Distribution of β Thalassemia Mutations and Its Correlation with α Thalassemia in Gujarati Families

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KEY WORDS β-thalassemia; genetic defects; Gujarati population; α-thalassemia

ABSTRACT We have characterized β-thalassemia mutations in 36 unrelated Gujarat (72 chromosome) families with at least one index case of severe anemia. The predominant mutations found were IVSI-5 (G-C) 61.1%, IVS I-1 (2.7%), –619bp (13.8%), CD8/9 (5.5%) and CD41/42 (8.3%). Among the less common mutations, only CD 15 (C-T) was found with a frequency of (1.3%). However, one allele of each was found for βE (CD 26 G-A) and βD (CD121 G-C) mutations. Using the amplification refractory mutation system (ARMS) technique we were able to identify mutations successfully in all the cases. We have also characterized common α-thalassemia mutations among the β-thalassemia subjects by using Gap PCR. Out of 35 thalassemia major patients 5 showed -α$^{3.7}$/αα genotype (13.8%) and 3 had -α$^{3.7}$/-α$^{3.7}$ (8.3%). These findings should prove useful for suggesting the first trimester prenatal diagnosis program based on direct mutation detection.

INTRODUCTION

β-thalassemia is an heterogeneous inherited disorder characterized by reduced or absent β-globin gene expression. This disease has a high frequency in the Mediterranean basin, Africa, Southeast Asia and the Indian subcontinent (Weatherall and Clegg 1981). The average incidence of the β-thalassemia trait in India is 3.3% with 1-2 per 1000 couples being at risk of having affected offspring each year (Varawalla et al. 1990). About 10,000 children with thalassemia major are born annually in India, constituting about 10% of the total number born in the world each year (Modell and Petrou 1983). The treatment of individuals with β-thalassemia

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major, which entails regular blood transfusions and expensive iron chelation regime, is yet not satisfactory. Thus the disease causes significant morbidity and mortality in affected individuals, making prenatal diagnosis an important option for couples at risk of having β-thalassemia major offspring. Prevention of the disease by genetic counseling and prenatal diagnosis has a particularly important role in this part of the world where there are limited resources for the medical care. Till date more than 200 mutations have been described in the β-globin gene, the success of prenatal diagnosis requires the adequate knowledge of spectrum of β-thalassemia mutations in the population. However, a subset of 5-6 mutations accounts for more than 90% cases in given geographic/ethnic area (Agarwal et al. 1994). A total of 17 different β-thalassemia mutations have been reported from Asian Indians settled abroad and in samples studied from different regions of the Indian subcontinent (North- West Pakistan, Sindh, Punjab, Tamil Nadu, Maharashtra and West Bengal and Uttar Pradesh). As few previous studies have taken into account the population of the Gujarat for the molecular characterization of the β thalassemia mutations. (Vaz et al. 2000; Parikh et al. 1994; Varawalla et al. 1991). The phenotypic severity in approximately 70% of patients with β-thalassaemia could be predicted from the nature of β-globin gene mutations. The milder picture of thalassemia major is related to a lesser imbalance of globin chain synthesis. Prenatal diagnosis based on type of β-globin gene mutations only can misdiagnose the phenotype. This milder phenotype may result from the inheritance of a mild β-thalassemia mutations resulting in a residual β chain output (Thein et al. 1993) or reduction of α chains synthesis due to

coinheritance of α-thalassemia (Camaschella et al. 1993). Therefore, an understanding of number of α gene along with the type of β-mutation provide better counseling to these patients, for prenatal diagnosis and patient care. This centre Kashiben Gordhandas Patel Children Hospital, Vadodara has been a very large for the blood transfusion for the thalassemic patients have not provided the molecular diagnosis for the patients previously. Till now, these centres have covered the near area for providing transfusion but as such no molecular diagnosis was given to the patient's. Keeping this in view we have in present paper discussed the 35 families of patients with thalassemia syndromes seen at the KGP children Hospital, Vadodara, Gujarat for DNA analysis.

MATERIALS AND METHODS

A total of 123 subjects including TM (n=36) patients, and their parents and siblings (n=87) from unrelated Gujarati families were included in this study.

Clinical Presentations: The patient presented with severe anemia and marked pallor and the mean age of presentation was 12.7 months. Most of these patients received transfusion 10 to 15 times per year, but few of them were transfused at irregular intervals due to economic and other constraints.

Hematological Anlysis: It was carried out on Sysmax-800 automated cell counter. HbA_2 levels were estimated by DE-Cellulose micro-column chromatography (Schleider et al. 1977). Alkali denaturation test (Betki et al. 1959) was used for quantitation of HbF.

Genomic DNA Analysis: DNA was extracted from the peripheral blood (Poncz et al 1989). Identification of β-thalassemia (β^{Th}) mutations was done by a PCR method based on allele specific priming, which is called the amplification refractory mutation system (ARMS) (Old et al. 1990). The common deletion α thalassemia mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$) were detected by Gap- PCR technique (Smetanina et al. 1996; Baysal et al. 1994). The primers were synthesized from the Genosys, USA.

RESULTS

β Thalassemia Mutations Analysis: A rapid detection of mutation by ARMS was performed in all the 123 samples for nine common mutations. The ARMS-PCR confirmed that among 123 subjects, 36 were the homozygous or compound heterozygous TM patients and 87 were identified as the carriers. We have taken only 72 β^{Th} and β variant (β^{var}) chromosomes in account, which were contributed by βTM patients. The IVS1-5 (G-C) mutation was found in majority of chromosomes (61%) and this was followed by IVS I-1 (2.7%), –619bp (13.8%), CD8/9 (5.5%) and CD41/42 (8.3).Among the less common mutations only CD 15 (-CT) was found in our Gujarati subjects and among beta chain variants β^E and β^D was found on one chromosome each. Thus, out of the 72 chromosomes, β^{Th} alleles were found on 70 and Hb variant were found on 2 chromosomes. By using ARMS-PCR, mutations were successfully identified in 100% (72 alleles) of subjects included in our study.

In total of 36 βTM patient 28 were homozygous, 6 were compound heterozygous for β^{Th} alleles and 2 were Hb variant/β-thal shown in table1. The predominant mutation in Gujarati population is IVS 1-5 (G-C) and majority of our studied patients (52.7%) were found homozygous for this mutation while, 9 (25%) patients were observed homozygous for 4 other common mutation viz: IVS1-1(G-T), -619 bp deletion, Co8/9(+G) and Co41/42(-CTTT). Rest of 8 (22.2%) were compound heterozygous for different β^{Th} mutations.

Table1: Distribution of β and α globin gene mutations in Gujarati patients

β-genotype of patients	No. (%)	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{3.7}/-\alpha^{3.7}$
IVS1-5 (G-C)/ IVS1-5 (G-C)	19	1	2
IVS1-5 (G-C)/ Co26	1	1	-
IVS1-5 (G-C)/-619bp	2	-	-
IVS1-5 (G-C)/Co15	1	-	-
IVS1-5 (G-C)/Co121	1	-	-
IVS1-5 (G-C)/Co41/42	1	1	1
IVS1-1(G-T)/ IVS1-1(G-T)	2	-	-
Co41/42/ Co41/42	2	-	-
-619bp/-619bp	4	1	1
Co8/9/Co8/9	1	-	-
Co8/9/ Co41/42 19	1	1	-

α-Genotype Analysis: Gap-PCR for common deletion mutation showed the presence of only $-\alpha^{3.7}$ kb deletion in Gujarati population. Out of 36 β-thalassemia major patients 5 showed $-\alpha^{3.7}/\alpha\alpha$ (13.8%) and 3 had (8.3%) $-\alpha^{3.7}/-\alpha^{3.7}$ genotype.

None of our subject was found positive for -α4.2 kb deletion. Rest of the individuals were found with a normal alpha genotype αα/αα.

DISCUSSION

Majority of beta thalassemia carriers and their parents included in our study were migrants from Pakistan who had settled in the western part of India. The area of Pakistan extending from the Karachi and Sindh in the west to the North–West Frontier province in the eastern part of Pakistan appears falls in the zone of the beta thalassemia gene. The disease is therefore quite prevalent in this belt. As expected, the most common mutation was IVS 1-5 (G-C), the only one in the β+ category (61.1%). This is in concordance with previously published reports which suggested 41 % and 60 % prevalence of this mutation (Varawalla et al. 1991; Garewal et al. 1994) in the population of Gujarati and Maharashtrians respectively. The present study describes the prevalence of five common mutations in 96% of β-thalassemia chromosome among Gujaratis, which is quite similar to the other reported figures viz: 88.1% (Thein et al. 1988), 89% (Parikh et al. 1990), 93.6% (Varawalla et al. 1991) and 88% (Vaz et al. 2000) in Gujarati population. However, this is the first study which describes the presence of less common mutations and structural Hb variant (β^D) in Gujarati population.

An important observation from our study shows that in Gujarat inspite of a lot of complexity, certain mutations are still confined to certain caste groups. For an example, the IVS I-1 mutation was mostly found in the Punjabi families whereas the -619bp del was common in the Muslim community. A study from Punjab (Garewal et al. 1994) also reported that in Punjabi Indians the IVS I-1 mutation accounted for nearly one quarter of β-thal genes. This suggests that inbreeding between the same castes, a system prevalent in India has kept the gene pools conserved without much outflow. A caste-based distribution of mutations is found to be helpful in providing diagnosis in individual families. But for a cosmopolitan population like that of Gujarati where there is rapid intermixing of the gene pools, the mutation detection should be a continuously ongoing project.

The table 1 shows presence of common α thalassemia deletion mutations, in heterozygous (-α3.7/αα) condition in 5 patients (13.8%) and homozygous (-α3.7/-α3.7) conditions in 4 patients (11%), but in none of them it was found to ameliorate the clinical severity as reported (Garewal et al 1994). Most of the previous studies have shown that the concomitant α gene mutations reduce globin chain imbalance leading to a mild phenotype. Patra et al. (1983) gave the frequency of α thalassemia (2.1%) in tribals and (22.9%) in non-tribals of Gujarat, Mukherjea et al. (1984) have reported 0.0%, 4.0%, and 6.5% prevalence of α thalassemia deletion mutations in Muslims, lower caste and higher caste of Baroda respectively. However the reported prevalence of α-thalassemia gene is very high (69.9%) by Mukherjea et al. (1984) in Gujarati tribe. According to Labie et al. (1989) the α-genotyping of the Gujarat tribes reveals the prevalence of about 95.0%.

Thus, the present study extends the observations of previous workers and provides information on the distribution of the β-thalassemia mutations among the carriers in the Gujarat state in India. This suggests the need to establish a program for genetic counseling and prenatal diagnosis of beta thalassemia for affected families and for initiating a control program by prospective screening of pregnant women as a multicentric study in Gujarat State. Such activities would eventually reduce the burden of this dreaded yet a common disease in the state and lead to its control. The application of the knowledge about mutation pattern was found to be beneficial since the mutations could be screened in the order in which they are present in our population. Hence it will not only help to reduce the screening cost but also to promote early genetic counseling and prevention of an affected child.

ACKNOWLEDGEMENTS

facilities for thalassemia screening program at SGPGIMS, Lucknow.

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