e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(11); 471-476

Original Research Article

Evaluation of Haemoglobin E and Haemoglobinopathies in Patients with Anaemia Attending a Tertiary Care Hospital of Assam, India

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Received: 19-08-2023 / Revised: 26-09-2023 / Accepted: 28-10-2023

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Conflict of interest: Nil

Abstract

Introduction: One of the common variants of haemoglobin (Hb) is haemoglobin E (HbE). The purpose of the present research is to assess the frequency of Hb E and other haemoglobinopathies among patients attending a tertiary care hospital of Assam, India.

Methods: Patients who visited the Advanced Clinical Biochemistry Laboratory of the Assam Medical College & Hospital in Dibrugarh, Assam, India between September 2021 and September 2022 were the subjects of the present time-bound cross-sectional study. Prior institutional ethics committee approval was obtained for this study. The cell counter (SYSMEX XN-500, Japan) was used to perform the Complete Blood Count (CBC). A High-Performance Liquid Chromatography (HPLC)-based D10 Haemoglobin Testing System (BioRad Laboratories, USA) was used to screen for different haemoglobin variants. The statistical package for the social sciences (IBM SPSS Version 16) was applied for the analysis of data. The difference between groups was statistically evaluated using the Chi-square test considering a p-value below 0.05 as significant.

Results: A total of 266 numbers of participants were included in the study. The mean age (±S.D) of the participants was 30.86 (±16.13) years. Hemoglobinopathies were detected in 51.5% of the participants and are common in 20-29 years age group (15.5%). HbE disease (21.1%) was the most frequently observed Hb variant followed by HbE trait (17.3%). Mean haemoglobin of HbE disease patients was 8.19± 2.23. Patients with β-thalassaemia trait shows the lowest mean haemoglobin level of 4.12±2.56. Substantial variation in hemoglobinopathies were observed among patients belonging to the native Assamese community and those of other immigrant communities (p-value<0.01). Also, prominent variation noticed within the various aboriginal groups (p-value<0.01); particularly predominance of HbE hemoglobinopathies among the tribal and other backward native Assamese groups.

Interpretation & Conclusions: Hemoglobinopathies are prevalent in the study population. Hb E disease is the commonest type of haemoglobinopathies among the study participants. Awareness towards haemoglobinopathies is necessary for better clinical utility.

Keyword: Anaemia; Haemoglobin; Haemoglobinopathies; High-Performance Liquid Chromatography; Sickle Cell Disease; Thalassaemia.

Introduction

A set of inherited conditions known as hemoglobinopathies impact the production of haemoglobin and are thought to be a major contributing factor to anemia [1]. Haemoglobin E (HbE) is one of the frequent Hb variations. HbE variant is the structurally defective haemoglobin resulting from mutations of beta globin gene due to changes in codon number 26 from glutamate to lysine. HbE is quite prevalent in the North-Eastern states of India, where the average allele frequency is 10.9% [2]. HbE is a β-haemoglobin variant and is extremely common in Asian countries. Despite the fact that HbE does not by itself result in any significant clinical issues, its interactions with different kinds of thalassaemia result in a very wide variety of clinical syndromes of varying severity [3]. Being clinically and haematologically identical, homozygous HbE disease and HbE/βthalassaemia are difficult to distinguish from one another [4]. Patients with doubly heterozygous HbE/β-thalassaemia may exhibit clinical symptoms of thalassaemia major and may experience serious problems if untreated [5, 6].The prevalence and burden of genetic disorders and haemoglobinopathies in Indian population is

complicated by the diverse ethnic, geographical, religious, and social variations [7].

The most prevalent single gene illnesses in India are hereditary haemoglobin (Hb) abnormalities. For both epidemiologic investigations and preventing thalassaemia major and clinically severe hemoglobinopathies, accurate and prompt identification of these illnesses is crucial [8].As per reports; an estimated 0.37 out of every 1,000 fetuses in India have Hb abnormality [9]. The National Health Mission produced guidelines for the management and prevention of sickle cell disease and thalassaemia major, two primary hemoglobinopathies for which the Indian government is currently putting a preventive and control program in place [10]. Although, incidence of HbE is common in north-east India, studies related to HbE and other hemoglobinopathies are very limited in this region.

Due to the movement of numerous races over the millennia and hence being the home to a varied range of socio-cultural, linguistic, and ethnically diverse populations, the native population of Assam is a rich pool of genetic divergence and haemoglobinopathies [11].Also, during the colonial era and thereafter, immigration from other parts of India to Assam including Bengali, Marwari, Bihari, Nepali and tea garden worker community etc. resulted a complex population dynamics of Assam [12]. As there is a marked genetic diversity among the complex population dynamics of Assam, variability in hemoglobinopathies may be postulated among the native Assamese population to the immigrant population accumulated over time to the state from other parts of the country. Hence, the present study aims to investigate the distribution of hemoglobinopathies among the patients with anaemia attending a tertiary care hospital of Assam and also to evaluate whether there is any difference in the distribution of hemoglobinopathies among patients belonging to the native Assamese population and those belonging to the inter-state immigrant communities.

Materials and Methods

Patients, who visited the Advanced Clinical Biochemistry Laboratory of the Assam Medical College & Hospital in Dibrugarh, Assam, India, were the subjects of the present time-bound cross-sectional study. This study has the prior approval of the institutional ethics committee. This study was conducted from September 2021 to September 2022.

The study covered 266 patients in total during the one year study period. Children below one year of age were not included in the study. Patients who had undergone blood transfusions within the preceding three months or women who were pregnant for more than thirty weeks were not included in the study. Jaundiced patients were not included either.

After obtaining the informed consent of each subject, 4 ml of venous blood was drawn into EDTA vials. Using the standard protocol, the Complete Blood Count (CBC) was carried out on the cell counter (SYSMEX XN-550, Japan). A High-Performance Liquid Chromatography (HPLC)-based D10 Haemoglobin Testing System (BioRad Laboratories, USA) was used to screen various haemoglobin variants.

The data were analysed using Statistical Package for the Social Sciences (IBM SPSS Version 16). The categorical data were presented as frequencies and percentages. The distribution of continuous variables was presented as mean and standard deviation (s.d.)

Results

The study included 266 participants, with women accounting for 69.9% of the total. The participants' average age (±s.d) was 30.86 (±16.13) years. Haemoglobinopathies were detected in 51.5% of the participants. Hb EE (21.1%) was the most frequently observed Hb variantfollowed by HbE trait(17.3%). During the study, β-thalassaemia trait was present in 4.9% cases5.3% and 3.0% of the cases, respectively, had sickle cell trait and disease. Most of the individuals in the study fell within the 20-49 age range (66.9%), and within this range, the 20-29 age group contributed the most to cases of haemoglobinopathies, accounting for 15.5%.

The frequency was least observed among paediatric as well as adult age group. Female participants contributed mostly towards abnormal haemoglobin types out of which homozygous and heterozygous HbE (30%) was mostly encountered (Table 1).

Table1: Demographic characteristics of the participants

Variables	Haemoglobinopathies						Total(n=266)
	Hb AA (n=129)	Hb AE (n=46)	Hb EE (n=56)	Hb AS (n=14)	Hb SS (n=8)	Hb β-thalassaemia (n=13)	
Age-group							
0-9	4 (1.5)	6 (2.3)	5 (1.9)	0	1 (0.4)	3 (1.1)	19 (7.1)
10-19	21 (7.9)	6 (2.3)	7 (2.6)	2 (0.8)	1 (0.4)	1 (0.4)	38 (14.3)

20-29	40 (15.0)	14 (5.3)	14 (5.3)	8 (3.0)	2 (0.8)	3 (1.1)	81 (30.5)
30-39	27 (10.2)	4 (1.5)	14 (5.3)	1 (0.4)	3 (1.1)	3 (1.1)	52 (19.5)
40-49	22 (8.3)	9 (3.4)	9 (3.4)	2 (0.8)	1 (0.4)	2 (0.8)	45 (16.9)
50-59	3 (1.1)	2 (0.8)	3 (1.1)	1 (0.4)	0	0	9 (3.4)
60-69	9 (3.4)	4 (1.5)	2 (0.8)	0	0	1 (0.4)	16 (6.0)
70-79	2 (0.8)	0	2 (0.8)	0	0	0	4 (1.5)
≥ 80 years	1 (0.4)	1 (0.4)	0	0	0	0	2 (0.8)
Sex							
Male	28 (10.5)	19 (7.1)	22 (8.3)	4 (1.5)	2 (0.8)	5 (1.9)	80 (30.1)
Female	101 (38.0)	27 (10.2)	34 (12.8)	10 (3.8)	6 (2.3)	3 (3.0)	186 (69.9)

HbE disease is observed to be the most prevalent abnormality in this study. The haematological data shows mean haemoglobin of 8.19± 2.23 among Hb E disease patients. A mean haemoglobin level of 5.85±1.62 was observed in sickle cell disease patients.Patients with β-thalassaemia trait shows the lowest mean haemoglobin level of 4.12±2.56. RBC, MCV, MCH and MCHC were lower than the normal range in all the hemoglobinopathies. The patients with β-thalassaemia trait and sickle cell disease were more prone to severe disease with lower levels of Hb and RBC levels (Table 2).

Table 2: Haematological data (mean±s.d.) in various hemoglobinopathies among the study participants

Haemoglobinopathies	Hb	RBC	MCV	MCH	MCHC
Hb AE	7.70±3.40	3.51±1.53	73.59±14.41	23.53±10.03	31.23±5.95
Hb EE	8.19±2.23	4.12±1.24	60.19±6.54	19.44±1.62	32.43±1.81
Hb AS	8.12±3.04	3.40±1.15	76.31±12.06	23.95±4.26	31.34±2.37
Hb SS	5.85±1.62	2.30±0.812	77.35±14.09	25.30±4.60	32.85±0.92
β-thalassaemia trait	4.12±2.56	2.10±2.17	81.02±22.04	25.65±8.37	31.35±2.42

Among the 266 participants, 161 (60.8%) belonged to the various native communities of Assam and 61 (22.9%) participants belonged to the tea-tribe community. Another 44 (16.6%) participants belonged to the other inter-state immigrant communities residing in Assam Substantial variation in hemoglobinopathies were observed among the communities (p-value<0.01). Among the participants belonging to the native Assamese communities, heterozygous and homozygous haemoglobin E wasfound to be predominant with 89 out of 161 participants. While among the tea-tribe community and other inter-state immigrant communities, sickle cell hemoglobinopathy was mostly encountered (Table 3).

Table 3: Distribution of Hemoglobinopathies among various communities

Hemoglobinopathies	Native Assamese communities	Inter-state immigrant communities	Tea garden labourer community	p-value for Chi-square test
Hb AA	58 (45.3%)	30 (23.4%)	41 (31.8%)	<0.01
Hb AE	41 (89.1%)	3 (6.5%)	2 (4.3%)	
Hb EE	48 (85.7%)	2 (3.6%)	6 (10.7%)	
Hb AS	3 (21.4%)	5 (35.7%)	6 (42.9%)	
HB SS	3 (37.5%)	1 (12.5%)	4 (50.0%)	
β-thalassaemia trait	8 (61.5%)	3 (23.1%)	2 (15.4%)	

As seen from Table 4, it was observed that ampledissimilarity exists in the distribution of hemoglobinopathies across the various native Assamese groups (p-value<0.01). Among the 161 native Assamese participants, 50 (31.0%) belonged to the general cast or forward casts out of which majority 28 participants had normal hemoglobinopathies followed by heterozygous HbE (13/50). Almost 33.0% of the participants belonged to the other backward classes mostly the "Ahom" and "Muttok" communities. The Hb E variants were mainly found (36/53) among those participants. The scheduled tribes of the study region mainly comprised of "Sonowal Kachari", "Bodo Kachari" and other similar tribes. Among the 38 participants belonging to the various scheduled tribes, majority 19 participants had homozygous E hemoglobinopathy. Sickle cell haemoglobin and thalassaemia trait were found to be less common among the participants belonging to the Assamese aboriginal.

Table 4: Distribution of hemoglobinopathies among participants belonging to various indigenous groups

Hemoglobinopathies	General castes (n=50)	Other Backward Classes (n=53)	Scheduled castes (n=20)	Scheduled Tribes (n=38)	p-value for Chi-square test
Hb AA (n=58)	28 (48.3%)	13 (22.4%)	7 (12.1%)	10 (17.2%)	<0.01
Hb AE (n=41)	13 (31.7%)	17 (41.5%)	5 (12.2%)	6 (14.6%)	
Hb EE (n=48)	6 (12.5%)	19 (39.6%)	4 (8.3%)	19 (39.6%)	
Hb AS (n=3)	0 (0.0%)	1 (33.3%)	2 (66.7%)	0 (0.0%)	
Hb SS (n=3)	1 (33.0%)	0 (0.0%)	0 (0.0%)	2 (66.7%)	
β-thalassaemia trait (n=8)	2 (25.0%)	3 (37.5%)	2 (25.0%)	1 (12.5%)	

Discussion

The most common haemoglobin variation in Southeast Asia and globally is haemoglobin E (HbE)[13-15].HbE by itself does not result in any severe clinical conditions except for mild anaemic conditions. But in instances, heterozygous β thalassaemia and HbE homozygous phenotype are comparable. Furthermore, when it is inherited in combination with a beta thalassaemia allele, the resulting condition, HbE/beta thalassaemia, can occasionally be characterized by a severe, transfusion-dependent thalassaemia major13. In India, HbE is most prevalent in north-east India and West Bengal [15]. The clinical diagnosis and treatment of these individuals, as well as prenatal diagnosis and genetic counselling, may be aided by the knowledge of this rather uncommon Hb variation in this region of India. Assessing the prevalence of hemoglobinopathies, such as HbE, among patients undergoing treatment at a tertiary care hospital in north-eastern India is the objective of the present study.

In the present study, hemoglobinopathies were detected in 51.5% of the participants. HbE disease (21.1%) was the most frequently observed Hb variant. The findings are comparable to a recent research including 9000 blood samples from upper Assam, which reported abnormal Hb fractions in 59% of the study participants with high incidence of Hb E variant11. Various studies have reported predominance of HbE in north-east India particularly among tribal population [16-18].According to a study conducted among indigenous people employed in the tea plantations of Assam, 34.8% of the population had haemoglobinopathies [19].

In the current study, sickle cell trait and disease were detected in 5.3% and 3.0% of the cases, respectively, while the β-thalassaemia trait was present in 4.9% of the cases. A different study has revealed a high incidence of β-thalassaemia and sickle cell anemia in the tribal population of Assam [19].In India, the prevalence of the β-thalassaemia trait ranges from 3% to 17%, while sickle cell anaemia varies from 1% to 44% [20,21]. A recent research reported prevalence of thalassaemia in endemic form in population of Assam [22].

The Majority of the participants in this studyare within the age groupof 20-49 years (66.9%) among whom hemoglobinopathies are mostly contributed by 20-29 years age group. Although, very little data is available on effect of age on clinical outcomes of hemoglobinopathies, however, the severity of anaemia is claimed to be worsen among adults with abnormal haemoglobin types particularly in those with Hb E/β-thalassaemia [23]. Abnormal haemoglobin types were found mostly among females. Homozygous and heterozygous HbEcomprised 30% of female participants. A study from Pakistan also reported female preponderance in cases with haemoglobinopathies in their findings [24].

In the present study severe anaemia was observed among patients with β-thalassaemia trait (Hb: 4.12±2.56) and sickle cell anaemia (Hb: 5.85±1.62). HbE trait is heterozygous state that is associated with target erythrocytes, mild erythrocytosis, and microcytosis and often clinically quiet while homogygosity of HbE shows prominent microcytosis and may result in reductions of red cell survival [15]. On the other hand, β-thalassemia can range in severity from having no symptoms at all to needing frequent blood transfusions [25].

In the present study, substantial variation was noted in the distribution of hemoglobinopathies among the native Assamese participants with those belonging to the other non-native communities (p-value<0.01). Also, the prominent variation noticed within the various aborigonal groups (p-value<0.01); particularly predominance of HbE hemoglobinopathies among the tribal and other backward groups are comparable to other similar studies [26,27]. Assamese marriage culture is mostly endogamous. Also, in recent times the State and central Governments are launching various schemes to provide financial aid to couples who marry across caste lines in order to reduce prejudice against the caste system in India. However, both endogamy and exogamy among these exposed communities may significantly impact on the genetic pool and alter the abnormal hemoglobinopathy distribution. Hence, pre-marital and pre-conceptual counselling is crucial to reduce

the burden of HbE and Hb beta thalassaemia in successive generations [26]. Hereditary hemoglobinopathies have long been recognized as a major public health concern, and prevention is key [28]. Although there has been a significant improvement in survival rates over the past 60 years in India, there has been little to no change in the available treatments methods for who have a severe form of thalassaemia [29]. According to a recent study, based on population projections through 2026, the requirement of annual blood for treatment will increase to 9.24 million units. This will come with an 86% increase in budgetary requirements, meaning that the blood would then account for more than 19% of the current National Health Budget [7]. Both heterozygotes and homozygotes of HbE have microcytic and hypochromic red blood cells are asymptomatic and only mildly anaemic. However, in the compound heterozygous condition HbE, the interaction of the βE allele with an β-thalassemia mutation results in a varied and occasionally severe anemia [13,14,30].Individuals with homozygous HbE illness presenting with severe anaemia should also be evaluated for compound heterozygous HbEβ thalassaemia as an alternate diagnosis [4].

Limitation

The limitation of the present study is that it included participants from only one tertiary health care centre of Assam and does not cover the whole population of the state. Analyzing the distribution of hemoglobinopathies in the study population may be aided by multi centric assessment.

Conclusion

Haemoglobinopathies are prevalent in the study population. HbE disease is the commonest type of haemoglobinopathies among the study participants. Substantial variation in hemoglobinopathies were observed between patients belonging to the native Assamese community and those of other immigrant communities (p-value<0.01). Also, prominent variation noticed within the various aborigonal groups (p-value<0.01); particularly predominance of HbE hemoglobinopathies among the tribal and other backward native Assamese groups. In order to raise awareness of HbE and other hemoglobinopathies and potentially improve their clinical utility and patient genetic counselling, it is imperative that basic sciences, prevention, and care delivery address the issues surrounding the interactions of hemoglobinopathies with various other infections.

Financial support & sponsorship: None

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