IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 3 Ver. II (May - Jun. 2015), PP 13-16
www.iosrjournals.org

Characterization of β-thalassemia mutations from north Maharashtra region

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Abstract: *Beta thalassemia is a serious public health problem in India. Due to lack of awareness among affected population and facilities for thalassemia testing, carrier frequency in some communities is high.In this report, the spectrum of β-thalassemia mutations were defined in patients of β-thalassemia major and carriers with varying disease severity in north Maharashtra region of India (mainly from the Dhule and Nandurbar districts) by the amplification refractory mutation system (ARMS). The six most common β-thalassemia mutations were detected, which included IVSI-5 (G–C), IVSI-1 (G–T), codon 8/9 (+G), codon 15 (G–A), codon 41/42 (−TCTT) and 619 bp deletion. These accounted for 85% in 93 patients of β-thalassemia major and carriers and 15% remained uncharacterized in these patients. The most common mutation was found to be IVSI-5 (G–C) in 10 (27.8%) out of 36 β-thalassemia major, and 21 (36.8%) out of 57 β-thalassemia carriers. Followed by codon 41/42 (–TCTT) and codon 8/9 (+G), the overall frequencies reported for these two mutations were 15.1% and 14.0% respectively. Fourteen cases did not show any of six mutations. The β-thalassemia mutation patterns revealed from this study not only help affected families but also health care system for making future plans to reduce the incidence by proper counseling.*
Keywords: *β-thalassemia major, β-thalassemia carrier, ARMS-PCR, globin gene, IVSI-5 (G–C), IVSI-1 (G–T)*

I. Introduction

In India, β-thalassemia is the most common autosomal recessive disorder characterized by the reduced or absent expression of the β-globin gene, leading to an imbalance of α and β-globin chains [1]. It has been estimated that the average incidence of β-thalassemia trait in India is 3.3% with 1-2 per 1,000 couples being at risk of having an affected offspring each year.

The disease is quantitative deficiency of β-globin chain production and is usually due to mutations on β-globin gene clustered on chromosome 11. More than 300 mutations of the β-globin gene have been characterized all over the world, though only a few specific of these mutations reported for each population. As the population of India is very diverse and ethnically complex, each region and ethnic group of the country has its own distinct set of mutations [2, 3]. Approximately there are twenty-two β-thalassemia mutations that have been documented in India and six common mutations viz. codon 8/9 (+G), codon 15 (G–A), codon 41/42 (–TCTT), IVSI-1 (G–T), IVSI-5 (G–C), and 619 bp deletion accounted for about 80%.

In this regard, the north Maharashtra region of the country is very poorly characterized. Here the sickle cell disease is very common along with β-thalassemia. Sickle cell disease in combination with β-thalassemia produces double heterozygous sickle/β-thalassemia, an uncommon form of the disease in this region. In order to characterize the spectrum of β-thalassemia mutations in the homozygous and heterozygous β-thalassemia, we have studied 93 patients.

The mutation analysis was performed by polymerase chain reaction (PCR)-based method of the amplification refractory mutation system (ARMS) for the five β-thalassemia mutations, namely codon 8/9 (+G), codon 15 (G–A), codon 41/42 (–TCTT), IVSI-1 (G–T) and IVSI-5 (G–C). This type of study definitely provides a sound platform to health care systems for genetic counseling.

II. Material and methods

The sample consisted of 93 clinically proved β-thalassemia major and minor patients reported from the Government Civil Hospital, Dhule. Of the total number of individuals studied, 36 were β-thalassemia major (mostly children with age ranges from 6 years to 15 years) patients who came here for blood transfusion and iron chelation from different parts of both the districts. Remaining 57 patients were β-thalassemia minor; who diagnosed under family study mostly. The diagnosis of carriers was based on HbA_2 estimation by HPLC, if it was >3.5% included in the study. Control blood samples were collected from different communities with informed consent.

DNA was extracted from peripheral blood lymphocytes by the kit and manual provided by Bangalore Genei. Analysis of mutations was carried out using the ARMS-PCR as described by Old et al [4]. Table 1 shows the list of primer including mutation-specific ARMS primers, control primers, and common primers used to

diagnose the six common β-thalassemia mutations in this study. There were four primers used in each reaction, of which two primers serve as internal control. Control C and control Dare internal control primers, which amplifies a part of β-globin gene at the 3' end. The 619 bp deletion is a common mutation in Indian subcontinent located between these two internal control primers, if the 619 bp deletion mutation is present it forms 242 bp PCR product with 861 bp control band in heterozygotes and without 861 bp control band in homozygotes.

For each PCR a total volume of 25 µl of reaction mixture was carried out, which contained 100 ng of genomic DNA, 1.0 µM of each primer, 1.5 mM $MgCl_2$; 200 µM deoxynucleotides triphosphates (dNTPs), and 2.5 U Taq DNA polymerase. The cycling was carried out on the thermal cycler with an initial denaturation at 95 ^{0}C for 5 min, followed by 30 cycles at 94 ^{0}C for 30 s, annealing at 65 ^{0}C for 1 min, and 72 ^{0}C for 1 min 30 s and the final extension at 72 ^{0}C for 5 min. The products were electrophoresed with 2.0% Agarose gel in 1X Tris-borate-EDTA buffer for 1 h at 100 volts. The gel was stained with ethidium bromide and visualized under a transilluminator. The evaluation of band patterns was performed by comparing it to the control. The following photograph shows the agarose gel electrophoresis pattern of ARMS-PCR for different mutations.

Table 1 Sequence of mutation specific, common and control primers

β-thalassemia mutation	Primer sequence 5'-3'	Used with common primer	Product size (bp)
IVSI-5 (G-C)	CTCCTTAAACCTGTCTTGTAACCTTGTTAG	A	285
IVSI-1 (G-T)	TTAAACCTGTCTTGTAACCTTGATACGAAA	A	450
Cd 8/9 (+G)	CCTTGCCCCACAGGGCAGTAACGGCACACC	A	225
Cd 41/42 (–TTCT)	GAGTGGACAGATCCCCAAAGGACTCAACCT	A	439
Cd 15 (G-A)	TGAGGAGAAGTCTGCCGTTACTGCCCAGTA	B	500
Common/control primer			
Common A	ACCTCACCCTGTGGAGCCAC	-	-
Common B	CCCCTTCCTATGACATGAACTTAA	-	-
Control C	CAATGTATCATGCCTCTTTGCACC	-	861
Control D	GAGTCAAGGCTGAGAGATGCAGGA	-	-

III. Results

A total of 93 β-thalassemia patients were included for the detection of common β-globin gene mutations, of which 36 were homozygous β-thalassemia and 57 were heterozygous β-thalassemia. Out of 36 homozygotes it was able to found mutations in 31 (86.1%) cases and remaining 5 (13.9%) cases were remained uncharacterized. In this group the most common mutation was IVSI-5 (G–C), a mutation of Asian-Indian origin was found in 10 (27.8%) subjects, followed by codon 8/9 (+G) an another Asian-Indian mutation at frequency of 19.4% in 7 cases. The mutation IVSI-1 (G–T) a Mediterranean mutation and codon 41/42 (–TCTT) were detected in 5 (13.9%) cases each, remaining four cases (11.1%) were detected to have codon 15 (G–A), mutation.

Of the 57 heterozygous β-thalassemia cases, it was able to detect mutations in 48 (84.2%) subjects; nine (15.8%) cases were remained uncharacterized. In this group, IVSI-5 (G–C) was the most common mutation detected in 21 (36.8%) cases, followed by codon 41/42 (–TCTT) mutation detected in 9 (15.8%) subjects. The mutations detected in remaining cases are presented in decreasing frequency: codon 8/9 (+G) in 6 (10.5%) cases, IVSI-1 (G–T) in 5 (8.8%), 619 bp deletion in 4 (7.0%) and codon 15 (G–A) in 3 (5.3%) cases. A 619 bp deletion was detected by the presence of 242 bp band along with 861 bp internal control band; presence of 861 bp internal control band represents the heterozygosity for the deletion. The PCR was repeated with internal control primers C and D only in case of 619 bp deletion for differentiation between codon 8/9 (+G) mutation as it produces 225 bpband. The mutational analysis results of both β-thalassemia major and carriers are depicted in Table 2, and Fig. represents the agarose gel showing band pattern of six common β-thalassemia mutations.

Table 2 Prevalence of β-thalassemia mutations in β-thalassemia major and carriers

Mutation	β-thalassemia major (36)		β-thalassemia carrier (57)		Total
	No. of subjects	Percentage	No. of subjects	Percentage	
IVSI-5 (G-C)	10	27.8	21	36.8	31 (33.3%)
IVSI-1 (G-T)	5	13.9	5	8.8	10 (10.8%)
Cd 8/9 (+G)	7	19.4	6	10.5	13 (14.0%)
Cd 41/42 (–TTCT)	5	13.9	9	15.8	14 (15.1%)
Cd 15 (G-A)	4	11.1	3	5.3	7 (7.5%)
619 bp deletion	-	-	4	7.0	4 (4.3%)
Uncharacterized	5	13.9	9	15.8	14 (15.1%)
Total	36	100	57	100	93 (100%)

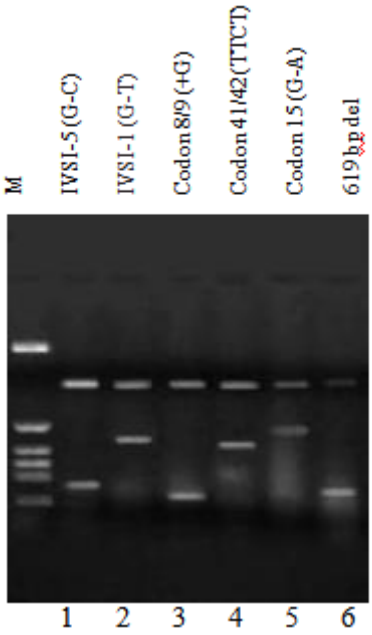

Fig. Ethidium bromide-stained agarose gel of ARMS-PCR for six common Asian Indian β-thalassemia mutations, lanes 1-6; mutation specific bands with 861 internal control bands which indicate proper reaction condition. M; molecular marker pBRHinf I digest

IV. Discussion

β-thalassemia is a common heterogeneous inherited disorder in India caused by the reduced or absent synthesis of β-globin. As no viable forms of treatment are available, identification of carriers and prenatal diagnosis remained the best form of management. The molecular basis of β-thalassemia in north Maharashtra region was investigated by ARMS-PCR. Five different β-thalassemia mutations were detected in 86.1% of the total 36 β-thalassemia major patients and six mutations in 84.2% of the total 57 β-thalassemia carriers (Table 2). Among all the β-thalassemia mutation detected, IVSI-5 (G–C) is the most common mutation, occurring at percentage of 27.8 in β-thalassemia major and 36.8 in β-thalassemia carriers. This is totally in agreement with previous studies, as the high frequency of IVSI-5 (G–C) mutation was reported in many parts of India such as Punjab, Gujarat, Maharashtra, and southern India [5-7]. The incidence of IVSI-5 mutation was reported to be 65% in north and south western Maharashtra [7, 8]. However, striking differences in frequencies between different regions of Maharashtra for these common Indian mutations were reported. The possible reason for this would be the different study population, practice of endogamy and affection towards consanguineous marriages in some groups.

The study has revealed the presence of codon 41/42 (–TCTT) mutation in five β-thalassemia major and nine β-thalassemia minor cases, which is comparatively common in many parts of the country. The frequency of this mutation was observed throughout India, 20 % in Bengal, 10 % in Tamilnadu, 7.2 % in Maharashtra, 9 % in Haryana, 3 % in Uttar Pradesh, 6 % in eastern India, 5 % in Punjab, and 2 % in western India [2, 9].

IVSI-1 (G–T) is another common mutation among Asian Indians[3]. It was observed in five cases of both β-thalassemia major and minor patients. The overall frequency (10.8%) was found to be quite comparable to the previous studies from different parts of India, Haryana (10 %), Uttar Pradesh (11 %), eastern India (11 %), western India 20 % and 9.52 % in south western Maharashtra [6, 10, 11].

Codon 8/9 (+G) was the second common mutation present in seven (19.4% %) β-thalassemia major patients and six cases of β-thalassemia minor subjects. The overall frequency (13.9%) of this mutation is comparable with the frequency reported from north India by Madan et al [12]. However, the study of Colah et al [7] has revealed the complete absence of this mutation from Khandesh region. This mutation has also been reported from different regions of the country like from western India it was 6.05% and from western Maharashtra it was 6.34% [8, 13].

In the present study, the overall prevalence of codon 15 (G–A) mutation was 7.5%, which was also reported in earlier studies, but the frequencies were quite variable due to different areas and population chosen. Earlier studies have reported the high prevalence (18.0%) of this mutation from north Maharashtra [7] and 5.65% in the population of Gujarat [13].

The present study has revealed the absence of 619 bp deletion from β-thalassemia major group and reported in four cases of β-thalassemia minor group. Earlier it has been shown that the prevalence of this

mutation was high in Vidharba region of Maharashtra as compared to Khandesh region [13]. Earlier studies from Gujarat also showed considerable high frequency of this mutation [13, 14].

V. Conclusion

Characterization of the mutation patterns revealed from such study will enable health authorities to know the burden of disease in north Maharashtra region and provide the basis for prenatal diagnosis and genetic counseling of affected individuals.

Acknowledgments

The authors are thankful to the Dean and faculty of Pediatric Department Govt. Civil Hospital, Dhule for their assistance. The authors are also thankful to the technical staff for their cooperation and University Grant Commission, New Delhi (Maulana Azad National Fellowship for minority students) for financial support.

References

[1]. D. J. Weatherall, andJ. B. Clegg, Inherited haemoglobin disorders: an increasing global health problem,Bull WHO,(79),2001, 704-712.
[2]. N. Y. Varawalla, J. M. Old, R.Sarkar,R. Venkatesan, and D. J.Weatherall, The spectrum of beta thalassemia mutation on the Indian subcontinent: the basis of prenatal diagnosis,Brit J Haematol,78(2), 1991,242-247.
[3]. N. Y. Varawalla, J. M. Old, andD. J. Weatherall, Rare β-thalassemia mutations in Asian Indians,Brit J Haematol, 79,1991, 640-644.
[4]. J. M. Old, N. Y.Varawalla, andD. J. Weatherall, The rapid detection and prenatal diagnosis of β-thalassemia in the Asian Indian and Cypriot populations in the UK,Lancet,336,1990, 834-837.
[5]. A. Bandyopadhyay,S. Bandyopadhyay, M. D. Chowdhury, and U. B. Dasgupta, Major β-globin gene mutations in Eastern India and their associatedhaplotypes,Hum Hered, 49, 1999, 232-235.
[6]. I. C. Verma, R. Saxena, E. Thomas, and P. K. Jain,Regional distribution of beta-thalassemia mutations in India,Hum Genet, 100(1),1997, 109-113.
[7]. R. B. Colah, A. Gorakshakar, S. Phanasgaonkar, E. D'Souza, A. Nadkarni, R. Surve, P. Sawant, D. Master, R. Patel, K. Ghosh, and D. Mohanty,Epidemiology of beta-thalassaemia in Western India: mapping the frequencies and mutations in sub-regions of Maharashtra and Gujarat, Brit J Haematol, 149(5), 2010, 739-747.
[8]. S. B. Satpute, M. P. Bankar, and A. A. Momin, The prevalence of β-thalassemia mutations in south western Maharashtra,Ind J ClinBiochem, 27, 2012, 389-393.
[9]. I. Panigrahi, R. K. and Marwaha, Mutational spectrum of thalassemia in India,Ind J Hum Genet, 13(1), 2007, 36-37.
[10]. E. F. Vaz, C. B. Thakur, M. K. Banerjee, andS. G. Gangal, Distribution of beta thalassemia mutation in the Indian population referred to diagnostic centre,Hemoglobin, 24, 2000, 181-194.
[11]. S. Agarwal, M. Naveed, U. R. Gupta, P. Kishore, and S. S. Agarwal, Characterization of beta thalassemia mutation in 57 beta thalassemia families seen at Lucknow,Ind J Med Res, 100,1994, 106-110.
[12]. N. Madan, S. Sharma, U. Rusia, S. Sen, and S. K. Sood, Beta thalassemia in northern India (Delhi),Ind J Med Res, 107, 1998, 134-141.
[13]. J. J. Sheth, F. J. Sheth, P. Pandya, R. Priya, S. Davla, C. Thakur, and V.Flavin, Beta thalassaemia mutations in western India,Ind J Pediatr, 6, 2008, 567-570.
[14]. N. Nigam, N. Munshi, M. Patel, andA. Soni, Distribution of beta thalassemia mutation and its correlation with alpha thalassemia in Gujarati families,Int J Hum Genet, 3(4),2003, 221-224.