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ORIGINAL ARTICLE

HEMOGLOBIN E DISORDERS IN SOUTH GUJARAT – A STUDY OF 35 CASES

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ABSTRACT

Background: Among the inherited disorders of blood, hemoglobinopathies and thalassemia constitute a major bulk of non-communicable genetic disease in India. Most commonly found abnormal hemoglobins in India are hemoglobin S (Hb S), hemoglobin E (Hb E) and hemoglobin D (Hb D). The distribution of Hb E ($\alpha_2\beta_2^{26Glu\square Lys}$) is mostly restricted to north-eastern India and it is relatively rare in rest of the country. Identification of this disorder is immensely important epidemiologically and aid in prevention of more serious hemoglobin disorder.
Aims: Thepurpose of the study is to highlight importance of identification of Hb E disorders and prevention of doubly heterozygous state for Hb E and β-thalassemia which may be falsely characterized clinically by thalassemia major.
Material and Method: This study is a part of the work done under Sickle Cell Anemia Control Programme, under which samples are tested for various routine as well as specific tests such as dithionite tube turbidity test (DTT Test), hemoglobin electrophoresis and High Performance Liquid Chromatography (HPLC) to diagnose Sickle cell disorders along with other hemoglobinopathies.
Result and Conclusion: Total 70308 cases were analyzed during the period ofJune 2007 to October 2011 out of these 35 cases of Hb E variant were identified. Among these 29 cases of Hb E trait, 1 case of Hb E disease and 5 cases of Hb E β-thalassemia were identified. Hb E trait and Hb E disease were asymptomatic while 5 cases of Hb E β-thalassemia were suffering from haemolytic anemia. Detection of this asymptomatic abnormal hemoglobin will help in the prevention of more serious doubly heterozygous hemoglobinopathy.

Key words: Hemoglobinopathy, Hb E, HPLC, doubly heterozygous, Hb E/β thalassemia.

INTRODUCTION

Hemoglobinopathies are genetically important haematological disorder affecting millions of people worldwide. The cumulative gene frequency of the three most predominant abnormal hemoglobins, i.e. Hb S, Hb D and Hb E has been estimated to be 5.35% in India.[1]The prevalence of hemoglobinopathies varies with geographic location and ethnic group.

Hb E is the second most prevalent hemoglobin variant worldwide. It is a slow moving β- chain variant ($\alpha_2\beta_2^{26\ glu\square Lys}$) and is common in south-east Asia with gene frequency in some countries ranging from 8% to 50-70%. In India, Hb E is mostly restricted to the north-east state of India

i.e. West Bengal, Assam, Andhra Pradesh, Nagaland, Manipur, Tripura, Meghalaya with average frequency of 10.9%.[2-4] Hb E disorder may be found in heterozygous Hb E trait, homozygous Hb E disease and compound heterozygous Hb E with other abnormal hemoglobinopathy or thalassemia with widely variable clinical phenotype.[2-5]

In this study, we have investigated & analyzed the occurrence of hemoglobinopathies, and to determine the clinical and haematological profile of different variants of hemoglobin among patients of government hospital & tribal communities from South Gujarat. The data so generated would help development of management, prevention and control programme for hemoglobinopathies in India.

MATERIAL AND METHOD

The present study was a part of the work done under *Sickle Cell Anemia Control Programme* run by Government of Gujarat in collaboration with non-government organizations. The present Sickle cell laboratoryhas been identified as tertiary center. In present study, samples were collected from (1) various camps in schools and colleges of Surat &Tapi districts, (2) DTT positive samples from primary health centers and community health centers, (3) tribal population attending New Civil Hospital, Surat, (4) clinically suspected cases of abnormal hemoglobin variant by clinicians and (5) samples referred from other NGOs.

Samples were tested for complete blood count, DTT and blood group, hemoglobin electrophoresis at alkaline pH on cellulose acetate and HPLC on BIORAD VARIANT (Beta thalassemia short programme). The Hb A_2/F calibrator and two levels of control (BIORAD) are analyzed at beginning of each run.Among the total 70308 cases screened, 35 cases of Hb E were detected during the period of June 2006 to October 2011.

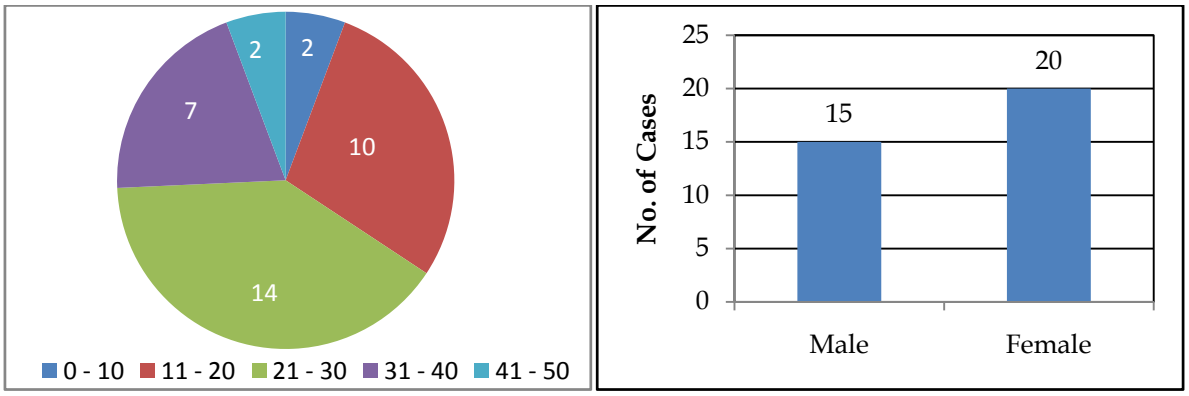

Fig 1: Age group & Sex wise distribution of Hb E cases

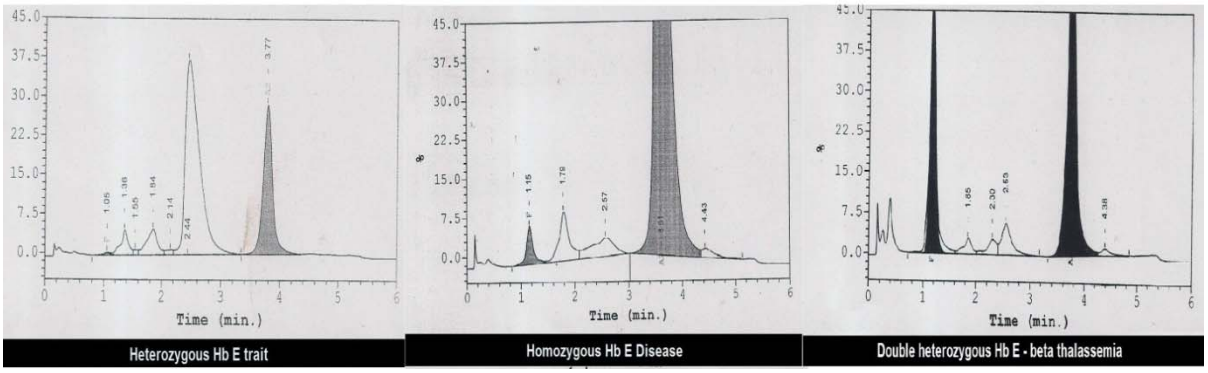

Fig 2 : HPLC Analysis of Hb E disorder

RESULT

Total of 70308 cases were analyzed. Out of these, 35 cases of Hb E variant were detected during period of June 2006 to October 2011. The Hb E variant included Hb E homozygous (1 case), Hb E heterozygous (29 cases) and Hb E/β thalassemia trait (5 cases).

The age and gender distribution of cases are shown in figure 1.

Hb E on electrophoresis present as thick band in A_2 region. On HPLC, Hb E present as raised peak in the A_2 window with retention times ranging from 3.68 to 3.79 minutes. Hb D Iran also elutes in same retention time in A_2 window. Differentiation with Hb E on HPLC relies mainly on the fact that, in heterozygous state Hb D Iran is usually more than 40% whereas Hb E is less than 40%. Alkaline electrophoresis shows migration of Hb D Iran in S/D/G region whereas Hb E migrates in the C/E/O (Hb A_2 region). In thalassemia trait Hb A_2 should be between 4.5 and 9 %.[6-10]

In present study, Hb E heterozygous cases had Hb E ranges from 21.2 to 39.9 %. Hemoglobin is slightly decreased. Hb E homozygous case had Hb E of 92.7 %. Hb E/β thalassemia caseshadHb E ranging from 19.5 to 85 % and HB F ranging from 13.1 to 37 % and low Hb 5 - 9.9 gm/dl. HPLC analysis of Hb E disorders have been shown in figure 2 & details are summarized in table 1.

All homozygous Hb E case and heterozygous Hb E cases were asymptomatic. 5 cases of Hb E/β thalassemia were suffering from symptoms of hemolytic anemia. Hematological parameters of all 5 Hb E/β thalassemia cases are shown in table no 2.

DISCUSSION

Hemoglobinopathies are a group of genetic disorders of hemoglobin. Inherited abnormities of the hemoglobin synthesis are divided into main two groups.

1. Structurally abnormal hemoglobin variant
2. Structurally normal hemoglobin but synthesized at reduced rate, known asThalassemia.[1,11]

Table 1: Hb E level in Hb E disorders

Cases	Hb E (%)	Hb F (%)
Hb E heterozygous	21.2 - 39.9	-
Hb E homozygous	92.7	-
Hb E/β thalassemia	19.5 - 85.0	13.1 - 37.0

Thalassemia, strictly speaking, is not part of hemoglobinopathies, but as its clinical presentation and propagation are same as hemoglobinopathies it is considered as a part of it. Thalassemia and hemoglobinopathy are autosomal recessive inherited disorder primarily affecting the globin moiety of hemoglobin molecule. In some disorders, there is both synthesis of structurally abnormal hemoglobin and a reduced rate of synthesis of the variant hemoglobin. Such diseases can be referred to as thalassemic hemoglobinopathies. [3, 11,12]

Table 2: Hematological parameters of all 5 Hb E/β thalassemia cases

Investigation	1st case	2nd case	3rd case	4th case	5th case
Hb (gm/dl)	5.6	5.6	5	6.7	9.9
Hct (%)	21.7	20.3	20.7	25.9	32.6
MCV (fl)	65	64	70.7	56.5	69
MCH (pg)	17	17	17.1	14.6	20.7
MCHC (gm/dl)	26	27	24.1	25.8	30.1
RDW (% CV)	29	21	32.5	28.9	16.9
PLT (x 10^9/l)	527	193	323	440	405
HPLC					
Hb A_2 (%)	51.2	68.9	85	63.3	19.5
Hb S (%)	0	0	0	0	0
Hb F (%)	37	25	22.2	32.6	13.1
Hb A (%)	4.5	4.6	9.3	6.4	63.8

Hb E is thalassemic hemoglobinopathies having reduced rate of structurally abnormal Hb E. It was first described by Chernoff and colleagues in 1954 and independently in the same year by Itano and colleagues. [3]Hb E is variant hemoglobin with a mutation in β globin gene causing substitution of glutamic acid for lysine at position 26 in β globin chain.Nuclear DNA is subject to spontaneous mutation. This may be a point mutation involving alteration of single nucleotide or a more extensive mutation, in which there is deletion, insertion or other alteration of more than one nucleotide. [12]

These disorders, which are mainly confined to certain geographical area, religions, castes and tribes, particularly with endogamous norms of marriage, are now widely prevalent all over the world. This is because of ever increasing migration of people from one place to another and mixing of different communities through marriage.[2] Hb E is most common in South-East Asia and second most prevalent hemoglobin variant worldwide. Its high frequency in South-East Asia is attributed to its mild thalassemic phenotype, which may impart positive selection in area where malaria is endemic.[3] Three chromosomal backgrounds containing the β^E

gene have been detected, suggesting multiple origin of the β^E mutation.[13]

Hemoglobinopathies and thalassemia are one of the major public health problems in India. It has been estimated that with a population of 1000 million at the year 2000 and a birth rate of 25 per thousand, there would be about 45 million carriers and about 15,000 infants born each year with hemoglobinopathies in India. The carrier frequency of hemoglobinopathy varies from 3 to 17 % in different population groups of India. The cumulative gene frequency of the three most predominant abnormal hemoglobins, i.e. Hb S, Hb D and Hb E, has been estimated to be 5.35% in India. They cause a high degree of morbidity, moderate to severe hemolytic anemia among vulnerable segment of society like infant and children, adolescent girls, pregnant woman etc. and several death in India.[1]

In India, Hb E is mostly restricted to the north-eastern states of India with average frequency of 10.9%, highest 22% in Calcutta (West Bengal) and 50 to 80% in Assam.[2] Although, Hb E is prevalent in Sri Lanka, it is not prevalent in southern India. It is thought to have reached Sri Lanka during migration from north-eastern India during the 5th century BC.[3] Hb E has been sporadically reported form a number of other Indian states such as Bihar, Orissa, Uttar Pradesh, Rajasthan, South Gujarat, Goa, Kerala, Tamilnadu, Delhi and Chandigarh.[14,15]

In different studies, prevalence of Hb E observed in Assam (23-52%), Tripura (41-46%), and Meghalaya (22-41%), Manipur (7%) and West Bengal (3-33%). High prevalence of Hb E in ten population of Assam (20-60%) and in three population of West Bengal (12-61%) has been studied by Deka et al and Das respectively in north-eastern India.[1]

Chatterges detected 526 cases of Hb E/β thalassemia and investigated in Calcutta among Indian Hindu and the regional distribution was Bengalees (508), Oriahs (10), Biharis (4), Assames (2), Punjabis (1), and South Indians (1). Sarkar et al detected 14 cases of Hb E/β thalassemia from Calcutta. *Dash et al* demonstrated a 1 case of Hb E/β thalassemia in Punjab. *Ghosh at al* described 7 cases of Hb E/β thalassemia from Punjab and 1 case from Rajasthan.[1]

An analysis of 50 British patients with hemoglobin E/β thalassemia (one-half of Bangladeshi origin, one quarter Indian/Pakistani and one-quarter originating in South-East Asia) gives an idea of the usual degree of severity of this condition. One-half of patients were regularly transfused and nearly one-half had required spleenectomy. A smaller number of United States patients had disease of similar severity, whereas, of 22 Canadian patients, 30 % were regularly transfused and 17% had required spleenectomy. In Thailand, patients are generally only transfused when this appear to be essential, so that the natural history of the untreated disease is more readily apparent, about one-half of affected individuals have a thalassemia major phenotype and one-half a thalassemia intermedia phenotype.[3]

In present study, we found 35 cases of Hb E disorders. Out of these 35 cases, few cases show origin of north-east India and were migratory mainly from Uttar Pradesh, Assam and Bihar, as Surat is business hub particularly for textile and diamond industry and huge manpower required for same. There is huge migration of people from various parts of the county including mainly form Assam, Bihar, Uttar Pradesh (eastern part of India) and south India. In Present study, one patient of Hb E/β-thalassemia failed to show any ancestral link with North-Eastern region of India. A study of mass migratory pattern in the olden times could be useful in explaining the occurrence of hemoglobinopathies such as the one presented here in regions that normally do not show the gene in the resident population. [4]

Hb E present in three forms, heterozygous state Hb E trait, homozygous Hb E disease and most important compound heterozygous Hb E/β-thalassemia or Hb E sickle cell anemia.[4, 5, 16]

In present study, 35 cases of Hb E variant have been identified. Out of these, 29 were Hb E heterozygous, 1 case was Hb E homozygous and 5 cases of Hb E/β thalassemia. The heterozygous state although clinically silent, is associated with microcytosis, slight erythrocytosis and target cells but no anemia. Hb E levels are usually 20 to 35%. In present study, 29 cases were heterozygous and had Hb E level range from 24 to 32 % and all patients were asymptomatic. [3, 4, 16]Homozygosity for Hb E is characterized by prominent microcytosis (MCV-55 to 65 fl) and significant morphologic alteration such as target cells but little or no anemia. No physical abnormalities are noted other than possible slight splenomegaly. Hb E account for 85 to 95% of the hemoglobin, Hb F is

normal. In present study, 1 case was of Hb E homozygous and had Hb E level 92 % and was asymptomatic. [3, 4, 16] Hb E trait may be co-inherited with β^0 or β^+ thalassemia. Double heterozygous Hb E/β thalassemia trait is important as clinical severity of disease is variable ranging from thalassemia minor through thalassemia intermedia to thalassemia major. The most severely affected individuals are transfusion dependent and have hepato-splenomegaly, intermittent jaundice, growth retardation, delayed sexual maturation and overexpansion of bone marrow cavity leading to facial deformity and malposition teeth.[3 - 5, 16] The Hb is 7-8 g/dl, MCV and MCH are more reduced than in Hb E trait. On HPLC Hb E representing 40 to 60 % of total Hb and Hb F is 30 to 60 %.[3] In present study, 5 cases were of Hb E/β thalassemia and were suffering from hemolytic anemia. On HPLC, they had Hb E ranging from 19.5 to 85 % and Hb F ranging from 13.1 to 37.0 %.

CONCLUSION

The incidence of sickle cell hemoglobinopathy and thalassemia in India are 0-40% and 3-17% respectively. In South Gujarat, sickle cell hemoglobin and thalassemia are widely distributed. The data pertaining to their occurrence and prevalence in South Gujarat are very sparse.[15]

Homozygous Hb E, heterozygous Hb E, Sickle cell trait and β-Thalassemia trait are asymptomatic. However, the identification of these individual is of crucial importance as they may be transmitters of abnormal gene giving rise various combination of hemoglobinopathiesand thalassemia in their progeny which are symptomatic and have high morbidity.[16] They are generally not curable but can be prevented by population screening, genetic counseling and prenatal diagnosis.[1]

These new insights into the knowledge of this disease are important because they are gradually becoming global health problem and impart diagnostic challenge to all the experts involved in the management of these patients. Findings must be supplemented by hemogram findings, family/sibling studies, hemoglobin electrophoresis, other confirmatory techniques and molecular studies based on HPLC findings and a case to case basis.[10]

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