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A study of beta-thalassemia mutations from Kathua Region of J&K

Suraya Partap Singh

Abstract: The study was designed to identify the beta-thalassemia mutations in Kathua region of UT of J&K. 46 unrelated patients with the history of beta-thalassemia were subjected to molecular analysis. Diagnosis of beta-thalassemia was made by conventional hematological procedures. Molecular analysis was carried out by ARMS-PCR to detect the seven frequent mutations found in north India. Of 46 patients, mutations could be detected in 39 patients only. These included 37 homozygotes and 2 compound heterozygotes. Mutations in the remaining 7 patients could not be characterized using this approach. Five different beta-thalassemia mutations could be detected in all 78 alleles. These are Fr 8-9(+G) (28 alleles, 35.9%), 619 bp del (20 alleles, 25.6%), IVS-1 nt(5) (G→C) (19 alleles, 24.4%), Fr 41-42 (-TTCT) (10 alleles, 12.8%) and Cap +1(A→C) (1 allele, 1.3%). The first four mutations account for 98.7% of the total number of beta-thalassemia chromosomes studied. This study will enable us to design an adequate approach to genetic counselling and prenatal diagnosis for couples at risk.

Key words: Beta-thalassemia; molecular analysis; ARMS-PCR; compound heterozygote; alleles.

1. Introduction

Beta-thalassemia is an autosomal recessive disorder characterized by acute microcytic, hypochromic, hemolytic anemia. It is caused due to deficient production or absence of the beta-globin chain. Clinical and hematological expression is quite heterogenous ranging from severe to intermediate beta-thalassemia or asymptomatic carriers. Advances in molecular genetic techniques have resulted in elucidation of the mutations causing beta-thalassemia. Till date more than 350 different beta-thalassemia mutations have been reported in the IthaGenes database. (Kountouris *et al.*, 2014).

Suraya Partap Singh (✉)

Department of Zoology,
Government Degree College, Reasi,
Jammu and Kashmir, India.
E-mail: suraya9@gmail.com

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Decrease in beta-globin production leads to excess of alpha-globin chains that get precipitated in RBC precursors, inhibiting their normal maturation and survival. This leads to severe anemic conditions in the sufferer.

Beta-thalassemia is one of the most common autosomal recessive disorders in India. The average prevalence of beta-thalassemia heterozygotes (carriers) varies between 3–4% which translates to 45 to 54 million carriers in our population of 1.35 billion people belonging to different ethnic, cultural and linguistic groups. Many of the ethnic groups have a much higher prevalence of beta-thalassemias in them ranging from 4–17%. (Madan *et al*, 2010; Coalh *et al*, 2017).

Around 80 different beta-thalassemia mutations have been reported among Indians so far by different groups of workers (Agarwal *et al.*, 2000; Edison *et al.*, 2010; Sheth *et al.*, 2008; Colah *et al.*, 2009; Sinha *et al.*, 2009; Patel *et al.*, 2014).

Beta-thalassemia constitutes a major health problem in India and Jammu & Kashmir is no exception. Little information on the molecular basis of beta-thalassemia in Kathua region of Jammu & Kashmir is available despite its high prevalence in the region. Here we report the characterization of beta-thalassemia mutations in

46 patients with beta-thalassemia major in Kathua. Five mutations have been detected and only 4 mutations account for 98.7% of beta-thalassemia genes. This is the first report of beta-thalassemia screening in Kathua region of J&K. Knowledge of the frequency and distribution of beta-thalassemia alleles that effect a given population facilitates molecular detection and also a proper genetic counselling.

2. Materials and Methods

Blood samples from 46 beta-thalassemia major patients were collected during the course of study. Diagnosis of beta-thalassemia was previously made by conventional hematological analysis in the Dept. of Hematology, Govt. Medical College, Jammu.

Hematological Analysis

Whole blood samples were collected with EDTA as anticoagulant. The complete hemogram including MCV, MCH, RBC count were obtained using automated cell counter. HbF and HbA_2 levels were determined by using HPLC.

Mutation Analysis

DNA was isolated from peripheral blood obtained in EDTA as anticoagulant by Phenol:Chloroform extraction (Sambrook et al. 1989) or by using DNA extraction kits (QIAamp DNA Blood Mini Kit, QIAGEN). The samples were tested by the method of Amplification Refractory Mutation System (Newton et al. 1989) for the seven known mutations previously reported in Indian subjects (Varawalla et al. 1991a b) using specific oligonucleotide primers. The list and the sequence of the primers used is given in table 1.

Table 1. Sequences of the primers (both mutant and normal) used for the detection of seven most common beta-thalassemia mutations taken up for the study.

Mutation	Primer Sequence	Control/ Common Used	Amplicon Size
IVS-1 nt(5) (G→C) **M**	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG	3	285
IVS-1 nt(5) **N**	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAC	3	285
Fr 8-9 (+G) **M**	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACC	3	215
Fr 8-9 **N**	CCT TGC CCC ACA GGG CAG TAA CGG CAC ACT	3	214
IVS-1 nt(1) (G→T) **M**	TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA	2	281
IVS-1 nt(1) **N**	GAT GAA GTT GGT GGT GAG GCC CTG GGT AGG	4	450
Fr 41-42 (-TTCT) **M**	GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT	3	443
Fr 41-42 **N**	GAG TGG ACA GAT CCC CAA AGG ACT CAA AGA	3	443
Cap +1 (A→C) **M**	ATA AGT CAG GGC AGA GCC ATC TAT TGG TTC	4	567
Cap +1 **N**	ATA AGT CAG GGC AGA GCC ATC TAT TGG TAA	4	567
-88 (C→T) **M**	TCA CTT AGA CCT CAC CCT GTG GAG CCT CAT	4	655
-88 **N**	TCA CTT AGA CCT CAC CCT GTG GAG CCT CAC	4	655
Del 619b bp **M**	CAA TGT ATC AGT CCT CTT TGC ACC	2	242
Control 1	CAA TGT ATC ATG CCT CCT TGC ACC		
Control 2	GAG TCA AGG CTG AGA GAT GCA GGA		
Common 3	ACC TCA CCC TGT GGA GCC AC		
Common 4	CCC CTT CCT ATG ACA TGA ACT TAA		

M : PRIMERS FOR MUTATION
N : PRIMERS FOR NORMAL ALLELES

PCR was carried out on a 25µl reaction mixture containing 12.5 µl of Premix, (providing a final concentration of 2.5 units of Taq DNA polymerase, 1.5 mM $MgCl_2$ and 200 µM of each dNTP), 5mol/ µl of each primer and 1 µg of template DNA. The thermal cycling was started with an initial heat activation step at 94 °C for 10 minutes followed by 35 cycles of Denaturation at 94 °C for 1 minute, Primer Annealing at 65°C for 1 minute and DNA extension at 72 °C for 1.5 minutes and then a final extension at 72 °C for 10 minutes. The PCR products were purified and electrophoresed in 1.5% Agarose Gel, stained in Ethidium Bromide and photographed under U.V.

Subjects with beta-thalassemia major found to be positive for any mutation were then tested for the presence of normal allele. Those found to have normal allele (compound heterozygotes) were further tested for other mutations. All the homozygotes and compound heterozygotes were counted as representing two thalassemic alleles.

3. Results

A total of 46 beta-thalassemia patients were analyzed for the presence of beta-thalassemia mutations. The methodology employed was designed to detect the most frequent mutations already reported from India. Using this approach mutation could be identified only in 39 patients, while mutations in rest 7 patients remained uncharacterized. Of 39 patients, 37 were found to be homozygotes and two were identified as compound heterozygotes. Thus a total of 78 alleles were studied and five different mutations could be detected in all 78 alleles. These are Fr 8-9 (+G), 619 bp del, IVS 1 nt(5) (G→C), Fr 41-42 (-TTCT) and Cap +1 (A→C). of 37 homozygotes, 13 were found to be homozygous for Fr 8-9 (+G), 10 for 619 bp del, 9 for IVS 1 nt(5) (G→C) and 5 for Fr 41-42 (-TTCT), while the remaining 2 patients were genetic compounds for two different mutations. One was compound heterozygote for IVS 1 nt(5) (G→C) and Fr 8-9 (+G) and the other was compound heterozygote for Fr 8-9 (+G) & Cap +1 (A→C). The results of agarose gel electrophoresis are shown in Figures 1-6. The distribution of beta-thalassemia mutations in Kathua is 28 alleles (35.9%) with Fr 8-9 (+G) mutation, 20 alleles (25.6%) with 619 bp del. mutation, 19 alleles (24.4%) with IVS 1 nt(5) (G→C) mutation, 10 alleles (12.8%) with Fr 41-42 (-TTCT) mutation and 1 allele (1.3%) with Cap +1 (A→C) mutation. Table 2 shows the beta-thalassemia alleles identified and their frequency.

Table 2. Showing beta-thalassemia alleles identified from Kathua District and their frequency

Mutation	Number of Alleles	%
Fr 8-9 (+G)	28	35.9
619 bp del	20	25.6
IVS-1 nt(5) (G→C)	19	24.4
Fr 41-42 (-TTCT)	10	12.8
Cap +1(A→C)	1	1.3
Total	**78**	**100**

4. Discussion

In terms of molecular defects, beta-thalassemia is a greatly heterogenous group and this study was sought to detect and characterize beta-globin gene abnormalities in Kathua population and has resulted in the identification of five different beta-thalassemia mutations in 84.78% of the individuals investigated, with 4 common ones Fr 8-9 (+G), 619 bp del, IVS 1 nt(5) (G→C) and Fr 41-42 (-TTCT), accounting for 98.7%. This is in agreement with the previous studies in the Asian-Indian population (Kazazian et al. 1984, Thein et al. 1988). Cap +1 (A→C) has also been detected in one of the individuals investigated. Except for two individuals, compound heterozygotes for two mutations, all other individuals studied had identical mutations reflecting the social practice of marriage with in each community. Mutations Fr 41-42 (-TTCT), Fr 8-9 (+G), 619 bp del. are β^{o}-thal mutations while mutations IVS 1 nt(5) (G→C) and Cap +1 (A→C) are β^{+}-thal mutations. Fr 41-42 (-TTCT) and Fr 8-9(+G) are frame shift mutations that result in the alteration in the ribosome reading frame and cause premature termination on RNA translation and hence no beta-globin chain is produced. 619 bp del. is a large deletion mutation also resulting in non-production of beta-globin chain of hemoglobin molecule.

Since no beta globin chain is produced in the mutations Fr 41-42 (-TTCT), Fr 8-9 (+G) and 619 bp del., the severity in individuals possessing these mutations in homozygous state is obvious. Mutation IVS1nt(5)(G→C) is β^{+}-thal mutation. This mutation alters the consensus sequence and reduces the efficiency of normal splicing of mRNA transcript, giving β^{+}-phenotype. In individuals with this mutation, beta-globin chain is produced but is defective and cannot form functional hemoglobin molecule along with alpha-globin chain, thereby causing severe disease in individuals possessing this mutation in homozygous state.

The patients, who were found to be compound heterozygotes, also had severe disease. One of these had two severe mutations, IVS 1

nt(5) (G→C) and Fr 8-9 (+G) responsible for severe beta-thalassemia, while the second patient had Fr 8-9 (+G) and Cap +1 (A→C) mutations in heterozygous state. Cap +1 (A→C) is a mild mutation but the phenotype of the individual with this mutation along with Fr 8-9 (+G) was highly severe. This is probably because of the presence of mild mutation in combination with severe beta-thalassemia allele. These observations have significant implication for setting up prevention and control of thalassemia in Kathua District of J&K.

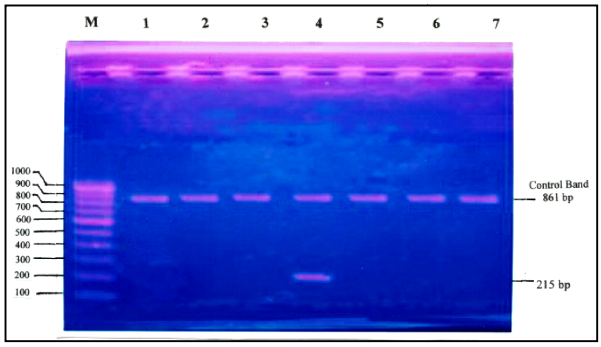

Fig. 1. Lane 4 : 215 bp fragment of Fr 8-9 (+G) beta-thalassemia mutation

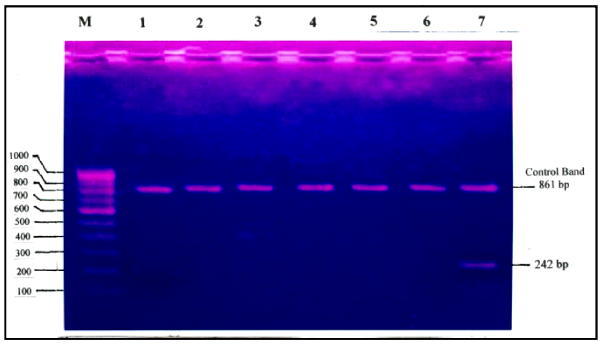

Fig. 2. Lane 7 : 242 bp fragment of 619 bp del. beta-thalassemia mutation

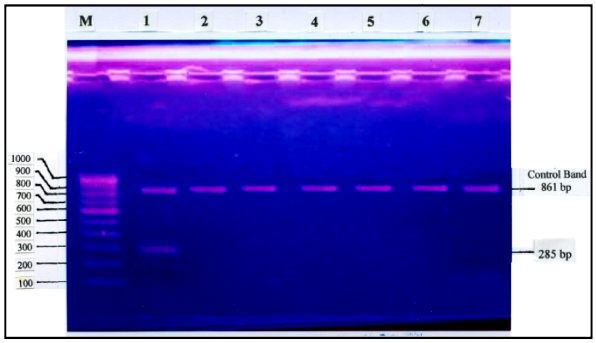

Fig. 3. Lane 1 : 285 bp fragment of IVS 1 nt(5) (G-C) beta-thalassemia mutation

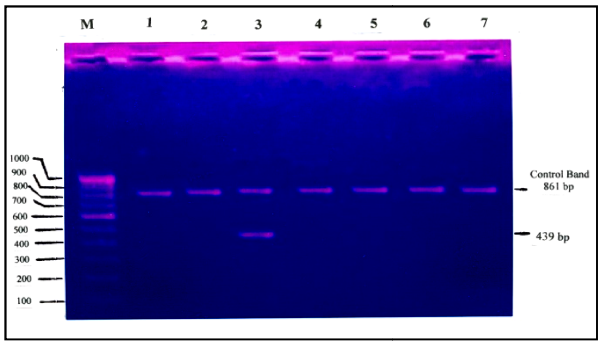

Fig. 4. Lane 3 : 439 bp fragment of Fr 41-42 (-TTCT) beta-thalassemia mutation

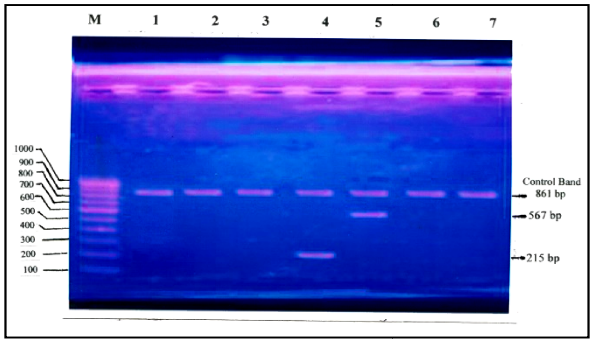

Fig. 5. Lane 4: 215 bp fragment of Fr 8-9 (+G) beta-thalassemia mutation; Lane 5 : 567 bp fragment of Cap +1 (A-C) beta- thalassemia mutation in, a case of Compound heterozygosity.

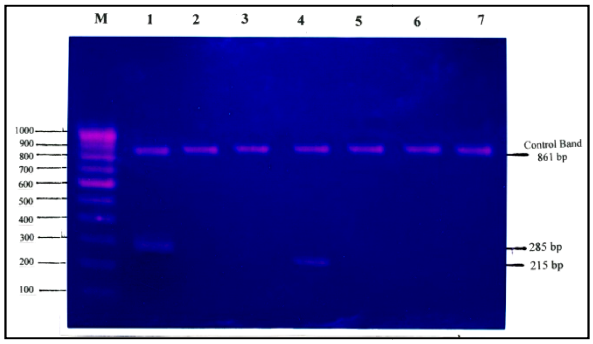

Fig. 6. Lane 1: 285 bp fragment of IVS 1nt(5) (G→C) beta-thalassemia mutation and Lane 4 shows 215 bp fragment of Fr 8-9 (+G) beta-thalassemia mutation, a case of Compound heterozygosity.

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