The Prevalence Of β -Thalassemia Mutations in South Western Maharashtra

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Abstract Thalassemia has been recognized by the World Health Organization as important inherited disorders principally impacting on the populations of low income countries. In this report, the prevalence of common β -thalassemia mutations in India was defined in 126 β -thalassemia carrier subjects in a western Indian population mainly from the south-western Maharashtra. The six most common β -thalassemia mutations were detected, which included IVS I-5 (G–C), IVS I-1 (G–T), codon 8–9 (+G), codon 41/42 (–TCTT), Codon 15 (G–A), and 619 bp deletion at 3' end of β -globin gene. These mutations accounted for 93.66 % in 126 β -thalassemia carrier subjects and 6.34 % remained uncharacterized. Out of 126, 82 (65.07 %) showed the most common (prevalent) type of mutation, IVS I-5 (G–C), followed by IVS I-1 (G–T) showed by 12 (9.52 %) subjects. Three (2.38 %) subjects showed 619 bp deletion, codon 8/9 (+G) and codon 15 (G–A) mutations were present in eight subjects each (6.34 %). Only five (3.96 %) subjects showed codon 41/42 (–TCTT). There were eight (6.34 %) subjects where

mutation was not any of the six mutations studied. This study provides the pattern of β thalassemia mutations from south-western Maharashtra, which will help to prevent β -thalassemia using prenatal diagnosis and proper counseling.

Keywords β -Thalassemia · South-western Maharashtra · Mutations · β -Thalassemia carrier · Prenatal diagnosis

Introduction

β -Thalassemia is a highly prevalent 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 prevalence of pathological hemoglobinopathies in India is 1.2/1,000 live births [2], and with approximately 27 million births per year this would suggest the annual birth of 32,400 babies with a serious hemoglobin disorders. Within this overall disease classification, 1989 WHO Working Group on, guidelines for the control of hemoglobin disorders estimated 3.9 % carrier frequency for β -thalassemia in India [3].

The β -globin gene families are clustered on chromosome 11 and are arranged over approximately 60,000 nucleotide bases. β -thalassemia is a quantitative deficiency of β -globin production, and are usually due to DNA mutations of the β -globin gene cluster and result from mutations affecting gene transcription, RNA processing, alter splice junctions or splice consensus sequences, mutations within exons and introns that create an alternative splice site. Majority of the mutations are point mutations and unlike α -thalassemia deletion mutations are relatively less frequent [4].

About the 3 % of the world's population carries the β -thalassemia gene. The bulk of this disorder is reported from

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Mediterranean, African and south-east Asian populations where the incidence of gene carriage may be as high as 10 % [4]. In India, β -thalassemia is the most common monogenic disorder. The average incidence of β -thalassemia trait in India is 3.3 % with 1–2 per 1,000 couple being at risk of having an affected offspring each year. The disease is characterized by its genetic heterogeneity at the molecular level, and more than 300 mutations of the β -globin gene have been characterized all over the world. Most are small nucleotide substitutions within the cluster. All mutations result in either absence of synthesis of β -globin chains (β^0 -thalassemia) or a reduction in synthesis (β^+ -thalassemia) [5, 6].

India has multiple geographical, ethnic, religious, and language divisions, [7] though each population seems to harbor only a few of these mutations. As the ethnic composition of the Indian population is varied, complex [8] and each region of the country have its own distinct set of mutations [3, 9, 10].

There are few β -thalassemia mutations which are common in India. Twenty-two β -thalassemia mutations have been documented by various authors in Indian patients with β -thalassemia. Six mutations, codon 8–9 (+G), Codon 15 (G–A), codon 41/42 (–TCTT), IVS I-1 (G–T), IVS I-5 (G–C), and 619 bp deletion at 3' end of β -globin gene, account for about 80 % of β -thalassemia mutation in Indian population [4].

Within each geographic population there are a few common mutations together with a few rare ones, which are responsible for over 90 % of β -thalassemia mutations. We undertook this study, because there was no study reporting the mutational spectrum from the south-western Maharashtra region. Identification of the exact mutation helps to define the severity of the phenotype, plan therapy and it was hoped that these observations might form the basis for a comprehensive diagnostic database that would be useful not only for genetic counseling but for prenatal diagnosis also.

Materials and Methods

A descriptive cross sectional study was carried out in the Department of Biochemistry, Government Medical College, Miraj from July 2010 to September 2011. A total of 126 β -thalassemia carrier subjects were included from south-western Maharashtra and adjoining region comprising Satara, Sangli, and Kolhapur districts from the state of Maharashtra and Belgaum & Hubali districts from the State of Karnataka. There are large number of patients who come from these places for medical treatment, to the Hospital(s) at Miraj and Sangli.

Subjects

This study was approved by the Institutional ethical committee, Government medical college, Miraj, Sangli, Maharashtra, India. The study sample consisted of 126 β -thalassemia carrier subjects. The parent of known β -thalassemia major patients were included in the present study and their β -thalassemia carrier status was confirmed by Hb A₂ level, if Hb A₂ was >3.5 % then the subjects were included for mutational analysis. Subjects with Hb A₂ if <3.5 % were excluded from this study. Family members other than parents of known thalassemia major child, who were β -thalassemia trait, were excluded from mutational studies.

DNA Analysis

Genomic DNA was extracted from peripheral blood lymphocytes by phenol chloroform extraction method [11]. Mutational analysis was carried out using the amplification refractory mutation system polymerase chain reaction (ARMS-PCR) as described [12]. The mutation-specific ARMS primers, control primers, and common primers used to diagnose the six common β -thalassemia mutations in this study are listed in Table 1. For each reaction there

Table 1 The mutation-specific ARMS primers, control primers, and common primers for six mutations included in the study

Sr. No.	Mutation specific/common/control primer	Primer sequence (5'–3')	Common primer to be used	Length of PCR product (bp)
1	Common A	CCCCTTCCTATGACATGAACTTAA	–	–
2	Common B	ACCTCACCTGTGGAGCCAC	–	–
3	Control C	GAGTCAAGGCTGAGAGATGCAGGA	–	861
4	Control D	CAATGTATCATGCCTCTTTGCACC	–	–
5	IVS I-5 (G → C)	CTCCTTAAACCTGTCTTGTAACCTTGTTAG	B	285
6	IVS I-1 (G → T)	TTAAACCTGTCTTGTAACCTTGATACGAAA	B	281
7	Cd 8/9 (+G)	CCTTGCCCCACAGGGCAGTAACGGCACACC	B	225
8	Cd 41/42 (–TCTT)	GAGTGGACAGATCCCCAAAGGACTCAACCT	B	439
9	Cd 15 (G → A)	TGAGGAGAAGTCTGCCGTTACTGCCAGTA	A	500

were total four primers used, two of these four primers serve as internal control. Control C and control D primers serve as internal control primers, which amplifies a part of β -globin gene at the 3' end. These internal control primers also encompasses the 619 bp deletion common in the Indian Subcontinent, if the 619 bp deletion mutation is present it forms 242 bp PCR product. The PCR was carried out in a total volume of 25 μ l of reaction mixture, containing 100 ng of genomic DNA, 1.0 μ M of each primer, 1.5 mM $MgCl_2$; 200 μ M deoxynucleotides triphosphates, and 2.5 U Taq DNA polymerase. Cycling was carried out on the thermal cycler with an initial 5 min denaturation step at 95 °C, followed by 30 cycles at 94 °C for 30 s, 1 min at 65 °C annealing temperature, and 72 °C for 1 min 30 s and the final extension at 72 °C for 5 min. Following amplification, 10 μ l aliquots were mixed with gel loading buffer and electrophoresed on 2 % agarose gel in Tris–borate–EDTA buffer for 1 h at 100 volts, gel was stained with ethidium bromide and visualized using UV transilluminator. The 3' region of the DNA is used as an internal control [13–15]. The experiment was performed twice for each group. The photograph below shows agarose gel electrophoresis pattern of ARMS-PCR for analysis of IVS I-5 mutation.

techniques (ARMS) we studied six common mutations from India.

Blood samples of β -thalassemia carrier patients were collected and subjected to DNA isolation and mutational analysis. Out of 126 subjects of β -thalassemia trait, the G > C substitution at IVSI-5, a mutation of Asian-Indian origin, is the most common mutation reported in this study, a total of 82 (65.07 %) subjects were reported as IVS I-5 (G–C) mutation, a Mediterranean mutation IVS I-1(G–T) of lower frequency, was found in 12 (9.52 %) subjects. Asian-Indian mutation, Codon 8/9 (+G) was found at low frequency present in eight subjects (6.34 %). Codon 15 (G–A) an another Asian-Indian mutation, clustered around countries of the Gulf, but also found in Syria and Israeli Arabs, found in eight (6.34 %) subjects. There were five (3.96 %) subjects who showed mutation of codon 41/42 (–TCTT). 619 bp deletion of Indian origin was found in three subjects (2.38 %). There were eight (6.34 %) subjects who did not show any of the six mutations studied. Tables 2 shows mutational analysis of subjects, carrier for β -thalassemia. And Fig. 1 shows the graphical presentation of the six common mutations of β -thalassemia carrier subjects from south-western Maharashtra.

Figure: Photograph showing Agarose gel electrophoresis of ARMS PCR using IVS I5 mutant specific primers. A product of 861 bp DNA fragment serves as internal control and indicative of proper reaction conditions. Presence of 285 bp fragment with IVS I5 mutant specific primer in the reaction mix indicates presence of the mutation.

Lane 1 to 3 – ARMS PCR reaction with different Test DNA samples

Lane 4 – ARMS PCR reaction with DNA sample positive for IVS I5 mutation

Lane 5 – ARMS PCR reaction with DNA sample negative for IVS I5 mutation

Lane 6 – Marker ladder, pBR 322 Hinf I digest

Results

In the present study, ARMS-PCR based analysis has been standardized for detection of common β -globin mutations. Mutational analysis was performed on 126 subjects who were proven carriers for β -thalassemia either during the screening or parents of known β -thalassemia major patients. DNA samples using PCR based

Discussion

β -Thalassemia is one of the most common single gene disorder in India and in number of other countries. In the present study, ARMS-PCR has been standardized and applied for detection of β -thalassemia mutations. The common mutations that are prevalent in the Indian subcontinent have been chosen for this study.

Table 2 Mutational analysis of β -thalassemia carrier subjects

Mutation	No. of subjects	Percentage (%)
IVS I-5 (G–C)	82	65.07
IVS I-1(G–T)	12	9.52
Codon 8/9 (+G)	8	6.34
Codon 15 (G–A)	8	6.34
Codon 41/42 (–TCTT)	5	3.96
619 bp deletion	3	2.38
Uncharacterized	8	6.34
Total	126	100

Fig. 1 The graphical presentation of the six common mutations of β -thalassemia carrier subjects from south-western Maharashtra

Out of the six mutations studied, 619 bp deletion at the 3' end of β -globin gene makes it nonfunctional. Frame shift mutations +1 codon 8/9 (+G) and –4 codon 41/42 (–TCTT) change the ribosome reading frame and causes premature termination of translation. Mutation IVS I-1 (G–T) causes change in splice junction causing ineffective RNA processing. IVS I-5 (G–C) destroys consensus sequences around splice junction which are essential for splicing. Mutation at codon 15 (G–A) causes a nonsense codon, terminating the synthesis of β -globin at a premature stage [16].

In the present study two mutations accounted for 94 subjects (74.60 %) out of total 126 subjects, these two mutations were IVS I-5 (G–C), and IVS I-1 (G–T). The other mutations namely, codon 8/9 (+G) and codon 15 (G–A) were present in eight subjects each, codon 41/42 (–TCTT) was present in five subjects and 619 bp deletion was found to be present in three subjects. There were eight (6.34 %) subjects who did not show any of the six mutations studied.

IVS I-5 (G–C) substitution which is β^+ mutation was found to be the most frequent mutation in our study. This is totally in agreement with previous studies, reported from India and the Indian sub-continent. In Maharashtra, incidence of IVS I-5 mutation was reported to be 54 % [13, 17]. The incidence of this mutation reported at various regions from Indian sub-continent is, from Sindh region 12 %, Punjab 38 %, Gujarat 41 %, Bengal 60 %, and from Tamilnadu 81 %. The incidence reported from Tamilnadu is the highest reported incidence from any of the regions. Similar results were observed from Haryana (57 %), Uttar Pradesh (58 %), and eastern India (72 %) which correlate with our studies [12, 18, 19].

IVS I-1 (G–T) is one of the common mutation among Asian Indians [13] and phenotype is of β^+ thalassemia. It is second common mutation which is observed in our study in 12 (9.52 %) subjects. The frequency was found to be similar to the previous studies from different geographic regions in India, Haryana (10 %), Uttar Pradesh (11 %), Eastern India (11 %), and southern India (5 %). In western India it was reported to be 20 % [20–22].

Codon 41/42 (–TCTT) mutation is one of the common mutations found in the Indian subcontinent. In our study the mutation was found in five subjects (3.96 %) out of 126 subjects. Incidence of this mutation was observed throughout India, from north-west Pakistan to Bangladesh and Bengal, and from Punjab to Tamilnadu. The incidence of this mutation was found to be 20 % in Bengal, 10 % in Tamilnadu, in Maharashtra 7.2 %, in Haryana it was 9 %, 3 % in Uttar Pradesh, 6 % in Eastern India, 5 % in Punjab, and 2 % in western India [12, 19].

Codon 8/9 (+G) mutation was present in eight (6.34 %) subjects out of total 126 subjects studied. This mutation has been reported from northern India (11 %) and from Gujarat (8.6 %) [15, 22, 23]. Varawalla et al. [12] reported incidence of this mutation from Sindh, Punjab, Gujarat, and Tamilnadu region.

In the present study, codon 15(G–A) mutation was detected in eight (6.3 %) subjects. The percent prevalence of codon 15 (G–A) was 2 % in Haryana, 11 % in Punjab, 6 % in Uttar Pradesh. In Gujarat it was 1.5 %, and in Maharashtra it was 18 % [12, 19, 20].

619 bp deletion was found only in three (2.3 %) of total subjects studied. This mutation was seen in Asian-Indians originally coming from Gujarat and Punjab [24]. Spectrum of 619 bp deletion mutation in India was found to be 16 % in Haryana, 14 % in Punjab, 5 % Uttar Pradesh, 2 % eastern India, 5 % in southern India and in western India it was found to be 8 % [20, 21]. Since this mutation originates from Punjab and Gujarat and the presence of these communities is relatively low and scattered in south-western Maharashtra region, incidence of this mutation may be low.

Since there is no proper screening program in place and usually the high risk couple (with a previous known thalassemia baby) approaches to the clinician for the sake of prenatal diagnosis at advanced stage of pregnancy, there is less time available for the mutational workup. Present study provides true picture of the spectrum of mutations in the subjects of β -thalassemia, which is vital from the view of prenatal diagnosis.

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