Distribution and ethnic variation of â- thalassemia mutations in Nepal

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ABSTRACT

This is the first study characterizing spectrum of β-thalassemia mutations in Nepalese population. Mutations were analyzed in 22 patients using 10 sets of allele-specific primers. Five of the mutations, namely F.S 41/42 (—TCTT), IVS1 nt5 (G—C), IVS1 nt1 (G—T), 619 bp deletion and F.S 8/9 (+G), were found to constitute 87.82% of total alleles studied. F.S 41/42 (—TCTT) was the commonest mutation. —88 (C—T), Codon 16 (—C) and Codon 15 (G—A), had a combined frequency of 12.18%. Distribution of mutations causing β-thalassemia in different ethnic Nepalese groups was analyzed. The mutational profile in Nepal share several similarities with that from the two neighboring countries, India and China. Detection of more than one mutation in three cases of thalassemia trait raises the likelihood of existence of multiple mutations in *cis* in Nepalese thalassemic carriers. Such possibility has to be carefully considered while developing prenatal screening program for Nepalese population.

Keywords: β-Thalassemia, Nepalese population, amplification refractory mutation system.

INTRODUCTION

β-thalassemia is a heterogeneous group of autosomal recessive disorder characterized by reduced expression of â-globin gene. The condition is associated with excess accumulation of β-globin chain, which dominates the clinical phenotype. Clinical severity depends on the extent to which synthesis of the affected globin is impaired, altered synthesis of other globin chains, and coinheritance of abnormal globin alleles.[1] Till date, over 300 â-thalassemia alleles have been characterized in and around the β-globin gene cluster.[2] In India thalassemia is the most common monogenic disorder with a frequency of carrier ranging from 2 to 17%.[3]

The State of Nepal, bordered on north by China and on south, east as well as west by India, has an estimated population of 22,736,934.[4] According to a WHO report on Global distribution of haemoglobin disorders there are 0.2-0.99 births per 1000 infants with a major haemoglobinopathy in Nepal.[5] In the South-east Asian countries, annual total birth with β-thalssaemia is 20420, out of 9983 are transfusion dependent and annual death because of lack of transfusion in these patients is around 9021.[6] One of the major reasons for high prevalence of genetic disorders is consanguinity. Nepal has a wide variation in ethnicity, the largest of the groups being *Chhettri* (15%) and the rest composed of *Brahman*-Hill (12.74%), *Magar* (7%), *Tharu* (6.6%), *Tamang* (5.5%), *Newar* (5.4%), *Yadav* (3.9%) and others (32.7%).[3] As there had been no study to characterize the mutations in the β-globin gene from Nepalese thalassemics, we undertook this investigation in order to analyze frequency of ten common different mutations in various ethnic groups from Nepal. Interestingly, we observed significant similarities and differences with the mutations reported from neighboring countries like India and China, consistent with the fact that a substantial population in Nepal had been migrants from these nations. As this is the first study profiling β-globin mutations in the Nepalese population, it is expected to give a useful insight on the genetic diversity and migrational inheritance in this ethnically versatile nation. Knowledge of relative frequency of mutations would also lead to effective prenatal diagnosis and genetic counseling strategies, reducing the burden of thalassemia in the Nepalese population.

MATERIALS AND METHODS

This study was carried out in B.P.Koirala Institute of Health Sciences (BPKIHS), Dharan from September 2005 to September 2007 in collaboration with Institute of Medical Sciences, Banaras Hindu University, India. Twenty two cases of β thalassemia, originating from different ethnic groups of Nepal, were enrolled in this study in compliance with the guidelines of ethical committee, BPKIHS, Dharan. Subjects were divided into three groups, namely β-thalassemia major, intermedia and trait, depending on the clinical presentation,

Table-1: Spectrum of mutations in β globin gene in Nepalese population

Types of β –globin mutations	Number of alleles			
	Total (n =41)	β Thalassemia major (n = 27)	β Thalassemia intermedia (n = 3)	β Thalassemia carriers (n =11)
F.S 41/42 (–TCTT)	13 (31.71)	8 (29.63)	1 (33.33)	4 (36.37)
IVS1 nt5 (G→C)	7 (17.07)	6 (22.22)	-	1 (9.09)
IVS1 nt1 (G→T)	6 (14.64)	3 (11.11)	-	3 (27.27)
F.S 8/9 (+G)	4 (9.76)	2 (7.41)	-	2 (18.18)
619 bp deletion	6 (14.64)	4 (14.82)	2 (66.67)	
–88 (C→T)	3 (7.32)	2 (7.41)	-	1 (9.09)
Codon15 (G→A)	1 (2.43)	1 (3.7)	-	-
Codon16 (–C)	1 (2.43)	1 (3.7)	-	-

'n' signifies the number of alleles.
The numbers within the parentheses denote the percentage of each allele.

transfusion frequency and hemoglobin electrophoresis profile.

Primers for the study were procured from Integrated DNA Technologies, Inc., USA. Reagents for PCR and DNA ladder were obtained from Bangalore Genei, India. All other reagents were of analytical grade. Autoclaved Milli Q water was used throughout the study.

Blood was collected from the subjects under informed consent and analyzed for complete hematological parameters as well as hemoglobin electrophoretic profile using routine procedures.[7-9] DNA was extracted from peripheral blood leucocytes using sodium perchlorate.[10] Mutations were analyzed by polymerase chain reaction (PCR)-based amplification refractory mutation system (ARMS),[11] where the 3' nucleotides of allele-specific primers anneal specifically with either mutant or normal sequences. Genomic DNA of selected individuals was studied for the presence of ten β-thalassemia mutations, namely IVS1 nt5 (G →C), IVS1 nt1 (G →T), F.S 8/9 (+G), F.S 41/42 (–TCTT), 619 bp deletion at the 3' end, Codon 30 (G→C), Codon 15 (G→A), –88 (C→T), Codon 16 (–C), and cap+1(A→C). In parallel, DNA from these subjects were also analyzed for the presence normal alleles (absence of mutations) using specific primers. The primer sequences and conditions for PCR-ARMS have been described elsewhere.[7,8,12,13] Amplicons were separated on 10% nondenaturating polyacrylamide gels and analyzed using the Alphaimager^{TM}2200 software (Alpha Innotech Corporation, USA).

RESULTS

We have characterized mutations from twenty two cases of â thalassemia from Nepal using ten sets of allele specific primers. Total of 41 alleles were identified using these sets of allele specific primers. The findings have been summarized in Table-1 and representative gel pictures are given in Fig. 1. Five of the mutations, namely F.S 41/42 (–TCTT), IVS1 nt5 (G→C), IVS1 nt1 (G→T), 619 bp deletion and F.S 8/9 (+G), were found to constitute 87.82% of total β thalassemia mutations in this population. F.S 41/42 (–TCTT) was the commonest mutation with 31.71% share. This was followed by IVS1 nt5 (G→C) (17.07%), IVS1 nt1 (G→T) (14.64%), 619 bp deletion (14.64%) and F,S 8/9 (+G) (9.76%), respectively. The remaining mutations, namely –88 (C→T), Codon 16 (–C) and Codon 15 (G→A), had a combined frequency of 12.18%.

To consider the influence of Nepalese ethnic variation on the nature of mutations, the target population was divided in three broad ethnic groups: group A (Aboriginal people of Nepal - *Newar*, *Tharu* etc), group B (*Brahmin*, *Chettri*, *Yadav*, *Muslims* and those originating from India) and group C (other castes originating from Central Asia and Tibet like *Rai*, *Tamang*, *Limbu*, *Sherpa* and *Bhotia*) (Table-2). F.S 41/42 (–TCTT) was found to have the highest frequency in each of these ethnic groups. In group A IVS1 nt1 (G→T) shared equal frequency with F.S 41/42 (–TCTT) and rest were contributed equally by F.S 8/9 (+G) and –88 (C→T). Incidences of IVS1 nt1 (G→T) and F.S 8/9 (+G) were significantly low in other two groups. In group B IVS1 nt5 (G→C), 619 bp deletion and IVS1 nt1 (G→T) constituted 16%, 16% and 12% of

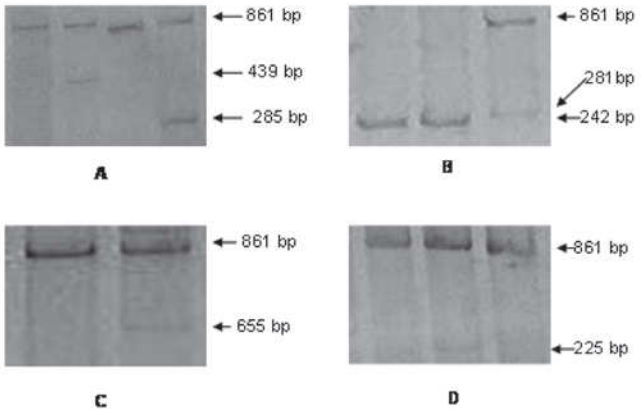

Fig. 1. Representative gel pictures analyzing mutations in β globin gene in the Nepalese population. PCR products were resolved on 10% nondenaturating polyacrylamide gels. Presence of 861 bp band signifies absence of 619 bp deletion. a, 439 and 285 bp bands represent F.S 41/42 (–TCTT) and IVS1 nt5 (G→C) mutations, respectively. b, 281 and 242 bp bands represent 619 bp deletion and IVS1 nt1 (G→T), respectively. Absence of 861 bp band reflects homozygosity of the deletion mutation. Bands corresponding to 655 bp (c) and 225 bp (d) represent –88 (C→T) and F.S 8/9 (+G) mutations, respectively

Table-2: Ethnic variation of β-thalassemia mutations in Nepal

Types of β –globin mutation	Group A (n=6)	Group B (n=25)	Group C (n=10)
FS41/42 (–TCTT)	2 (33.33)	8 (32)	3 (30)
IVS1 nt5 (G→C)	-	4 (16)	3 (30)
IVS1 nt1 (G→T)	2 (33.33)	3 (12)	1 (10)
FS8/9 (+G)	1 (16.67)	2 (8)	1 (10)
619 bp deletion	-	4 (16)	2 (20)
–88 (C→T)	1 (16.67)	2 (8)	-
Codon15 (G→A)	-	1 (4)	-
Codon16 (–C)	-	1 (4)	-

'n' signifies the number of alleles.
The numbers within the parentheses denote the percentage of each allele.

the mutations, respectively. The striking finding in this group was the presence of rare mutations like Codon 16 (—C), Codon 15 (G→A) and —88 (C→T) (Table-2). In the ethnic group C IVS1 nt1 (G→T) shared similar frequency with F.S 41/42 (—TCTT), followed by 619 bp deletion. It is worth mentioning that, the latter mutation was completely absent in group A subjects (Table-2).

Interestingly, we recorded more than one mutation per subject in 59% of target thalassemics from Nepal (13 out of 22 cases studied) by using primers against normal as well as mutation-specific alleles. Out of these 5 cases were minors, where multiple mutations were supposed to be organized in *cis*. In one interesting case of thalassemia major we detected four mutations. The patient was heterozygous for F.S 8/9 (+G) and homozygous for IVS1 nt5 (G→C), whereas other mutations present in this case were Codon 16 (—C) and —88 (C→T).

DISCUSSION

In our earlier studies we have documented β thalassemia mutations in the North Indian states bordering Nepal.[8,12] Here we have characterized the mutations in twenty two Nepalese subjects with varying ethnicity and found interesting comparisons between the two neighboring nations. The mutation F.S 41/42 (—TCTT) had the highest frequency (31.71%) among the Nepalese, whereas it varies between 2 to 15% in the Indian population and the commonest mutation is IVS1 nt5 (G→C) with frequency ranging from 27 to 85%,[8,13] while this had an overall frequency of 17% among the Nepalese (Table-1). This mutation was absent in aboriginal people of Nepal but present in other groups which are mainly the migratory population from India or Central Asia and Tibet. On the other hand, F.S 41/42 (—TCTT) remains one of the commonest mutations in China.[14] As the frequency of F.S 41/42 (—TCTT) was high across all the ethnic Nepalese groups studied, this can be an important demographic document.

The 619 bp deletion also had a distribution profile similar to IVS1 nt5 (G→C). It was characteristically absent in the aboriginal Nepalese, whereas its frequency was high (20%) among the people originating from Central Asia and Tibet. In India its frequency varies between 0 to 16%.[8,13] IVS1 nt1 (G→T) had a high frequency (33.33%), similar to F.S 41/42 (—TCTT), among aboriginal people of Nepal, with an average frequency of 14.64% across all the ethnic groups. Its incidence varies between 0 to 14% among Indian population.[13]

Our study highlights significant differences in the nature of β globin gene mutations between the Nepalese aboriginal group (group A) and the migrated population (groups B and C) (Table-2). Rare mutations like —88 (C→T), Codon 15 (G→A) and Codon 16 (—C) with respective reported frequencies of 7.32%, 2.43% and 2.43% were mostly prevalent among the group B Nepalese population. Interestingly, these mutations are extremely rare in the Indian context.[8,13] Thus, distribution of mutations in Nepalese thalassemics has unique characteristics, though it shares several similarities with their Indian and Chinese counterparts.

This is the first detailed report on the Nepalese population providing significant inputs on mutational as well as demographic profile of this country. Detection of more than one mutation in three cases of thalassemia trait raises the possibility of existence of multiple mutations in *cis* in a large percent of Nepalese thalassemic carriers. PCR-ARMS has been employed for prenatal screening/diagnosis program in different Asian Indian populations in the world. While developing such a program for Nepalese population, the likelihood of multiple mutations in *cis* in the fetus has to be carefully considered. Further, the information is expected to lead to effective prenatal diagnosis and genetic counseling strategies, reducing the burden of thalassemia in the Nepalese society.

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