

Original article:

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

Aims & Objective: This study was done to screen and molecularly characterize β -thalassaemia mutations in the parents and siblings of thalassaemia major index cases using amplification refractory mutation system polymerase chain reaction for the common Indian mutations 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.

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

Observation & Results: 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: Screening of siblings of thalassaemia major cases is necessary and facilitate detection of carriers ultimately helps in prevention of birth of Thalassaemic child.

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

1.0 Introduction

Thalassaemia and haemoglobinopathies, a group of autosomal-recessive inherited human disorders, are prevalent in many parts of the world. Heterozygote screening and genetic counseling are essential for the prevention and control of severe thalassaemia diseases. (1) At molecular level, β -thalassaemia represents a great heterogeneity as more than 380 mutations have been identified for the β -globin gene responsible for this disease (OMIM, 2005).(2) Despite this heterogeneity, each at-risk population has its own spectrum of common mutations, usually from 5 to 10, a finding that simplifies mutation analysis.(3) As the ethnic composition of the Indian

population is varied and complex (4), each region of the country has its own distinct set of mutations (5). Homozygosity for β -thalassaemias usually results in transfusion-dependent thalassaemia major and, rarely, in mild non-transfusion-dependent conditions. Heterozygote screening and genetic counselling are essential for the prevention and control of severe β -thalassaemia disease. Since they can be controlled cost-effectively by programmes that integrate treatment with carrier detection and genetic counselling, WHO has recommended global development of these services.(6) Carriers are easily detected by routine haematological methods and can be forewarned of their reproductive risk.(7) 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.(7) Retrospectively informing parents with affected children of their 25% recurrence risk allows them to limit family size and, where average family sizes are typically large, this approach can significantly reduce affected birth prevalence.(8) Population screening is not the only useful strategy: family studies can be cost-effective where consanguineous marriage is common(9) or carrier prevalence is low.(10). Five mutations, 619 bp deletion at 3' end of β -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 β -thalassaemia mutations in India.(11-13). In this study, samples from parents and siblings of β -thalassaemia major child were collected and checked for possible five common Indian β -thalassaemia mutations and to study the percentage of thalassaemia affected carriers in Thalassaemic families.

2.0 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 samples were collected including father, mother and siblings of thirty thalassaemia major's families.

2.1 DNA Isolation

DNA isolation from the buffy coat cells of EDTA-anticoagulated blood samples was done as described (14). The genomic DNA was extracted from peripheral blood samples by standard proteinase K/sodium dodecyl-sulphate (SDS) digestion followed

by phenol-chloroform extraction (15) or salt precipitation method.(16)

2.2 Detection of Mutations

The mutations were characterized by the PCR method employing allele specific priming technique (AMRS) described by Newton et al (14) which has been adopted to study the thalassaemia mutations(13, 14, 17). Five mutations, namely IVS-I-5 (G \rightarrow C), IVS-I-1(G \rightarrow T), CD 41/42 (-TCTT), CD 8/9(+G) and Δ 619 bp deletion from the 3' end of the β -globin gene were screened. All the primers used were procured from HiMedia. 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).

3.0 Results

In this study, molecular characterization of all samples collected from parents and siblings of thalassaemia major index cases was done to identify the proportion of thalassaemia carriers as well as the incidence of β -thalassaemia mutations present in them. Two groups were made one constituted samples from 50 parents including 29 samples from father and 21 from mothers of β -thalassaemia index cases. The second group covers samples from 33

siblings of homozygous β -thalassaemic index cases; there were 22 (66.6%) males and 11 (33.3%) females. The mean age of siblings group was 7.2 (range: 1 year and 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 i.e. IVS-I-5 (G \rightarrow C), IVS-I-1(G \rightarrow T), CD 41/42 (-TCTT), CD 8/9(+G), Δ 619 bp deletion using ARMS PCR. Gap-PCR was used to simultaneously amplify the β -globin gene from genomic DNA and to detect the Δ 619bp deletion mutation (18). Among the parents, the main heterozygous mutation identified was IVS 1-5 (G \rightarrow 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. The IVS 1-5 (G \rightarrow C) is observed with 30.3% frequency in the siblings followed by CD 41/42 (-TCTT) 27.7%. Among all the carriers, the frequency of IVS 1-5 (G \rightarrow C) mutations was found to be 36.1% followed by 25.3% of CD 41/42 (-TCTT) mutation & then IVS-1-1(G \rightarrow T) with 16.8%, Δ 619 bp deletion with 12.04% and CD 8/9 (+G) with 4.8%. 5% mutations were still remains uncharacterized in the study.

4.0 Discussion

Preventive screening programmes to identify carriers are being used by many countries where thalassaemia is a common disease. The incidence of β -thalassaemia has decreased significantly after the introduction of screening programmes.(19) The various approaches used are population screening, high-risk group screening, antenatal screening, and extended family screening (cascade screening). 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.(20) 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 (4, 15, 21). However, for characterization of rare β -thalassaemia mutations; gene sequencing, DGGE and single strand conformation polymorphism techniques are reliable tools (22). But these techniques are very costly. 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 (4, 17). The method is developed for the detection of 9 core mutations, i.e. IVSI, 5(G \rightarrow C), 619 bp del, FS8/9(+G), IVSI,1(G \rightarrow T), FS 41/42(-CTTT), C15(G \rightarrow A), FS16(-C), C30(G \rightarrow C) and C5(-CT), which are prevalent in the Indian population.(23). 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).(24). This study showed 76% siblings as carriers of β -thalassaemia mutations. Therefore it is extremely important to screen the siblings of thalassaemia major child to prevent the birth of another thalassaemic child in the family.

5.0 Conclusion

This study concludes that screening of immediate family members of thalassaemia patients is more

effective than screening in other groups, and, hence, has the potential to lead to a nationwide programme for control of thalassaemia and related haemoglobinopathies. Because haemoglobin disorders are commonly a point of entry for genetic approaches into health systems,(25) services should be designed to provide a foundation for more comprehensive community genetics services.(26).

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Conflict of Interest: NIL

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