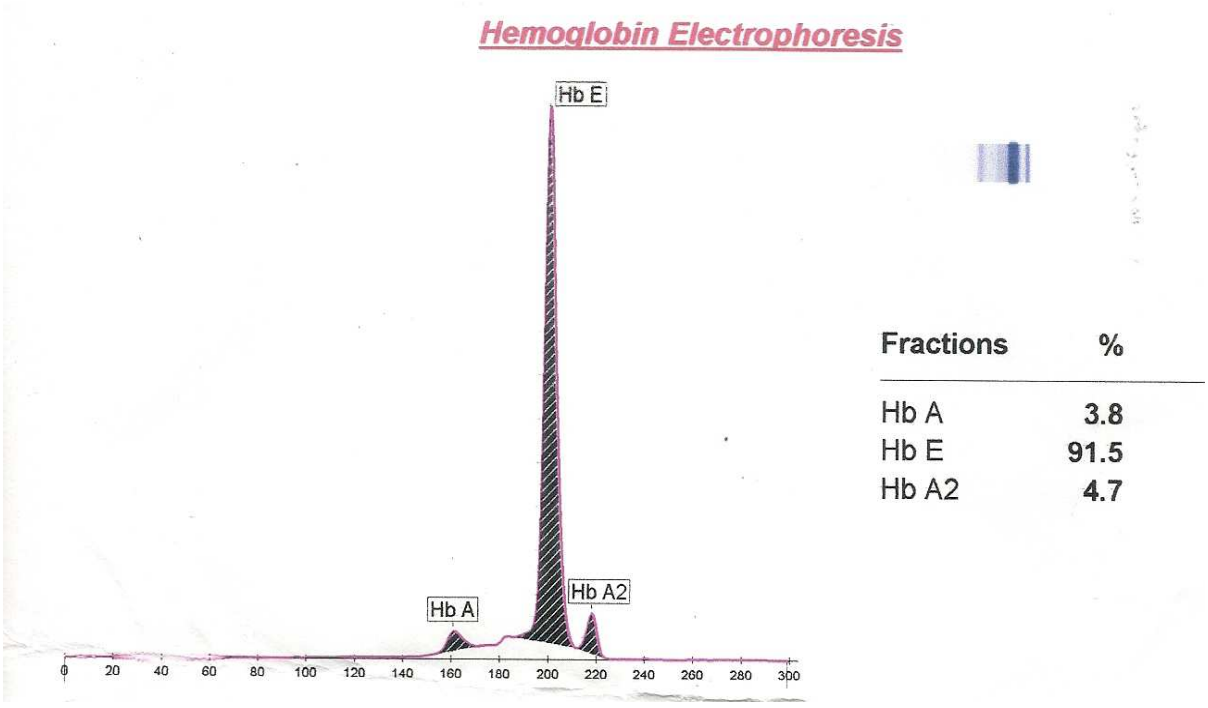

Figure-4: HbE disease on capillary electrophoresis

Range of HbA_2 as determined by capillary electrophoresis: Haemoglobin A_2 values in HbE trait ranged from 1.0%-3.8% with a mean of 3.1%±0.4 which was slightly raised than normal, while in HbE disease it was all the more raised, ranging from 4.4%-5.7% with a mean of 4.8%±0.4. (Figure 2,3,4)

Discussion

Haemoglobinopathy is a very important group of diseases in tropical countries. There are many different types of haemoglobinopathies and among them some types are frequently encountered in this part of the world (North Eastern India). Its severity can range from congenital haemolytic anemia, to anemia in children or adult; with many symptoms to completely asymptomatic carriers. HbE constitutes an important haemoglobinopathy in north-eastern states of India, including Assam.

In the present study abnormal haemoglobins were detected in 61 cases out of the 100 cases examined. Out of these, HbE (HbE trait= 35, HbEE disease=10) was detected in 45 cases. The present study showed high incidence of HbE in this region, in which 35 cases were HbE trait and 10 cases of HbE disease. This was in confirmation with earlier studies by different workers. According to Singh et al [5], HbE is the most popular haemoglobin variant in Southeast Asia as well as in Northeast India. Prevalence of HbE in high frequencies (0.6455) among the BodoKachari and other Tibeto-Burman speaking populations of Assam is a mind-boggling subject now.

Clinical Findings: Pallor was present in 71.4% of patients with HbE trait and all the patients of HbE disease. Icterus was found in 8.5% of HbE trait & 40% case of HbE disease. Splenomegaly was found in 2 cases of HbE disease in the present study. In the study by Aggarwal et al 2011[6] out of the 10 cases of HbE disease and 16 cases of HbE trait from a total of 60 cases, 8 cases of HbE disease and 10 cases of HbE trait presented with variable signs and symptoms of anemia, the rest being diagnosed incidentally while they were investigated for some other ailment/illness. In a study by Vichinsky, HbE disease patients were occasionally found to have splenomegaly.[7] Clinically HbE is a mild type of disorder both in homozygous and heterozygous states therefore Hb E individuals are minimally anemic and asymptomatic.[12]

Haematological Findings: HbE trait: The average value in the present study for haemoglobin was 12.2 ±1.0 g/dl, 5.25 ± 0.6 million/cumm for RBC count, 35.0 ± 3.9 for HCT, 71.3 ± 5.7 fl for MCV, 23.7 ± 2.5 pg for MCH and 32.6 ± 1.2 g/dl for MCHC. Aggarwal et al in 2011, found mean value of Hb to be 11.1g/dL in HbE trait [6]. Fairbanks et al 1980[8], Cunningham 1982[9], Craft 1983[10] found a normal haemoglobin, PCV (HCT), mild erythrocytosis and microcytosis in HbE trait. In another study by Winichagoon et al 2008[11] HbE heterozygote groups had higher RBC concentrations and lower mean corpuscular volume and mean corpuscular haemoglobin ($P < 0.05$). Findings in the present study are more or less consistent with the earlier findings.

Peripheral blood film in the present study showed mild anisocytosis and poikilocytosis and target cells. Microcytosis on peripheral blood has been described by other authors also. Aggarwal et al also [6] found microcytic hypochromic picture with aniso-poikilocytosis in HbE trait.

HbE disease: The present study showed that the patients with HbE disease had mild reduction in haemoglobin with a mean Hb value of 11.2±0.7 g/dl. The RBC count was slightly increased, with a mean value of 5.74±0.5 million/cumm. The Haematocrit value (PCV) was reduced for most of the patients with a mean of 35.2 ± 2.2%. The MCV value was reduced for most of the patients. The mean value was 57.6 ± 4.7fl. The MCH was also reduced with a mean value of 22.0±1.1 pg. MCHC was normal with a mean of 32.9±2.2 g/dL. The WBC and platelet counts were also normal for all patients. According to a study by Vichinsky, the red cell morphology show targeting, and Hb EE individuals are asymptomatic with very mild anemia and microcytosis [7]

Lachant (1987)[12] described HbE homozygous to have on an average of 1g/dl less haemoglobin concentration than that seen in heterozygotes; and MCV averages about 5-10 fl less than HbE trait.

Iron profile of the patients with HbE disease and trait : Several studies have shown in the past that the HbE trait and disease patients usually do not develop a state of iron overload. In the study by Winichagoon et al (2008)[11] it was found that the HbE heterozygotes did not differ from the control subjects in any of the iron indices. In the study by Aggarwal et al (2011)[6], Iron profile was normal in all patients with HbE trait and disease. In our study also iron profile was normal in all patients as none of the patients needed blood transfusion and did not have a state of iron overload, which was consistent with the findings of above studies.

Range of HbE (%) in HbE trait and disease as determined by capillary electrophoresis: In our study, HbE values in HbE trait ranged from 10.4%-30.2% with a mean of 22.7±3.9% while in HbE disease it ranged from 80.5%-94.6% with a mean of 89.4 ±4.2%. Moiz et al 2012[13] found Haemoglobin E values ranging from 16.4 to 30.6% (mean ± SD; 25.9 ± 4.3) in HbE trait and a high HbE (> 78%) in HbE disease.

In the study by David et al (2009)[14], the mean HbA_2 of patients with HbE trait was 3.4% (SD, 0.4%), which was significantly higher ($P < .001$) than the 2.6% (SD, 0.4%) for the control group. Seven samples were assayed from subjects who were homozygous for HbE and their mean HbA_2 was 4.4% (SD, 0.4%), which was significantly greater ($P < .001$) than the HbA_2 values for HbE heterozygotes.

Range of HbA_2 as determined by capillary electrophoresis: An interesting property unique to capillary electrophoresis is its ability to separate HbE from HbA2, a feature that was not seen in many of the widely used methods before. HbE migrates with HbC, HbO and HbA2 in alkaline electrophoresis and HPLC. Even at acid electrophoresis, HbE is still inseparable from HbA2. Although this seldom causes any diagnostic problem, the ability to separate HbE from HbA2 does give additional information such as the actual levels of HbA2 and HbE in HbE heterozygous and homozygous cases.[15].

In our study HbA_2 value in HbE heterozygotes ranged from 1-3.7% with a mean of 3.1±0.4 and that of HbE homozygotes was raised ranging from 4.4-5.7% with mean of 4.8±0.4 making it consistent with the observation of raised HbA_2 levels. The raised HbA_2 value is due to the β-thalassemic nature of the HbE mutation which not only causes a structural change by substitution of glutamine by lysine at codon 26 of β-globin gene but also cause a decreased production of the variant β-globin chain raising A_2 levels. Due to the raised HbA_2 levels such cases may be confused with double heterozygous

HbE-Thalassemia cases and so need to be differentiated from them as double heterozygotes also have elevated HbA_2 levels (usually >6%) increased along with raised HbF levels. In a study by Prasing and Pornprasert, levels of HbA2 measured by CE in HbE-β thalassemia were significantly higher than those of homozygous HbE disease.

Moreover, all HbE-β thalassemia patients had HbA2 levels higher than 6% while only 1 of 19 (5%) homozygous HbE patients had HbA2 levels higher than the cutoff. These results suggest that the occurrence of α and δ-globin chains in HbE-β thalassemia disease was higher than that in homozygous HbE [16].

Conclusion

Haemoglobin E (both disease and trait) constitutes an important haemoglobinopathy in people of lower Assam with patients presenting clinico-haematologically with asymptomatic to symptomatic phenotype, a decrease in MCV on CBC, microcytosis and target cells on PBF and a normal iron profile. An important finding on Capillary Electrophoresis in patients of HbE disease (HbEE) is the elevated HbA_2 value of >3.5% (which earlier could not be known as the traditional electrophoretic methods could not separate HbE from HbA_2). Increased HbA_2 level on Capillary electrophoresis is due to the β-thalassemic nature of the HbE mutation. Due to the raised HbA_2 levels such cases may be confused with double heterozygous HbE-Thalassemia cases and so need to be differentiated from them as double heterozygotes also have elevated HbA_2 levels [usually >6%] along with raised HbF levels.

Therefore, a proper diagnosis is essential to distinguish it from other haemoglobinopathies and so that genetic counseling and other preventive measures can be undertaken to reduce the burden of HbE in this part of the world.

Acknowledgements: We acknowledge the technical staff of Haematology lab of Silchar Medical College and Hospital.

Funding: Nil, **Conflict of interest:** None initiated,
Permission from IRB: Yes

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How to cite this article?

Bhargava P, Datta D, Bhattacharjee S. A Clinico-Haematological study of hemoglobin E disease and trait. *Int J Med Res Rev* 2016;4(9):1543-1550.doi:10.17511/ijmrr.2016.i09.05.

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