Original Article

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HbE Variants: An Experience from Tertiary Care Centre of Eastern India

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ABSTRACT

Background: HbE is the second most common structural haemoglobin disorder after sickle cell haemoglobin (HbS). Haemoglobin E (HbE) is variant haemoglobin with a mutation in the globin gene causing substitution of glutamic acid for lysine at position 26 of the globin chain.

Aims & Objectives: 1)To Know demographical parameters of HbE variants and 2) To know the Hematological parameters of HbE variants.

Materials & Methods: The present single-centre, retrospective, cross-sectional study was carried out on consecutive 2035 Hb-E variants who were screened by CE-HPLC for hemoglobinopathies in the Thalassemia control unit (TCU) in our tertiary care referral centre after obtaining the proper approval from ethical committee of the institution and informed consent from the patients.The evaluation was done in Bio-Rad Variant Haemoglobin Testing System (Hercules, California, USA) using variant b-thalassemia short program pack. All the analyses were done using IBM SPSS statistics software, version 19 and MedCalc software, version 12.3.0.0.

Result: Among 2035 subjects' majority (80%) were diagnosed as HbE carrier.Age ranged from 1 year to 75 years with mean of 19.1±13.2 years. Study population mostly comprised of antenatal mothers (23.2 %) followed by premarital (20.2%), children (18.1 %), post marital (17.4%), %), family member of affected person (16.6%), suspected patient (3.5 %) family member of carrier (0.2%) and others (0.8%).

Conclusion: HbE disorders are paradox: its behaviour ranges from good, bad to ugly and the value of HbE does not correlate with the severity of the disease

Keywords: *HbE Disease, HbE Carrier, HbE Beta Thalassemia, HPLC.*

Introduction

Southeast Asia is the region with heterogenous group of haemoglobinopathies among which HbE is the hallmark.[1] Highest prevalence is noted in in some parts of Thailand and Cambodia and in Laos followed by in Sri Lanka, North Eastern India, Bangladesh, Pakistan, Nepal, Vietnam, and Malaysia.[3-5] Structural abnormality resulting from substitution of glutamic acid for lysine at position 26 of the globin chain resulting in this divergent condition with a wide spectrum of clinical manifestations. Heterozygous state (genotype AE or haemoglobin E trait), Homozygous state (EE or haemoglobin E disease) are mostly asymptomatic. On the contrary, Compound heterozygous states such as haemoglobin E/β Thalassemia (E/β thal), sickle cell/haemoglobin E disease (SE genotype) reveals clinical presentation ranging from that of thalassemia minor to major.[6] Globally the compound heterozygous forms of beta thalassemia rarely led to major health burden apart from the case of haemoglobin E beta thalassemia. Sparse literatures are available on HbE variants from eastern part of India. Therefore, we have conducted this study to know demographical variables and the haematological parameters of HbE variants.

Methods & Materials

Study Design

The present single-centre, retrospective, cross-sectional study was carried out on consecutive 2035 Hb-E variants among all cases screened by CE-HPLC for hemoglobinopathies in the Thalassemia control unit (TCU) in our tertiary care referral centre. Proper approval from the ethical committee of the institution was taken and informed consents were obtained. The study population included respondents from different districts of our state along with adjacent states too. Data of all the subjects during the study period of 5 years and 6months starting from January 2014 to June 2019 were retrieved from our database which was maintained and organized by the Linux-based Thalamon software (Venus IT Solutions). Although no absolute exclusion criteria were used but sampling was deferred for at least 4 weeks after or just before next transfusion in patients requiring blood transfusions. Common haematological parameters were

measured with an automated haematology analyser (KX-21, Sysmex Corporation, Japan). Red cell morphology and platelet counts were crosschecked with well-prepared peripheral blood films. In addition to haematological profile, relevant demographic factors like age, sex and respondent categories were analysed. All the abnormal variants were corroborated with red cell morphology, indices, ethnicity and family history.

Blood Sampling and HPLC Procedure

The evaluation was done in Bio-Rad Variant Haemoglobin Testing System (Hercules, California, USA) using variant II Beta-thalassemia short program pack consisting of elution buffer 1 (sodium phosphate), elution buffer 2 (sodium phosphate), whole blood primer (lyophilized human red blood cell hemolysate with preservatives), haemolysis reagent (deionized water), wash solution (deionized water), HbA2/F calibrator/diluent set (lyophilized human red blood cell hemolysate with deionized water), sample vials, ROM (read-only memory) card, cation exchange analytical cartridges and CD-ROM. The Bio-Rad Variant II is a fully automated CE-HPLC system to separate and determine area percentages for haemoglobin A2 and F and to provide qualitative determinations of abnormal haemoglobins. 1–2 ml of whole blood samples were collected in EDTA vials and were stored at 2–8 ^{0}C. EDTA anticoagulated 5 µlwhole blood samples were mixed with 1.0 ml of haemolysis reagent to each sample vial and were analysed in batches. The prepared samples were injected sequentially into the analysis stream at 6.5-min intervals and separated by the cation exchange cartridge using a phosphate ion gradient generated by mixing two buffers of different ionic strengths to elute the different haemoglobins. HbA2/F calibrator and two level controls were analysed at the beginning of each run. A dual-wavelength filter photometer analysed the haemoglobin elution from the cartridge by detecting the absorbance changes at 415 nm and the secondary filter at 690 nm corrected the baseline for effects caused by mixing buffers with different ionic strengths. Different peaks of different haemoglobins in defined windows with their retention time, relative percentage and area displayed in a chromatogram of absorbance versus time (Fig. 1). Total acceptable area of each analysis ranged from 1 to 3 million volts.

Statistical analysis: Continuous variables were expressed as mean ± standard deviation (SD). Categorical variables were presented as frequencies and percentages. All the analyses were done using IBM SPSS statistics software, version 19 and MedCalc software, version 12.3.0.0.

Result

A total of 2035 subjects were included among which 1628 (80%) were diagnosed as HbE carrier, 118 (5.8%) were HbE disease and 289 (14.2%) were diagnosed as E-beta thalassemia. over a period of 5 years and 6 months. Female preponderance was documented with male: female ratio of 1.8:1. But on the contrary HbE disease and E beta thalassemia are more frequent among Males. An uneven year wise distribution was noted specially in the number of HbE carriers. Age ranged from 1 year to 75 years with mean of 19.1±13.2 years. Most of the abnormal cases were first time presented in 2nd decades and HbE-beta thalassemia revealed early disease manifestation (13.40±12.51 years). Study population mostly comprised of antenatal mothers (23.2 %) followed by pre marital (20.2%), children (18.1 %), post marital (17.4%), %), family member of affected person (16.6%), suspected patient (3.5 %) family member of carrier (0.2%) and others (0.8%).[Table 1]

MCV, MCH and MCHC were inversely proportional to HbE levels but RDW is directly proportional. All haematological parameters revealed significant difference between three categories. [Table 2] Haemoglobin level is markedly diminished in E beta thalassemia, moderately decreased in HbE disease or mildly decreased or normal in HbE carrier. HbE disease and E beta thalassemia are more frequent among Males. Among total study population (2035) HbE carriers are of 81.4%, HbE disease shows 4.2% and E beta thalassemia are 14.4%. [Figure1-3]

Discussion

HbE is most prevalent in South East Asia which includes North East India with a carrier frequency of 40% in some region.[6] It is an autosomal recessive disorder resulting from substitution of glutamic acid by lysine at codon 26 of beta globin gene. Although a single point mutation is responsible for development of this hemoglobinopathies but being an autosomal recessive disorder it can manifests with a remarkable phenotypical heterogeneity. At one pole, the homozygous and heterozygous form are majorly asymptomatic in contrast to the compound heterozygous form which present with clinical picture indistinguishable from thalassemias major.[7] Previous articles suggest that oxidative stress and high temperature leads to precipitation of unstable HbE which resulting in red cell damage.[8] But isolated HbE produces minimal morphological abnormality and thus does not contribute in overt clinical manifestation. Now it is accepted that coinheritance of α or β thalassemia with HbE in the form of compound heterozygous cause clinical syndrome of variable severity specially in South East Asia due to complex interaction. [9,10] Higher frequency was reported from North-east India including West Bengal apart from little sparse literature from other part of India. [11-15]

Complete haemogram along and high-performance liquid chromatography helps in exact categorisation of

Table 1: Distribution of the study population (n = 2035) depending on various demographic factors.

Parameters	Total	E Carrier	E Disease	E-Beta Thalassemia	P Value
No Of Cases In Each Year					
2014	396	317	19	60	<0.001
2015	350	291	16	43	
2016	333	266	10	57	
2017	211	166	9	36	
2018	464	386	16	62	
2019	281	231	15	35	
Age					
Mean±Sd	19.10±13.27	23.28±11.14	24.97±13.35	13.40±12.51	<0.001
Minimum	1	1	1	1	
Maximum	75	75	61	70	
Sex					
Male	763	547	61	155	0.0484
Female	1272	1081	57	134	
Respondant Category					
Antenatal Mother	475	452	13	10	0.0016
Children	369	212	21	136	
Post Marital	353	287	29	37	
Premarital	410	357	23	30	
Suspected Patient	70	18	6	46	
Family Member of Affected Person	338	286	24	28	
Family Member of Carrier	3	3	0	0	
Other	17	12	2	3	

One way ANOVA test was performed

Table 2: Assessment of haematological parameters in different groups of patients.

DIAGNOSIS	NUMBER	HB LEVEL (g/ dl)	HCT %	RBC COUNT	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW
E-Beta Thalassemia	**289**	**6.2 ± 1.5**	**22.3 ± 4.0**	**3.15 ±**	**61.5 ± 10.0**	**17.8 ± 3.1**	**29.6 ± 2.1**	**28.2 ± 5.5**
HBE Disease	**118**	**9.5 ± 2.7**	**28.4 ± 6.1**	**4.66 ±**	**62.7 ± 5.8**	**20.1 ± 2.5**	**30.5 ± 2.1**	**19.8 ± 3.9**
HB E Carrier	**1628**	**11.4 ± 1.7**	**35.2 ± 5.5**	**4.68± 0.67**	**80.0 ± 5.7**	**24.6 ± 2.5**	**32.9 ± 1.7**	**15.82± 6.1**
P value		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

One-way ANOVA test was performed

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	6.0*	---	1.07	96818
P3	---	5.3	1.78	82663
Ao	---	9.2	2.18	144022
A2	84.2*	---	3.59	1248044

Total Area: 1,571,547

F Concentration = 6.0* %
A2 Concentration = 84.2* %

*Values outside of expected ranges

Analysis comments:

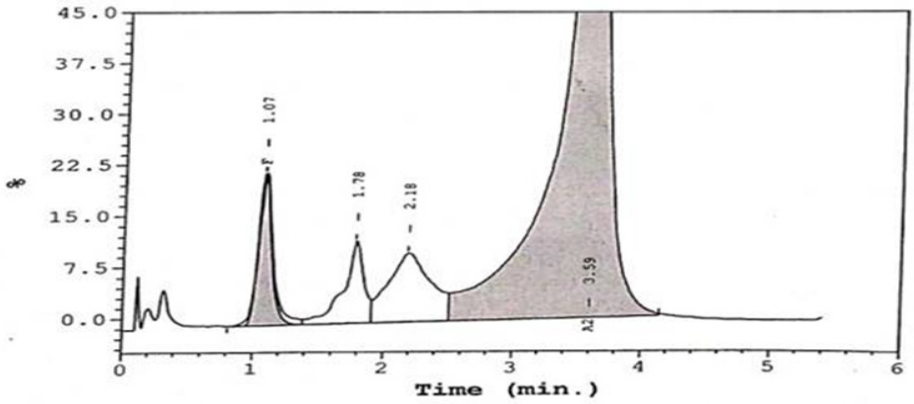

Fig. 1: CEHPLC chromatogram of HbEE disease.

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	1.6*	---	1.06	24366
Unknown	---	0.9	1.17	13045
P2	---	2.5	1.31	36243
P3	---	2.1	1.66	30865
Unknown	---	2.1	1.79	30033
Ao	---	64.3	2.40	940643
A2	28.4*	---	3.55	386950

Total Area: 1,462,144

F Concentration = 1.6* %
A2 Concentration = 28.4* %

*Values outside of expected ranges

Analysis comments:

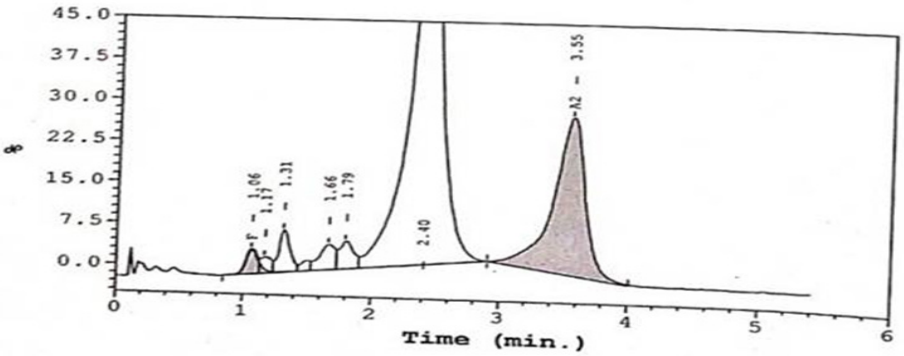

Fig. 2: CEHPLC chromatogram of HbE carrier.

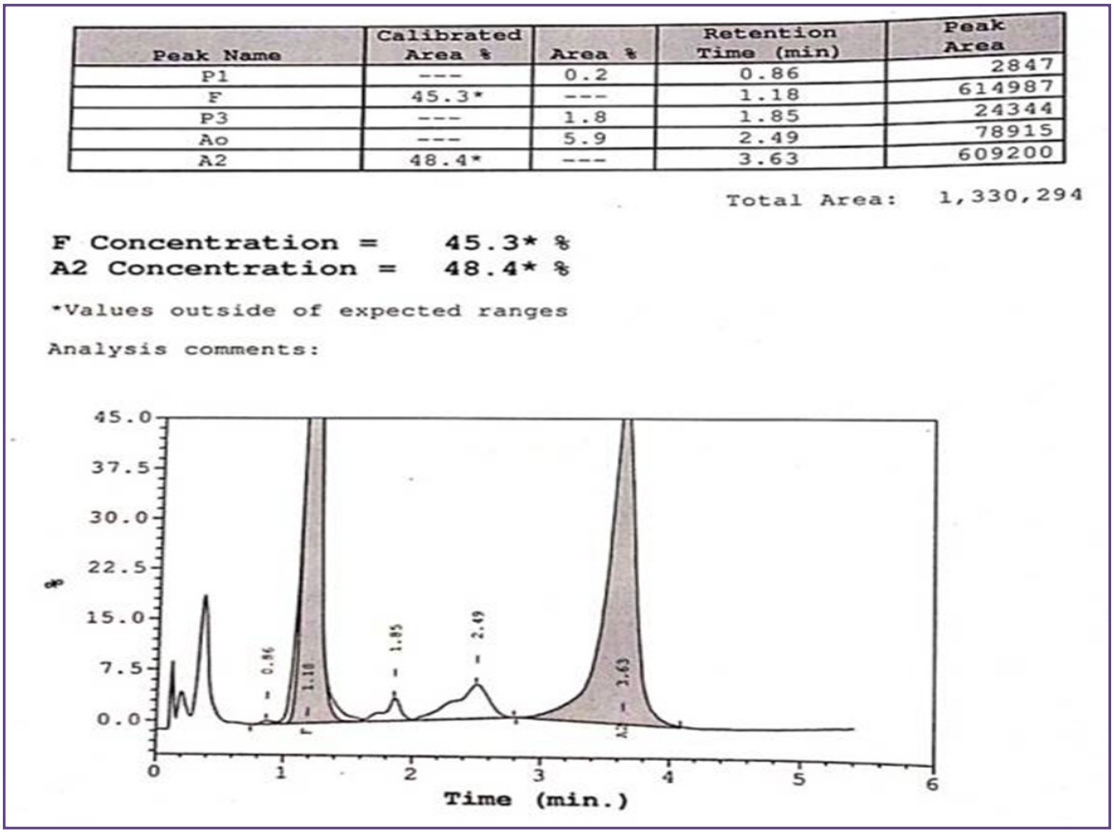

Fig. 3: CEHPLC chromatogram of HbE-beta thalassemia.

haemoglobinopathies. Mild anaemia and minimal reduction in red cell indices were observed in both homozygous and heterozygous forms. The compound heterozygous form demonstrated moderate to severe reduction in haemoglobin level and red cells parameters. Automated cell counter Discrimination indices are rapid and easily accessible modality to suspect beta thalassemia trait. But in case of HbE, there are no such indexes available to differentiate it from normal individual on complete blood count. Only HPLC with special emphasis on HbA2/E level along with HbF value is the reliable tool for identification of each category. Literatures are available regarding correlation studies of HbE/F level with RBCs parameters like MCV, MCH and RDW but those were non-contributory. [16]

HbE disorders can coexist with α or β thalassemias and iron deficiency anaemia. HPLC study can diagnose HbE/ β thalassemia but not α thalassemias. DNA study is required for further analysis of this case. Like other hemoglobinopathies iron study is essential to rule out falsely low values of RBCs parameters specially in rural and antenatal population.

So HbE disorders are paradox: it behaviour ranges from good, bad to ugly and the value of HbE does not correlate with the severity of the diseases. Homozygous form with highest HbE level is asymptomatic majorly in contrast to the coinheritance with other haemoglobinopathies.

Conclusion

To conclude, RBC indices, HPLC findings and family study are sufficient to detect and differentiate between the HbE variants. However, we need to be aware of the limitations and problems associated with the diagnostic methods in order to avoid false negative cases. Genetic studies are very helpful in borderline cases. The present study reflected the magnitude of the burden of HbE variants in our tertiary care hospital based population and can be of help in increasing awareness among the health care providers as well as the general population.

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Competing Interests

Nil

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