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Mutational analysis of thalassemia in transfusion-dependent beta-thalassemia patients from central India

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Abstract:

BACKGROUND: Thalassemia and hemoglobin (Hb) disorders are the most common genetic disorders among humans. These disorders entail huge morbidity, economic, and psychological burden on the families of the affected. Genetic counseling and prenatal diagnosis are the steps, which helps to reduce this burden. At present, there is paucity of data on the mutational spectrum of thalassemia from the central Indian region.

METHODS: Blood samples were collected from 62 transfusion-dependent patients, demographic and relevant data were collected and screened for the two rare mutations – 88 (C-T) and CAP + 1 (A-G) using amplification refractory mutation system-polymerase chain reaction (PCR) and GAP PCR technique. PCR was performed for rare Hb disorders such as Hb Lepore and δ β chain disorder by GAP PCR in addition to five common Indian beta-thalassemia mutations IVS1-5 (G-C), IVS1-1 (G-T), Cd41/42 (–TCTT), Cd8/9 (+G), 619 bp deletion.

RESULTS: Overall 93.5% of the mutations could be identified. Among the abnormal Hb, sickle cell and HbE were found at 4% and 3% of all the loci studied. We also reported two loci with Hb δ β and one locus with Hb Lepore in the present samples. IVS I-5 (G–C) was the common mutation (46%) followed by IVS I-1 (G–T) (12%) and 619 bp (9%).

CONCLUSION: The identification of the genotypes helps to define the severity of the phenotype, plan therapy and form the basis of the comprehensive diagnostic database that would be useful not only for genetic counseling but prenatal diagnosis as well, contributing to the current focus of the National Policy to prevent and control hemoglobinopathies.

Keywords:

B-thalassemia, central India, transfusion dependent

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Introduction

Thalassemia and other hemoglobinopathies are one of the most common genetic disorders among humans that result in significant morbidity and mortality worldwide.[1] Beta-Thalassemia is a highly prevalent autosomal recessive disorder typified by the decreased or absent expression of the β-globin gene. It leads to a disproportion of α and β globin chains causing changes in red cell osmotic fragility and severe anemia. Besides, a few abnormal hemoglobin traits such as sickle cell (HbS) in homozygous condition or combination with β thalassemia mutations can lead to transfusion dependence and result in complications.[2] It has been anticipated that the frequency of pathological hemoglobinopathies in India is 1.2/1000 live births[3] and with approximately 22 million births per year,[4] this would suggest the annual birth of 26,400 babies with a serious

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Hb disorder. The WHO Report on guidelines and management of Hb disorders estimated a 3%–4% carrier frequency for β-thalassemia in India, encompassing all types of β-thalassemia trait.[5,6] However, in the absence of more comprehensive, quantitative epidemiological information, it continues to be widely cited as the baseline national prevalence for β-thalassemia in India. At the same time, our survey on 5045 blood donors in Central Indian city of Bhopal indicated a prevalence of β-thalassemia carriers to be 9.59% by Bayesian estimation.[7] The study underscored the importance of screening of carriers in the population and need for identifying the common mutations to serve as a platform for future prenatal diagnostic programs. Carrier screening along with prenatal diagnosis have successfully reduced the burden of thalassemia and other hemoglobinopathies in Mediterranean countries.[8] In India, the centers for prenatal diagnosis are mainly concentrated in the major metropolitan cities which may not be convenient for people living in remote areas. Previous studies conducted in various parts of the country have identified common as well as some rare mutations in the population. In general, the mutations IVS I-5 (G→C), Codon 41/42 (−TCTT), 619-bp deletion and FS 8/9 (+G) make up for most of the mutations in India.[9,10] However, location-specific studies have sometimes reflected changes in the mutational spectrum showing a predominance of rare mutations in a certain community or region. Similarly, the importance of screening of abnormal Hb traits is of equal importance as these in combination with β-thalassemia mutation lead to transfusion dependence. At present, there is a paucity of mutational data on thalassemia from Central India region, which prompted us to undertake this study in a tertiary care hospital to determine the frequencies of β-thalassemia and abnormal Hb mutations in transfusion-dependent patients.

Methods

The study was approved by the Institutional Ethical Committee. Sixty-two transfusion-dependent patients were enrolled in the study after taking informed consent. Data pertaining to age, sex, frequency of transfusion, age at first transfusion, and splenectomy was collected from the recruited subjects.

A volume of 5 ml of blood was collected in Ethylenediaminetetraacetic acid-vacutainers. DNA was extracted by phenol-chloroform method. The quality of DNA was analyzed by running on 1% agarose gel, and the quantification was done by UV visible spectrophotometer. More than 400 mutations are known to be responsible for β-thalassemia. Amongst them, IVS1–5 (G→C), IVS1-1 (G→T), 619-bp deletion, Codon 41/42 (−TCTT), and Codon 8/9 (+G) mutations are responsible for more than 80% of β-thalassemia cases in India. In the first phase, screening for the five common Indian beta-thalassemia mutations IVS1–5 (G-C), IVS1–1 (G-T), Cd41/42 (−TCTT), Cd8/9 (+G), 619 bp deletion and two rare mutations −88 (C-T) and CAP + 1 (A-G) was done. Except for 619 bp pair deletion, the mutations were screened by amplification refractory mutation system (ARMS) PCR. Gap polymerase chain reaction (GAP PCR) was done for 619 bp deletion. For ARMS PCR, identification of heterozygous and homozygous conditions was done by running two parallel PCRs; one for normal and another for mutation-specific primers. In heterozygous condition, the sample tested positive for both the primers, whereas in homozygous conditions, the normal primer did not yield a result. As a screening strategy, we first looked for the most common mutation in the Indian scenario, i.e., IVS1–5 (G-C) followed by the lesser common mutations. The genotype that did not test positive for the above mutations were further analyzed for Hb Lepore and δβ thalassemia trait by GAP PCR method. The primer list and PCR conditions for ARMS and GAP PCR for the five common and two rare mutations were followed as described by Old *et al*.[11] The primer sequence and PCR conditions for delta beta mutation and Hb Lepore mutation were used as described by Craig *et al*.[12] Due to constraints in primer availability, we were not able to detect either one or both mutations in around 15 samples. For these samples, high-performance liquid chromatography (HPLC) (BIORAD, VARIANT)-based analysis of abnormal Hb traits in both the parents of the affected children was done.

Results

Of 62 transfusion-dependent patients enrolled for the study, 42 were male and 20 female. The average age of the subjects was 8 years. The age at first diagnosis of the disease ranged from 6 months to 3.5 years. The frequency of transfusion was in the range of 15 days to 1 month in all the cases studied. The G-C substitution at IVSI-5, a mutation of Asian-Indian origin, was the most common mutation in this study. The mutation was present in 59 (47.5%) of the 124 chromosomes studied [Figure 1]. The second common mutation a Mediterranean mutation IVS I-1 (G–T) of lower frequency, was found in 15 loci (12%) followed by 619 bp Deletion which was found in 11 loci (9%) [Figure 2]. Of the other two common Indian mutations Codon 8/9 (+G) was seen at around 7% of the loci and CD44/42 (−TCTT) was present at 4% of the loci studied. The two rare mutations −88C/T and capsite A/G occupied 2.4% and 1.6% of all the loci studied. Two samples were heterozygous for δβ thalassemia trait [Figure 3] and one for Hb Lepore trait [Figure 4]. After molecular screening for the above-mentioned traits, there were around 14 loci that remain unidentified.

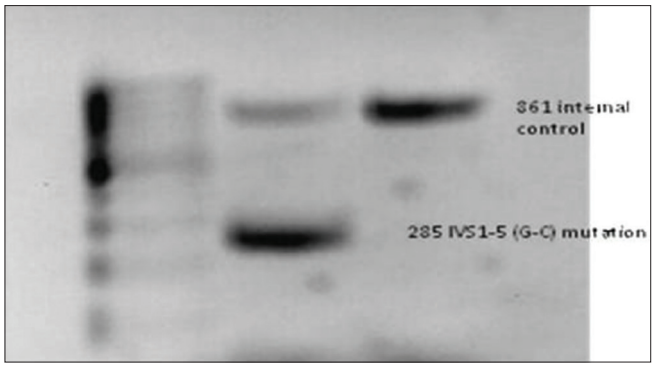

Figure 1: Gel picture showing IVS1-5 mutation by amplification refractory mutation system polymerase chain reaction

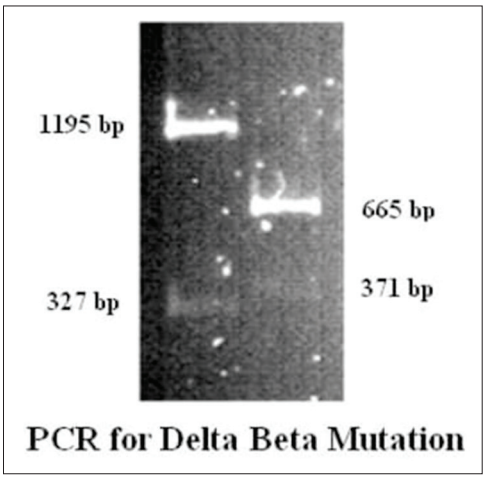

Figure 3: Gel Picture showing delta beta mutation

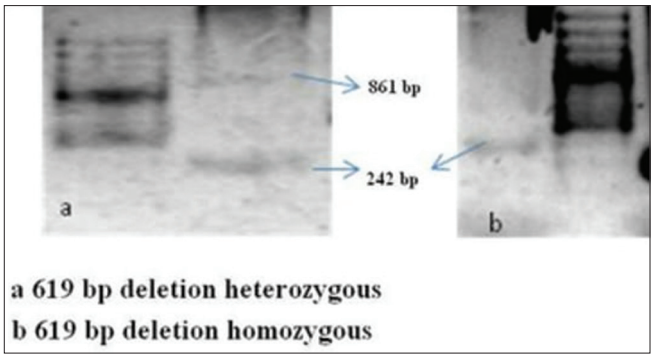

Figure 2: Gel picture showing 619 bp mutation

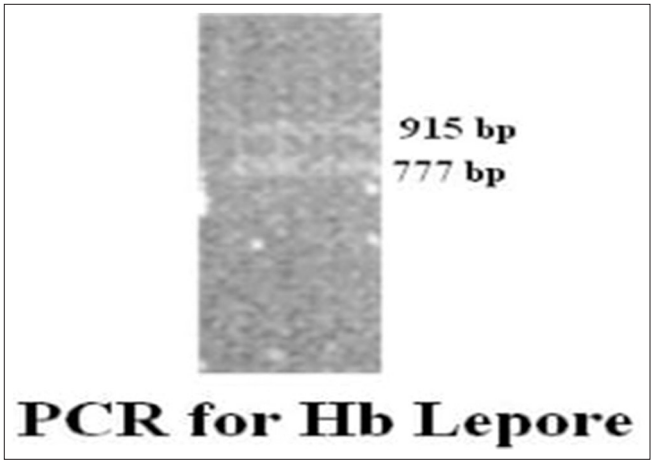

Figure 4: Gel Picture showing hemoglobin Lepore mutation

Out of the cases where HPLC was done in patients, in two cases, both parents were HbS or sickle cell trait, thus proving sickle cell homozygosity. Besides, one child was heterozygous for HbS/β that trait. We also identified two cases of HbE trait amongst the parents screened for HPLC-based Hb distribution. At the end of our study, mutations at around 8% of the loci remain unidentified. The genotypes that were found in more than one patient have been listed in Table 1. Most cases in which both loci had β thalassemia genes presented symptoms of the disease before the completion of 1 year, and the frequency of transfusion was less than a month. In case of heterozygosity of abnormal Hb such as HbE and β thalassemia or homozygosity for HbS, the age at first diagnosis was usually more than 2 years. The frequency of transfusion was around 1 month in case of homozygosity for HbS and heterozygosity of HbE/β thalassemia.

Table 1: Genotypes present in the patients

Mutations Identified	No. of patients
IVSI-1-5 homo	17
IVS1-5/IVS1-1	10
IVS1-5/CD41/42	2
IVS1-5/CD8/9	3
IVS1-5/-88 capsite	2
IVS1-5/610	2
619 bp deletion-88 capsite	2
cd8/9/619 bp deletion	2
sickle cell homozygous	2

Discussion

About 400 mutations are known in the beta-globin gene that can lead to reduced or absence of Hb production. Besides, there are mutations that can result in the production of abnormal forms of Hb such as sickle cell (HbS) and Hb E. 64 β-globin mutations are reported in Indians as documented in the Thailand database till date.[13] It is noteworthy that each population has a mutational spectrum where certain mutations are common in a population than the others.[14] The development of PCR-based assays to detect the common mutations in a population is an easy and cost-effective method for the prenatal diagnosis of this disease. ARMS PCR is an effective tool to identify point mutations. The concept relies on the fact that in a PCR reaction a single change in the 3′ end of the primer will prevent annealing to the template. This technique was further adapted by Old *et al*. for the development of thalassemia mutation detection and is commonly used

worldwide.[15] Various studies have been conducted on the Indian population before, the first being from Verma *et al*.[10] which identified seven common mutations in the Indian population including-Frameshift/8–9 (+G), Nonsense codon 15 (TGG-TAG), Frameshift/41-4 (−TCTT), Frameshift 414 (TCTT), Frameshift/16 (−C), IVS-1 nt 5 (G-C), 619-bp deletion 13 and 25 nt deletion, at 3′ end of the gene.[16] Although the seven common mutations were shown to comprise more than 90% of the mutations that were studied, further studies pointed out the regional difference in frequency of the mutations or reporting of new mutations. A case in point is the 619 bp deletion which has a prevalence rate of 14.2% in Western Indian states of Gujarat and Maharashtra, whereas in the southern states its prevalence is <2%. Other community-based studies have shown the dominance of certain mutations in a community for example, the high rate (35.3%) of Codon 15 (G > A) mutation in Maharashtra, the high percentage of −88 (C > T) alleles in Jat-Sikh community and the high prevalence of Codon 5 (−CT) in Gujarat (79.7%) is associated with the Lohana and Prajapti communities in that state.[17] The present study consisted of 62 transfusion-dependent children in a cosmopolitan city of the central India with immigrants from various parts of the country besides the native population. Although community-specific studies provide a better picture of specific mutation, our preliminary study helps to provide an overall view, which can be further investigated at the community level. Overall, we report the dominance of IVS 1–5 (G-C) as the most common mutation attributing to almost half of all the chromosomes studied. Overall, the five common mutations comprised almost 80% of all the loci. This implies that the mutational spectrum is similar to the one that is known for Northern India. This information can be applied to set up a basic prenatal diagnosis center where almost 70%–80% of the cases can be identified. Amongst the abnormal Hb HbS or the sickle cell trait was the most common, which was not unexpected as our previous study estimated the sickle cell trait in the population to be around 1.0%.[7] Another interesting observation made during this study was the presence of abnormal Hb such as Hb Lepore, delta beta trait, and HbE in the affected children. These mutations for abnormal Hb in combination with beta-thalassemia mutations lead to transfusion dependence, thereby making it imperative to include them in the prenatal diagnosis program. Although our study was incomplete as we could not identify all the mutations which could have probably reported some rare or new mutations, we believe this preliminary study can form the basis of further community-specific studies. Another observation made in this study was the lower number of female children in the patients enrolled for the study. This point out that either a lesser number of females was affected by this condition or it could be due to lack of medical attention for the female child.

Through this study, we also tried to relate the genotype to the phenotype of the patients and observed that in children heterozygous for beta-thalassemia and abnormal Hb trait or homozygosity for sickle cell Hb had less severe conditions.

Conclusion

Our study helps in identifying the mutational spectrum of the region and serve as the platform for setting up a prenatal diagnosis center in the region. The information strengthens the commitment of the Government of India to prevent and control hemoglobinopathies and focus in prevention to reduced morbidity, mortality, economical, psychological, and social burden of thalassemia to the society. A national policy for preventing and control of hemoglobinopathies has been framed emphasizing the need to focus on these genetic disorders on a priority basis.[18] Considering the magnitude of the problem and cost implications of the management, suitable control measures are needed with the identification of carriers and preventing the birth of affected children through prenatal diagnosis.

Ethical statement

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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Conflicts of interest

There are no conflicts of interest.

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