

Research Article

Screening and Molecular Characterization of β -Thalassaemia Mutations in Parents and Siblings of Thalassaemia Major Patients

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Abstract

Background: Hemoglobinopathies are priority genetic diseases for prevention programs. Beta thalassaemia major is one of the single gene blood disorders worldwide. It is also a major health concern in India. Screening of carriers all the way through diverse screening approach is the only way to prevent birth of thalassaemia major child.

Objective: This study was done to screen and molecularly characterize common Indian beta thalassaemia mutations in the parents and siblings of thalassaemia major index cases using ARMS polymerase chain reaction and also as an alternative approach to population based screening program for identifying thalassaemia carriers to prevent birth of thalassaemic children in the family members of a thalassaemia index family.

Methods: Blood samples were collected from thirty families of thalassaemia index cases. Fifty samples from parents and thirty samples from siblings were collected for thalassaemia carrier screening and molecular characterization of five common Indian β -thalassaemia mutations using amplification refractory mutation system polymerase chain reaction.

Results: The results showed that seventy five (90%) cases of heterozygous beta thalassaemia were detected in the survey of 83 samples of parents and siblings having beta thalassaemia major children.

Conclusion: The study concludes that screening of siblings of thalassaemia major cases is necessary and facilitates detection of carriers which ultimately helps in prevention of birth of a thalassaemic child.

Keywords: ARMS PCR; β -thalassaemia mutations; family members; molecular classification

1. Introduction

Thalassaemia and haemoglobinopathies, a group of single gene autosomal-recessive inherited human disorders, are widespread in many parts of the world. Parental screening and genetic counseling are needed for the prevention and control of severe thalassaemia diseases.¹ There are more than 380 mutations in β -globin gene have been known for causing β -thalassaemia disease.²

As the ethnic composition of the Indian population is varied and complex³, each region of the country has its own distinct set of mutations.⁴ Due to homozygosity for β -thalassaemias, it results in transfusion-dependent thalassaemia major and, rarely, in mild non-transfusion-dependent conditions i.e thalassaemia intermedia. Heterozygote screening and genetic counselling are essential for the prevention and control of severe β -thalassaemia disease. Thalassaemia and other haemoglobinopathies can be controlled cost-effectively by carrier screening and genetic counseling and hence WHO has recommended development of these services globally.⁵ Carriers are easily detected by routine haematological methods and can be counseled for their reproductive risk.⁶ A policy of detecting carriers and informing them of their risk, and possibilities for reducing it, usually leads to a fall in births and deaths of affected children.⁶ Retrospectively, screening of parents and genetic counseling with affected children could eventually allow them to limit family size and, where average family sizes are typically large, this approach can significantly reduce affected birth prevalence⁷. Prevalence studies along with extended family screening and high risk caste-wise studies can be cost-effective where consanguineous marriage is common⁸ or carrier prevalence is low.⁹ Five mutations, 619 bp deletion at 3' end of beta-globin gene, IVS1-5 (G→C), IVS 1-1(G→T), FS 8/9 (+G) and FS 41/42 (-CTTT) account for most of the beta-thalassaemia mutations in India.¹⁰⁻¹² In this study, samples from parents and siblings of β -thalassaemia major child were collected and tested for possible five common Indian β -thalassaemia mutations and to study the percentage of thalassaemia affected carriers in thalassaemic families.

2. Subjects and Methods

The parents were confirmed β -thalassaemia carriers as they had an affected child with transfusion-dependent β -thalassaemia. 50 samples from parents including 29 paternal and 21 maternal samples were taken for screening of five common Indian β -thalassaemia mutations from 30 families. Thirty three samples including 22 males and 11 females were also collected from their second or third child i.e. total 83 blood samples were collected including father, mother and siblings of thirty thalassaemia major's families. 1 mL of blood was used for genomic DNA isolation. Informed and written consent was taken from the parents and siblings.

2.1 DNA Isolation

DNA isolation from the buffy coat cells of EDTA-anticoagulated blood samples was done by standard proteinase K / sodium dodecyl-sulphate (SDS) digestion followed by phenol-chloroform extraction¹³ or salt precipitation method.¹⁴

2.2 Detection of Mutations

The mutations were characterized by the PCR method employing allele specific priming technique (AMRS) described by Newton *et al.*¹⁵ which

has been adopted to study the thalassaemia mutations.¹⁶ Five mutations, namely IVS-I-5 (G→C), IVS-I-1(G→T), CD 41/42 (-TCTT), CD 8/9 (+G) and Δ 619 bp deletion from the 3' end of the β-globin gene were studied for β-thalassaemia. All the primers used were procured from IDT. A total of 7 different primers were used. In this technique; presence of amplification product indicates mutation.

2.3 Validation of ARMS PCR

The ARMS PCR was validated by using negative and positive control samples. Fragments were separated in ethidium bromide stained agarose gels and results were documented in a Gel Documentation system (Perkin Elmer). PCR assays were reproduced at least twice for confirmation.

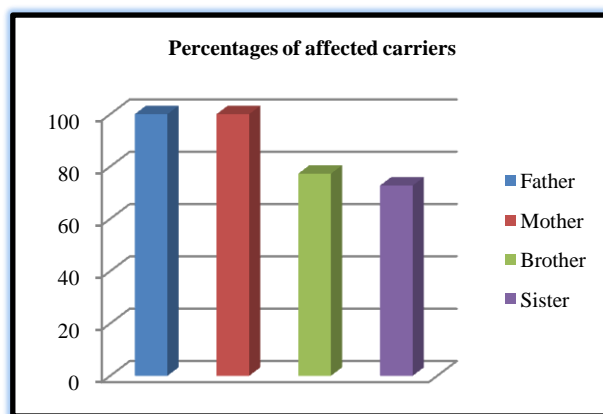
2.4 Data analysis

Presence of bands in both wild-type and mutant PCR assay was inferred as a heterozygous mutant for the particular mutation concerned. Frequency distribution was calculated and comparisons were made in Excel spreadsheets (Microsoft).

3. Results

In this study, molecular characterization of all samples collected from parents and siblings of thalassaemia major index cases was made to identify the proportion of thalassaemia carriers as well as the incidence of mutations present in them. Two groups were made one constituted samples from 50 parents including 58 % (29) samples from father and 42 % (21) from mothers of β-thalassaemia index cases. The second group covers samples from 33 siblings of homozygous β-thalassaemic index cases; there were 66.6% (22) males and 33.3% (11) females. The mean age of siblings group was 7.2 (range: 1 year to 20 years). The mean family size was 1.88 ± 3.8 (ranging between 2 and 4) children per family. Mutation screening done for the five common Indian β-thalassaemia mutations IVS-I-5 (G→C), IVS-I-1(G→T), CD 41/42 (-TCTT), CD 8/9 (+G), Δ 619 bp deletion using ARMS PCR. Gap-PCR was used to amplify the β-globin gene to detect the Δ619bp deletion mutation.¹⁷ Among the parents, the main heterozygous mutation identified was IVS 1-5 (G→C) 40% followed by CD 41/42 (-TCTT) 24%. Among the siblings, 24.2% (8) were identified as normal, whereas 75.7% (25) were reported as β-thalassaemic carriers (Fig.1). The IVS 1-5 (G→C) was observed with 30.3% frequency in the siblings followed by CD 41/42 (-TCTT) 27.7%. Among all the carriers including parents and siblings, the frequency of mutations observed was IVS 1-5 (G→C) 36.1% followed by 25.3% CD 41/42 (-TCTT) then IVS-1-1(G→T) 16.8%, Δ 619 bp deletion with 12.04% and CD 8/9 (+G) 4.8%. 5% mutations were still remains uncharacterized in the study. Eighty percent of samples studied were diagnosed as β-thalassaemia minor clinically only on the basis of red blood cell parameters as reported by the study subjects while after molecular diagnosis i.e. ARMS PCR ninety percent of them were thalassaemia carriers.

Fig.1: Percentages of β-thalassaemia affected carriers in the study



4. Discussion

Prevalence studies, antenatal screening, high-risk group screening, carrier screening and extended family screening (cascade screening) to identify carriers are very important strategies for prevention of β-thalassaemia in India. These strategies are being used by many countries to prevent thalassaemia. Studies showed that the incidence of β-thalassaemia has decreased considerably after employing screening programmes.¹⁸

Immediate family screening is a way forward as evidenced by identification of 62.2% of siblings being carriers as opposed to 5-8% carriers in the general population.¹⁹ This study demonstrates that screening of parents and their siblings is one of the important strategies for prevention of birth of thalassaemic child in the family. ARMS PCR is the widely used method in India due to its specificity and rapidity.^{3,13,19} However, for characterization of rare mutations of β-thalassaemia gene sequencing, DGGE and single strand conformation polymorphism techniques are reliable tools.²⁰ Dr. Old and colleagues pioneered the ARMS method for mutation identification and prenatal diagnosis of β- Thalassaemia in the Cypriot and Indian populations in UK.^{3,16} The method is developed for the detection of 9 core mutations, i.e. IVS 1-5(G→C), 619 bp del, FS 8/9(+G), IVS 1-1(G→T), FS 41/42(-CTT), CD 15(G→A), FS 16(-C), CD 30(G→C) and CD 5(-CT), which are prevalent in the Indian population.²¹ Our results shows that 95% of mutations can be characterized using ARMS PCR. It can be efficiently utilized by present and future laboratories for molecular characterization of β-thalassaemia mutations. In theory, when the prevalence of carriers is 5 percent in the general population and 31 percent in families with an affected member, a person with an affected relative would have an approximately 1.6 percent chance of entering a marriage at risk for producing affected children (31 percent of 5 percent).⁸ This study shows that 76% siblings as carriers of beta thalassaemia mutations and hence if consanguineous marriage take place there is an immense probability of having a thalassaemia major child. Consequently it is extremely needed to screen the siblings of thalassaemia major child to prevent the birth of a thalassaemic child in the family.

5. Conclusion

Thalassaemia and other haemoglobinopathies are common single gene genetic disorders and their prevention is very important. Awareness about the disease and its consequences allows a range of options, including limiting of family size, ensuring that at-risk infants are tested at birth, and requesting antenatal or prenatal diagnosis. Prevention programmes and awareness camps should be scheduled into health systems. High risk population should be regularly checked and screened for possible β-thalassaemia mutations. Screening of immediate family members of thalassaemia patients is more effective strategy in controlling the disease. Cascade screening and extended family screening programmes have the potential that can lead to an effective measure for controlling thalassaemia in India. There is also a need to make the screening more readily available and to motivate high-risk groups through awareness-raising programmes. The study concludes that screening of siblings of thalassaemia major cases is necessary and facilitate detection of carriers ultimately helps in prevention of birth of a thalassaemic child.

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