Original article

A cross sectional study of haemoglobin variants in north east India

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Abstract

Study of abnormal haemoglobin has been given an important status in human biology . Haemoglobin variants are genetic haematological abnormalities affecting millions of people all over the world . Among the inherited disorders of blood , haemoglobinopathies and thalassaemia constitute a major bulk of non communicable genetic disease in India .Most common abnormal haemoglobin in India are haemoglobin E (HbE) , haemoglobin S (HbS) and haemoglobin D (HbD) .

The distribution of HbE ($\alpha_2\beta_2^{26Glulys}$) and HbS is mostly restricted to north eastern states of India which is relatively rare in rest of the country . Identification of this disorder is immensely important epidemiologically and aids in prevention of more serious haemoglobin disorder .

Key Words : Haemoglobinopathy , HbE , Electrophoresis , thalassaemia .

Introduction

Study of abnormal haemoglobin has been given an important status in human biology and has interested physiologist , geneticist ,clinician , biochemist , public health scientist and anthropologist alike . Abnormal haemoglobin variants are a group of autosomal recessive disorders characterized by the synthesis of a structurally abnormal globin chain . Inherited disorders of haemoglobin are the most common gene disorders with 7% of the world population being carriers[1] .Haemoglobin variants are genetic haematological abnormalities affecting millions of people all over the world . Inherited abnormalities of haemoglobin include a myriad of disorders ranging from thalassaemia syndromes to structurally abnormal haemoglobin variants[2] .Out of the many methods for separation of haemoglobin components , electrophoretic analysis remains the most effective and widely used technique .The method of agarose gel electrophoresis introduced in recent years enabled confirmatory diagnosis of some of the abnormal haemoglobin like S and D . Pauling and associates discovered that the sickling phenomenon could be demonstrated by electrophoresis.

Most commonly found abnormal haemoglobin in India are HbS , E , D , G , H , I , K , L , N ,Q .Cumulative gene frequency of haemoglobinopathies in India is 4.2%[3,4] It has been estimated that around 50 million people in South Asia alone carry the gene for HaemoglobinE(HbE)[5,6] . HbE is mostly redistributed to north eastern India , Bangladesh , Indonesia ,Malaysia ,Myanmar , Singapore and Thailand[5] .The prevalence of haemoglobinopathies varies with the geographic location and ethnic groups .Among the common Hb variants HbE and β thalassemia are commonly found in the North Eastern states of India ie Assam , Arunachal Pradesh , Nagaland , Manipur , Tripura and Meghalaya and the average allele frequency of HbE in north east regoion is 10.90%[4,7] . Identification of this disorder is immensely important epidemiologically and aid in prevention of more serious haemoglobin disorder . HbE (β 26

Glu-> lys) is 2nd most prevalent haemoglobin variant worldwide of the estimated 30 million persons with abnormal haemoglobin . More than 80% live on the south east mainland .North East India lies in the South East Asia mainland . Different haemoglobin patterns are seen in different population in North East .

Present study has been undertaken to

(a) Know the different types of haemoglobin in various communities of north east region of india

(b) Associated disorders due to abnormal haemoglobin

Limitation of study

A wide range of semi automated and automated equipments are available nowadays for measuring different haematological parameters .The equipments , which count cell are used mostly . Automated cell counters count large number of cells when compared to manual methods , thus allowing greater precision.Manual methods of cell counting have been time consuming and tedious and have been associated with low degree of precision .In this study manual methods are used for different investigations , so full precision cannot be achieved .

Materials and method

This study has been cleared by the Institutional Ethical Committee of Gauhati Medical College and Hospital , Guwahati . This is a hospital based cross sectional study in which a total of 100 patients were taken to find different types of haemoglobin .This study was carried out in N F Railway Hospital Maligaon , in the Department of Pathology . Patients attending medicine out patient department with history of anaemia and other complaints were traced and blood samples were taken after proper consent . Selected cases were from different parts of north east (Assam , Manipur , Arunachal Pradesh , Tripura , Meghalaya ,Mizoram , Nagaland)

Methods:

From each patients 8ml of blood was collected in EDTA for complete haemogram , alkali denaturation test and electrophoresis . Blood smears and sickling test were done from the last few drops of blood during collection .Blood smears stained with leishman stain were examined for abnormal morphology of the red cells and also for the presence of abnormal cell bodies.Haemoglobin estimation was estimated by acid haematin method with random comparison with cyan methaemoglobin method for standardization purpose.Other haematological test total RBC count ,PCV , MCV, MCH, MCHC test were done . Osmotic fragility test and sickling test was done . Alkali denaturation test was done for HbF

Electrophoresis of Haemoglobin :Several electrophoretic technique using different stabilizing medias and buffer system are at presently available for the detection of normal and abnormal human haemoglobins .For routine screening purpose , electrophoresis and agarose gel electrophoresis are quite adequate and suitable .While cellulose acetate electrophoresis and starch electrophoresis are more refined method and most satisfactory for accurate determination of the proportion of HbA_2.

Nowadays agarose gel electrophoresis is virtually replacing paper electrophoresis of haemoglobin .

Results and observation:

Out of 100 subjects , 40 were Assamese , 10 from Meghalaya , 10 from Arunachal Pradesh , 10 from Nagaland ,10 from Manipur ,10 from Tripura and 10 from Mizoram . Out of 40 Assamese people 10 were Ahom , 9 were Boro , 3 were Karbi , 3 were missing , 5 were Kalita, 6 were tea garden , 4 were Koch and Rabha .

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Out of 40 Assamese people 18 are HbA , 08 are HbE (disease) , 08 are HbE (trait) , 04 are HbE (thal) , 02 are HbS (disease) .

Fig 1 shows distribution of haemoglobin variants in Assam ,

Fig 1)

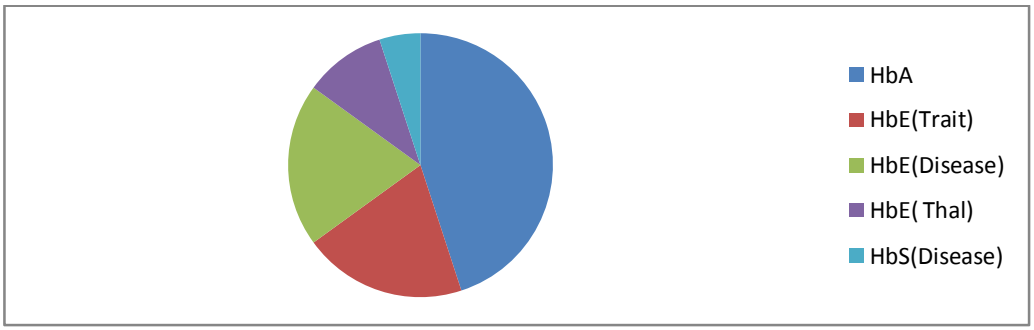

Fig 2 Meghalaya

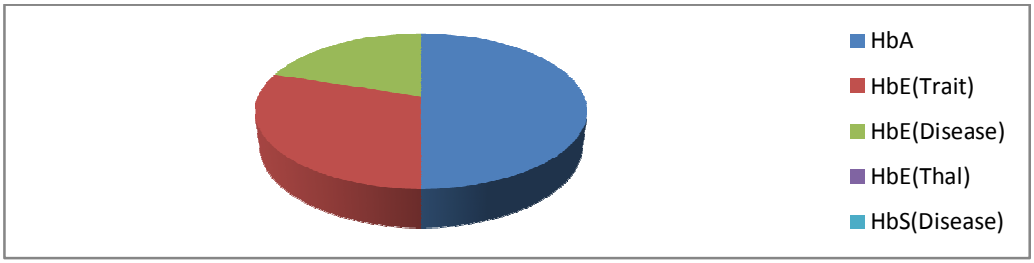

Fig 3 Arunachal Pradesh

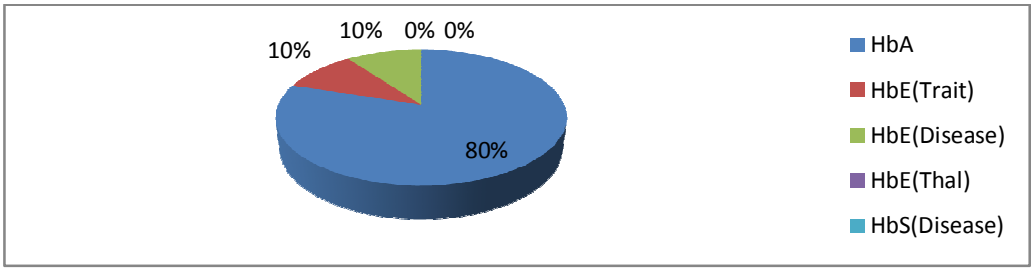

Fig 4 Manipur

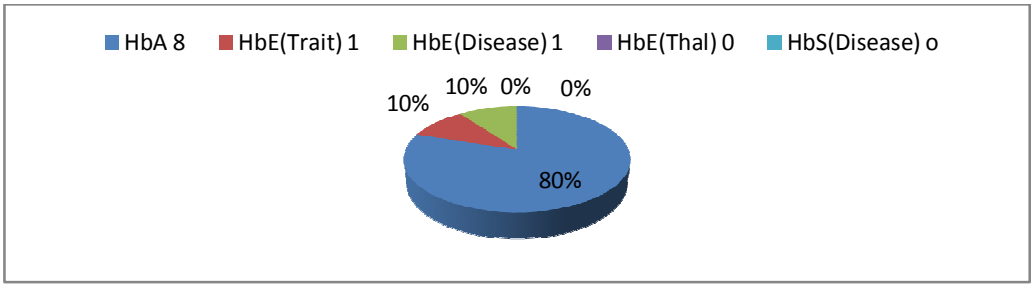

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Fig 5 Nagaland

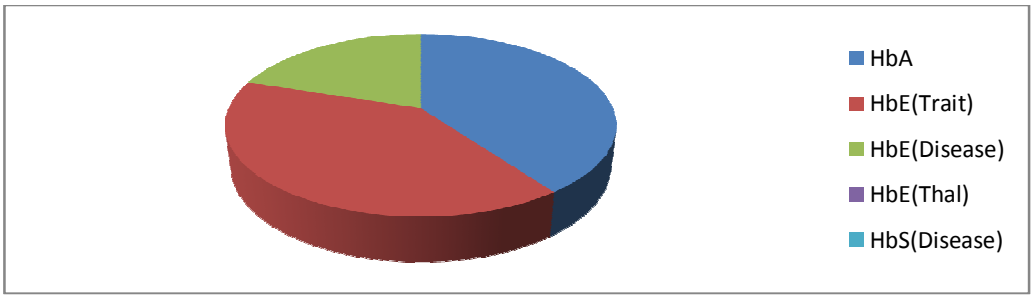

Fig 6 Tripura

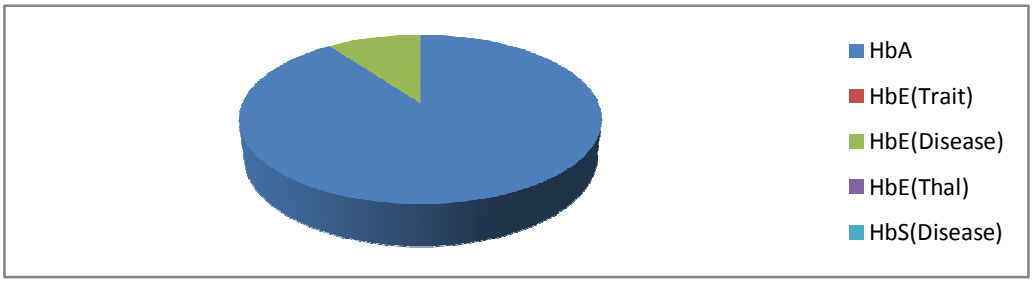

Fig 7 Mizoram

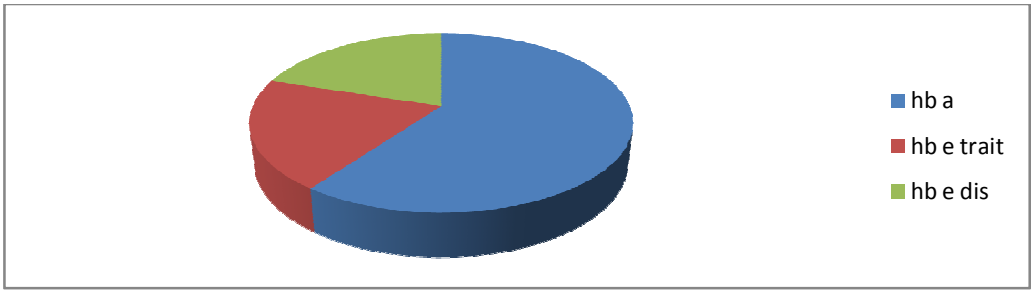

Table 1 shows percentage of different haemoglobin variants in north east India .

Percentage of different haemoglobin variants in north east

Hb types	No.	Percentage
HbA	55	55%
HbE (T)	21	21%
HbE (D)	17	17%
HbE (Thal)	5	5%
Hb S (Disease)	2	2%

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Table 2 shows haematological value of HbA :Haematological value of HbA

	Range	Mean
Hb in gm	11-14	12.5
RBC in mill/cumm	4.5-6.5	5.5
PCV in %	36-47	41.5
MCV(fl)	76-96	86
MCH (pg)	27-32	28.5
MCHC %	32-38	35

Table 3 shows haematological value of HbE(trait):Haematological value of HbET

	Range	Mean
Hb in gm%	3.7-10.6	7.31
RBC in mill/cumm	1.6-4.2	3.13
PCV in %	13-40	25.11
MCV(fl)	65.8-96.8	80.7
MCH(pg)	18-26.6	23.6
MCHC(%)	23-37%	29.25

Table 4 shows haematological value of HbE(thal):Haematological value of HbEThal

	Range	Mean
Hb in gm%	4.5-7.81	5.83
RBC in mill/cumm	2.2-3.5	2.75
PCV in %	17-26	22.25
MCV(fl)	64.3-80	74.5
MCH (pg)	18-24.1	24.42
MCHC%	22.5-32.2	27.35

Table 5 shows haematological value of HbE(disease)

	Range	Mean
Hb in gm%	3.4-9.3	6.83
RBC in mill/cumm	1.12-4.2	2.7
PCV in %	12-35	23.19
MCV(fl)	64.2-96.87	77.97
MCH(pg)	16.2-31.6	24.40
MCHC%	21.3-39	25.74

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Table 6 shows haematological value of HbS(disease)

	Range	Mean
Hb in gm%	3.91-6.09	5.3
RBC in mil/cumm	1.62-6.9	2.26
PCV in %	13-22	18
MCV(fl)	75.86-83	74.95
MCH(pg)	20.3-26.5	22.9
MCHC%	26.8-32	29.4

No of Assamese people 40

Discussion

In the present study of different types of haemoglobin in northeast out of 100 cases 55 were normal adult haemoglobin , 21 were HbE trait ,17 were HbE disease , 5 were HbE thalassaemia and 2 were sickle cell anaemia . It is similar with other workers .In different studies , prevelance of HbE observed in Assam (23% - 52%) , Tripura (41-46%) , Meghalaya (22 -41%) ,Manipur (7%) ,and West Bengal (3- 33%) . High prevelance of HbE in ten population of Assam (20 – 60%) and in West Bengal (12 – 61%) has been studied by Deka et al and Das respectively in north eastern India[8] .In another study done by B M Jha et al . where 35 cases of Hb E disorder were detected in south Gujrat[9].

Haemoglobinopathies are a group of genetic disorders of haemoglobin . Inherited abnormalities of haemoglobin synthesis are divided into 2 groups

(1) Structurally abnormal haemoglobin variants

(2) Structurally normal haemoglobin but synthesized at reduced rate , known as Thalassaemia

Thalassaemia , strictly speaking , is not part of haemoglobinopathies , but as its clinical presentation and propagation are same as haemoglobinopathies it is considered as a part of it . Thalassaemia and haemoglobinopathy are autosomal recessive inherited disorder primarily affecting the globin moiety of haemoglobin molecule . In some disorders there is both synthesis of structurally abnormal haemoglobin and a reduced rate of synthesis of the variant haemoglobin .Such diseases are referred to as thalassaemic haemoglobinopathies .HbE is thalassaemic haemoglobinopathies having reduced rate of structurally abnormal HbE . It was described by Chernoff and colleagues . HbE is variant haemoglobin 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 .

These disorders which are mainly confined to certain geographical area, religions castes , tribes , particularly with endogamous norm 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 marriages[10] . HbE is most common in South East Asia and second most prevalent haemoglobin variant worldwide . Its high frequency in South East Asia is attributed to its mild thalassaemic phenotype ,which may impart positive selection in area where malaria is endemic[11] . The compound heterozygous state for HbE β is common in Thailand and occur in South

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East Asia stretching from Indonesia to Sri Lanka , Northeast India and Bangladesh [14, 15, 16]

Haemoglobinopathies and thalassaemia 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 thousands , there would be about 45 million carriers and about 15000 infants born each year with haemoglobinopathies in India .The carrier frequency of haemoglobinopathy varies from 3-17% in different population groups of India . The commutative gene frequency of the three most predominant abnormal haemoglobin ie HbS , HbD and HbE has been estimated to be 5.35% in India .They cause a high degree of morbidity and moderate to severe haemolytic anaemia among vulnerable segments of society and several deaths in India[8] . In India HbE is restricted to the north eastern states of India ie West Bengal , Assam , Andhra Pradesh , Nagaland , Manipur , Tripura ,Meghalaya with average frequency of 10.9% [10] . In a study by suprio Roy Choudhury et al found 74.33% normal Haemoglobin and 25.6% showed some abnormality [12]

In another study it was found that HbE heterozygous is the most common of Hb variants (23.5%) followed by β thal trait (18.12%) .They found compound HbE – β thalassaemia trait (9%) , HbE homozygous(6.5%) , HbS trait (3.35%) , β thal major (2.13%) , HbS disease (2%) , α thalassaemia (0.63%) , compound HbS – β thalassamia(0.12%)[13] . HbE has sporadically reported from other states of India such as Bihar , Orissa , Uttar Pradesh , Rajasthan , South Gujrat , Goa, Kerala , Tamil Nadu , Delhi and Chandigarh .

Conclusion

The present study deals with 100 cases . Among this 55 cases were normal adult haemoglobin HbA , 21 were HbE trait , 17 were HbE disease , 5 HbE thalassaemia and 2 sickle cell disease were detected .

Haematological parameters showed that varying degrees of anaemia is seen in abnormal haemoglobin cases . Findings were supplemented by haemogram findings, family and sibling studies , haemoglobin electrophoresis ,and other confirmatory techniquies . Genetic haemoglobin disorder with severe anaemia cause considerable pain and suffering to the patients and their families and are major drain on health resources in India . Frequencies found in the present study showed that haemoglobinopathies and thalassaemia are public health problem in North East Region of India emphasizing the need for genetic counselling program . Nationwide government sponsored programme can effectively reduce the occurrence of new cases of serious haemoglobin variants as well as thalassaemia major cases . The incidence of haemoglobin E haemoglobinopathy and thalassaemia in India are 10.9% and 3-17% . In North East India HbE and thalassaemia are widely distributed . Heterozygous HbE , Homozygous HbE , Sickle cell trait and β thalassaemia are asymptomatic .However identification of these individuals is of crucial importance as they may be transmitter of abnormal gene giving rise to various combination of haemoglobinpathies and thalassaemia in their progeny which is symptomatic and has high morbidity . They are not curable but can be prevented by population screening , genetic counselling and prenatal diagnosis . Since in this present study cases were 100 ,a definite conclusion could not be made . It is true that normal adult haemoglobin percentage is more but there is also haemoglobinopathies and thalassaemia which is common among north east people and is found in almost all ethnic group .

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References

1. Weatheral DJ Genetic disorders of haemoglobin In: Hoffbrand AV , Lewis son , Tuddenham EGD , editors . Postgraduate Haematology . 4th ed. London UK : Arnold Publishers ; 2001: 91-119
2. Sachdev R Dam AR , Tyagi G. Detection of Hb Variants and Haemoglobinopathies in Indian population using HPLC : Report of 2600 case .Indian J Pathol Microbiol 2010; 53:57-62.
3. Sarnaik SA .Thalassemia and related haemoglobinopathies . Indian J Pediatr 2005 ; 72: 31924
4. Patne SC , Shukla J Hemoglobin E disorders in Eastern Uttar Pradesh . Indian J Pathol Microbiol 2009 ; 52 : 110-2
5. Fuchareon S Ketvichit P , Sirinaratkul N ,et al . Clinical manifestation of beta thalassaemia/ haemoglobin E disease . J . Pediatr Hematol Oncol 2000 ; 22 : 552-7 .
6. Balgir RS . Prevalence of abnormal haemoglobin E gene in the Dhelki Kharia tribal population Current Science 2003 ; 85(11) : 1604 – 1608 .
7. Balgir RS . Genetic epidemiology of the three predominant abnormal haemoglobin in India 1996 ; 44 : 25 – 8 .
8. R.S . Balgir . The burden of hemoglobinopathies in India and challenges ahead . Current Science 2000 ; 1536 – 47 .
9. B M Jha , et al HaemoglobinE Disorders in South Gujrat --- A study of 35 cases . National Journal of Community Medicine Vol 3 Issue 1 Jan March 2012
10. Shashi C . U . Patne , Jyotishukla Hemoglobin E disorder in eastern Uttar Pradesh . Indian Journal of Pathology and Microbiology 2009 ; 52 : 110 – 12
11. Barbara J Bain . Hemoglobinopathy diagnosis , 2nd edition Black Well Publishing
12. Supriob Ray Choudhury , Himasu Roy , Nirmal Bhattacharrya , Palash Kumar Mondol , Pranab Kumar Biswas and Sumit Roy -- Spectrum of haemoglobin Variants in Eastern Indian Population a study of 14,145 cases .
13. Mauchumi Saikia Pathak et al Int J Biol Med Res . 2014; 5(1) : 3841 – 3846
14. Mujawar Quais , Ukkali Sadashiva , Malagi N N Jholis AN , Haemoglobin E – β + thalassaemia – A case report from Bijapur , South India AI . Ameen J Med Sc . 2009; 2(1) : 82—84
15. Krishnamurti L , Few reports of HaemoglobinE beta thalassemia in Northeast India underdiagnosis or complete exclusion of beta thalassemia by haemoglobin E (Pediatr Hematol onol 2000; 22(6): 558-63
16. Agarwal S , Gulati P , Singh K . HaemoglobinE beta thalassaemia in Uttar Pradesh . Indian Pediatr 1997 ; 34 : 287- 292 .