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Phenotypic-Genotypic Correlation of Hemoglobinopathies in North East India

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ABSTRACT
Hemoglobinopathies, or hereditary haemoglobin abnormalities, are common in India, with varied prevalence across areas. The purpose of this study, which was undertaken in Assam, Northeast India, was to look at the phenotypic-genotypic connection of hemoglobinopathies in a group of 25 people. Several mutations have been discovered in beta-thalassemia and Hb E variations. IVS1-5(G>C) was the most frequent beta-thalassemia mutation and it was linked to severe types of the illness. The c.79G>A mutation was the most common among Hb E variations. This study emphasizes the genetic variety of hemoglobinopathies in the region, as well as the significance of early diagnosis in predicting clinical severity. Larger, more diversified investigations are needed to corroborate these findings and assist diagnostic procedures in nations where hemoglobinopathies are common.

INTRODUCTION

Hemoglobinopathies are the most common genetically inherited illnesses worldwide. The world health organisation (WHO) estimates that roughly 5% of the world's population has hereditary haemoglobin (Hb) abnormalities.

Hemoglobinopathies affect roughly 4.2% of the population in India[1]. However, the prevalence of these illnesses varies greatly across the country. For example, HbS is quite frequent in Orissa, but HbE is the most common hemoglobinopathy in Northeast and East India[3,4]. Furthermore, Hb D-Punjab is most common (2%) among Sikhs in Punjab[5]. According to an Indian council of medical research (ICMR) research, HbE is notably prevalent in assam (23.9%) and kolkata, west Bengal (3.92%)[6].

Hemoglobinopathies range in severity from asymptomatic illnesses like beta-thalassemia mild to severe disorders like thalassemia major, which require regular blood transfusions and considerable medical care. Symptoms may include generalised weakness, bone abnormalities, aberrant facial characteristics and growth retardation[7]. Patients with transfusion-dependent thalassemia are at high risk of iron overload, which can lead to iron buildup in important organs such as the liver, myocardium and others. Cirrhosis, liver fibrosis, myocarditis, cardiomyopathy, arrhythmias, growth retardation and other major health issues might arise. Iron chelation therapy should be started as soon as possible to avoid iron excess and its accompanying toxicity. In certain circumstances, splenectomy can relieve symptoms and lead to a higher overall survival rate[7,8].

MATERIALS AND METHODS

From February 2021 to December 2022 the research was carried out in the department of clinical haematology at guwahati medical college in Guwahati, Assam. The study was approved by the institutional ethics committee and all participants provided signed informed permission.

A total of 25 participants were enrolled and diagnoses were made using a multifaceted approach that included clinical history, physical exams and pertinent investigations. Personal histories were recorded in detail, with an emphasis on characteristics such as parental consanguinity, family history of hemoglobinopathies, family history of endocrine disorders, number of monthly blood transfusions and disease duration. Anthropometric measures, evaluations for pallor, jaundice the presence of hemolytic facies, organomegaly and a history of splenectomy were all performed.

Each subject provided 3ml of venous blood in EDTA vials for the laboratory tests. A complete blood count, peripheral smear analysis, reticulocyte count and High-Performance Liquid Chromatography (HPLC) were among the tests performed. To diagnosis beta thalassemia trait, HbA2 values more than 4% were employed as a threshold.

The bio rad D 10 haemoglobin testing system was used to undertake quantitative measurement of several haemoglobin components, including Hbs A, F, E/A2 and Hb S. Approximately 1-2 mL of whole blood was taken in EDTA vials and kept at temperatures ranging from 2-8°C. In each sample vial, these EDTA-anticoagulated whole blood samples were combined with 1.0 mL of hemolysis reagent and then analysed. At 6.5-min intervals, prepared samples were injected sequentially into the analysis stream and separated by a cation exchange cartridge, which used a phosphate ion gradient formed by combining buffers with varying ionic strengths to elute the distinct haemoglobins.

Calibration was done at the start of each run using a HbA2/F calibrator and two level controls. To analyse haemoglobin elution from the cartridge, a dual-wavelength filter photometer was utilised, measuring absorbance changes at 415 nm with baseline correction at 690 nm to account for fluctuations induced by buffer mixing with variable ionic strengths.

Direct DNA sequencing was chosen as the primary approach for identifying uncommon and new mutations. The DNA sequence analysis included the whole HBB, HBA1 and HBA2 regions.

RESULTS

We examined the features of hemoglobinopathies in Assam, India and investigated the genotype-phenotype link in 25 people ranging in age from one year to 68 years. The average haemoglobin concentration was 9.40.77 gm dL^{-1} (Table 1).

The most common mutation found in beta thalassemia was IVS1-5 (G>C) in Intron 1, which was found in 10 cases. This mutation was linked to a severe kind of thalassemia. One patient, however, had a compound heterozygous mutation with c.92+5G>C at exon 1 and c-18-122C>T in the promoter region. Surprisingly, for this patient, this combination resulted in a non-transfusion-dependent phenotype.

Eleven of the research participants had a c.79G>A mutation at exon 1, corresponding to a Hb E variation. Four of the 11 patients had a compound heterozygous mutation including IVS1-5(G>C) at Intron 1, resulting in a severe transfusion-dependent HBE/beta thalassemia phenotype. Another patient with beta thalassemia had a homozygous mutation at IVS 11-1(G>A) (HBBc.315+1G>A), resulting in a less severe phenotype.

Furthermore, one patient had a homozygous mutation that resulted in a 619 bp deletion, resulting in transfusion-dependent beta thalassemia.

Table 1: Features of hemoglobinopathies in Assam

	Phenotype	
Genotype	TDT	NTDT
Mutational profile (N = 25) (34.3%)	(N = 13) (52%)	(N = 12) (48%)
HBB, c.79G>A (EXON 1) (N = 11)		
Homozygous		5
Heterozygous		2
Compound heterozygous with IVS 1-5 (G>C)	4	
Severe TDT		
IVS 1-5 (G>C) (INTRON 1)/ c.92+5G>C (N = 10)		
Compound heterozygous with c.79G>A(EXON 1)	4 Severe TDT	
Compound heterozygous with c-18-122C>T (Promoter)		1
Compound heterozygous with codon 30(G>C)	1	
Homozygous	3	
Heterozygous		1
Homozygous for 619 BP DEL (N = 1)	1	
Homozygous for C.314G>A AT HbA2 (EXON 3) (N = 1)		1
Compound heterozygous CAP site +1(A>C) and CODON 30(G>C) (N = 1)	1	
Homozygous at c.314G>A at exon 3 of HB A2(N = 1)		1
Homozygous IVS 11-1(G>A)(N = 1)	1	
Codon 8/9(+G) (N = 2)		
Homozygous	1	
Compound heterozygous with IVS 1-1(G>C)	1	
Homozygous for HBS		1

Furthermore, one infant was discovered to have a homozygous mutation, c.314G>A at exon 3 of HB A2, leading in intermediate-severity alpha thalassemia, also known as Hb Sallanches.

DISCUSSIONS

A prominent centre for hemoglobinopathies and thalassemia is found in India's North Eastern area, notably in Assam, where several ethnic groups have historically migrated. Since Assamese people have linguistic and cultural links to communities in Southeast Asia, it has been shown by several writers that HbE is common among Assamese people[9].

Significant difficulties in managing HbE-thalassemia arise from its phenotypic heterogeneity and the lack of knowledge about how it develops naturally. From moderate, asymptomatic anaemia to life-threatening diseases needing transfusions from infancy, clinical symptoms can range[10].

Understanding the genetic basis of thalassemia and HbE problems and their consequences for disease severity depends on the molecular information on hemoglobinopathies. Studies carried out in different parts of India have discovered both common and uncommon mutations in the population. In general, IVS I-5 (GC), IVS 1-1(GT), Codon 41/42 (TCTT), 619-bp deletion and Codon 8/9 (+G) are the five mutations that are most common in India[11,12]. The missense SNP mutation c.79G>A (p.Glu27Lys), which involves the substitution of guanine for adenosine at exon 1, was the most often found in our patient cohort. This mutation causes a single amino acid change from glutamic acid to lysine. This is consistent with the Hb E variation, which was found in 11 cases.

Numerous studies[13,14] have consistently identified the mutation c.79G>A at exon 1 as the most prevalent mutation among Hb E variants. The compound heterozygous mutation IVS1-5(G>C) in Intron 1 was present in four of these eleven cases, resulting in a severe transfusion-dependent phenotype of HBE/beta thalassemia, which is consistent with research by Shah *et al*.[15].

This study's findings that IVS1-5(G>C) in Intron 1 was the most common beta thalassemia mutation are consistent with those of Saikia *et al*.[16], who reported a 63.09% positive rate for this mutation in 105 patients in Northeast India. Intriguingly, compared to other parts of India, just one patient had the highly uncommon IVS1.1 mutation, a significant finding among Indo-Asian populations[17], which is consistent with a study by Sinha *et al*.[18].

A research by Oppenheim *et al*.[19] supports the hypothesis that the homozygous mutation at IVS II-1(G>A) (HBBc.315+1G>A) in one beta thalassemia patient caused a less severe phenotype, possibly as a result of enhanced HBF production. Furthermore, one infant had the very uncommon homozygous mutation c.314G>A at exon 3 of HB A2, which results in intermediate-severity alpha thalassemia, commonly known as Hb Sallanches, with only sporadic case reports in the literature[20,21].

It is important to recognise that the study's very small sample size (N = 25) may restrict how far the findings may be applied. The genotype-phenotype association and its potential for early clinical severity prediction are nonetheless highlighted. Furthermore, the study was restricted to one community and the incidence of certain mutations may differ between various ethnic groups or geographical areas. To confirm and build upon these findings, more research with bigger sample sizes and various demographics is required. Prenatal diagnostic programmes and

neonatal molecular marker screening programmes have the potential to improve outcomes, particularly in underdeveloped nations where hemoglobinopathies are common.

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