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Spectrum of β-Thalassemia Mutations in Punjabis

Gurjeewan Garewal and Reena Das

Department of Haematology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

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ABSTRACT In the present paper the prevalence of the β-thalassemia has been reported. It has been assaved that the trust is 3.5% and nearly half of these individuals belong to the Khatri-Arora castes. These families have migrated from the South-west districts of the undivided Punjab, now in Pakistan. In thalassemia major families, the common five mutations account for 93.5% of the alleles with another 3.35 % with CAP+1 and–88 (C-T), facilitating the prenatal diagnosis of thalassemia in this population.

INTRODUCTION

Thalassemia is the most commonly inherited disorder worldwide. In India, β-thalassemia affects different ethnic and geographical groups, with prevalence of 2-14% in different regions in an ICMR study (1993). In Punjab the prevalence reported by Sukumaran and Masters was 6.5% (1974). The population of Punjab is approximately 15-18 million and is heterogeneous in its caste structure. Marriages most often take place within the same caste and it is important to know the prevalence of β-thalassemia trait amongst the different castes as well as the distribution of the various mutations amongst the different castes for proper counseling and prenatal diagnosis. We have studied the prevalence of β-thalassemia and the β-gene mutations in Punjabis belonging to different castes. This data is useful for planning cost effective screening programmes as well as providing genetic counseling and prenatal diagnosis.

MATERIALS AND METHODS

β-thalassemia Trait: Screening and confirmation of β-thalassemia trait (BTT) was carried out in 780 parents of thalassemia major of Punjabi origin by performing complete blood counts on a fully automated blood cell counter (Sysmex K-1000) and quantitation of HbA_2 was done by micro-column chromatography using DE 52 (Dacie and Lewis 1991). The castes of both parents were recorded and the data analyzed to document the prevalence of thalassemia in different caste groups of Punjab.

Detection of β-thalassemia Mutations: This was done on 352 alleles of β-thalassemia major and the method used was the ARMS-PCR method (Newton et al. 1989) and the primer used were as those reported previously. (Varawalla et al. 1990).

RESULTS

The results of the caste-wise distribution of BTT are shown is Table 1. Almost half the individuals belonged to the Khatri-Arora castes of Punjab (47.8%). The remaining 52% were distributed amongst 13 other caste groups.

Mutational Analysis of β-thalassemia: This was done on 352 alleles of thalassemia major and the results are recorded in Table 2.

Table 1: Caste structure of 780 individuals with β-thalassemia trait

Castes	*Number of cases*	*Percentage (%)*
Khatri-Arora	372	47.8
Jats + Jat Sikhs	110	14
Brahmin	62	8
Baniyas	60	7.7
Harijans	38	4.9
Kamboh	16	2
Sunar	10	1.2
Rajput	10	1.2
Lobana	8	1
Lohar	8	1
Ramgarhia	8	1
Zeer	8	1
Suni	8	1
Bazigar	6	0.75
Total	780	100

DISCUSSION

The prevalence of the β-thalassemia trait in Punjabis has been variously reported as 3-6.5%. In a recent study by us, we found that it is 3.5% amongst the voluntary blood donors of Punjabi origin (under publication). Since the Punjabis

Table 2: Spectrum of β-gene mutations in 352 Thalassemia major alleles

Mutations	*Number*	*Percentage (%)*
IVS 1,5 (β^+)	112	31.8
619 bp del (β^0)	66	18.7
Fr 41/42 (β^0)	54	15.3
Fr 8/9 (β^0)	52	14.8
IVS 1,1 (β^0)	45	12.8
Cap+1 (β^{++})	9	2.5
-88 M (β^{++})	3	0.85
Uncharacterized	11	3.1
Total	352	100

comprise of various castes/ethnic groups and marriages usually take place within the same caste, it is logical that data on the distribution of a genetic disorder like thalassemia in different castes will help in control of the disease by genetic counseling and prenatal diagnosis. Our study on 780 individuals shows that the Khatris and particularly their subcaste the Aroras, comprise nearly half (47.8%) of the affected population. This is significant. In an earlier study reported by one of us (G.G.), in which 74 individuals were studied, 65% belonged to the Khatri-Arora castes, (Garewal et al. 1994). In the current study, 780 individuals were studied and therefore, these figures are a better reflection of the distribution of β-thalassemia trait amongst different castes in Punjabis. With a prevalence of nearly 50% in a particular ethnic community, that otherwise forms 10-15% of Punjabi population, a screening programme in this particular community would be very cost effective as by covering 15% of the Punjabi population, one would be covering 50% of the population at risk of having thalassemia major children.

We had in our earlier study (Garewal et al. 1994), documented that 80% of these families had migrated from areas of Punjab that are now in Pakistan. Most of these families originated from the South West districts of the undivided Punjab. In fact, most of the Punjabis in Delhi and other parts of India with β-thalassemia are also migrants from West Punjab, now in Pakistan. The fact that in Punjabis, the prevalence of β-thalassemia trait is high amongst the migrants from West Pakistan has also been documented in other studies from Delhi (Verma et al. 1997; Madan et al.1998). Since the migration of not only Punjabis, but other ethnic groups continues not only in India, but across the globe, it would be important, while dealing with thalassemia families to look at the ethnic group in more details to define a mutation and offer genetic counseling. Study of the geographical area alone may not be sufficient because of the migration factor. In fact, with inter-caste marriages becoming more common, it would be advisable to look at caste structure, ethnic background of both parents when dealing with an autosomal recessive genetic disorder like β-thalassemia.

Analysis of the β-gene mutations in patients of thalassemia major revealed that the common five Indian mutations accounted for 93.5% of the alleles and another 3.35% were due to the two mild mutations CAP+1 and –88 (C-T) mutations (Table 2). In the latter case (mild β+mutations), the other mutation was a β^0. We have observed consistently that if a mild mutation is coinherited with a β^0 mutation then the presentation is of thalassemia major, unless there is another interacting factor e.g. concomitant α-thalassemia. This is important for genetic counseling. From this study it becomes apparent that by screening for the common five Indian mutations, we can offer prenatal diagnosis to most of the families of thalassemia major patients in this region. In fact, if we screen for the CAP+1 and –88 (C-T) mutations, we are left with only 3% of the alleles in the thalassemia major group being contributed by other β-gene mutations where we need to resort to restriction fragment length poly-morphism (RFLP) or proceed for DGGE/ sequencing in order to offer prenatal diagnosis.

The distribution and prevalence of the common five mutations confirm that IVS 1-1 (C-T) and the 619 bp deletion are more prevalent in Punjabis as compared to other population groups from other regions of India (Verma et al. 1997). It is also relevant that this β^0 mutation IVS1- 1 (G-T) in the Indian context is seen predominantly in individuals from Punjab, Sind and Gujarat. Varawalla et al. (1991) noted in their study of individuals of Asian Indian origin that 97% of IVS1-1 (G-T) out of 700 alleles belonged to individuals of these regions. In Punjab the prevalence of this mutation varies from 12-31%, (Garewal et al. 1994; Verma et al. 1997; Madan et al. 1998), whereas from other states it has not been reported or when reported the prevalence is low (Verma et al. 1997) and is probably due to the Punjabis settled in other states and also due to the Gujarati and Sindhi populations spread all over the country. It is also worth mentioning that

this common mutation i.e. IVS1- 1 (G-T) in homozygous form may present as thalassemia intermedia, albeit relatively severe as compared to the thalassemia intermedia group as a whole. This is due to the fact that there is a very high association of this mutation with the Xmn-1 polymorphism (Ho et al. 1998) that confers mildness by virtue of increasing the γ chain output with resultant more HbF, thus having an ameliorating effect.

To summarize in Punjabis, the prevalence of the β-thalassemia has trait is 3.5% and nearly half of these individuals belong to the Khatri-Arora castes. These families have migrated from the South-west districts of the undivided Punjab, now in Pakistan. In thalassemia major families, the common five mutations account for 93.5% of the alleles with another 3.35 % with CAP+1 and –88 (C-T), facilitating the prenatal diagnosis of thalassemia in this population.

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