

β -Thalassemia

Introduction

β -thalassemia is a common autosomal recessive, monogenic blood disorder. Because of this disease, hemoglobin contained in every red blood cell (RBC) is abnormally synthesized or completely suppressed (Khateeb *et al.*, 2000; Hafeez *et al.*, 2007). This leads to a drop in blood Hb level, resulting in anaemia, microcytosis, hypochromia, hematuria, splenomegaly, and jaundice with elevated Hb-A2 and Hb-F levels. In addition, dwarfism, hypothyroidism, hypogonadism etc., might also be associated with thalassemia major (Jensen *et al.*, 1998).

The spectrum of clinical manifestations can be divided into asymptomatic and symptomatic forms (Cao *et al.*, 1996). So the diagnosis of β -thalassemia relies upon the measurement of red blood cell indices. However, molecular analysis of the gene encoding the β -globin chain is the best diagnostic approach.

The β -globin gene cluster on chromosome 11p15.4-11p15.5 spanning 45 Kb and consists of globin like five genes: 5'- ϵ -G γ -A γ - δ - β -3' (Olivieri, 1999; Das and Talukder, 2002). Mutations in various regions of β -globin gene result in either the absence or reduction of the synthesis of globin chains, while some mutations produces highly unstable β -globin products (Higgs *et al.*, 2001). The common defects in β -thalassemia are because of point mutations, small deletions or insertions in the β -globin gene (Mansoor *et al.*, 1998).

To date over 300 mutations of β -globin genes have been identified worldwide (<http://www.uwcm.ac.uk/uwcm/mg/hgmd.html>; <http://globin.cse.psu.edu>), while only 20 mutations being most common. Generally, the mutations are known to be population specific. In any particular ethnic group there are usually four to six common mutations, which account for more than 90% of β -thalassemia cases, and variable number of rarer ones (Mansoor *et al.*, 1998; Fakher *et al.*, 2007).

Identification of both common and rare β -thalassemia mutations has proved essential for the implementation of screening and prenatal diagnosis programs. In addition, these mutations can be used as genetic markers to study the origin and spread of β -thalassemia genes, revealing historical relationships between populations (Makhoul *et al.*, 2005). Therefore, the spectrum of β -thalassemia alleles has been determined in a

wide variety of populations including Greeks and Italians, Sicilians, Turks, Spaniards, Asian Indians and Chinese (Kazazian *et al.*, 1984b; Chehab *et al.*, 1987; Kazazian and Boehm, 1988; Varawalla *et al.*, 1991; Benito *et al.*, 1996; Cao *et al.*, 1996; Loukopoules, 1996).

In Iran, more than 23 different mutations have been identified for β -thalassemia (Fakher *et al.*, 2007). Among these, most common mutations like IVS-I-5 (G-C), IVS I-110 (G-A), IVS-I-6 (T-C), IVSII-1 (G-A), IVS- I-1 (G-A) , IVS-II-745 (C-G), FSC-5, FSC-8/9 (+G) FSC-44 (-C) , codon 30 (G-C), codon 39 (C-T) and IVS -I-25 (-25del) were reported (Mahboudi *et al.*, 1996). Similarly various mutations were also identified from different Indian regions, of which five common ones accounted for 91.8 to 93.6%; namely the ones at IVS -1 - 5 (G-C), FSC - 8/9 (+G), IVS-1 - 1 (G-T), FSC- 41/42 (-CTTT) and the 619 bp deletion at the 3' end of the gene (Varawalla *et al.*, 1991; Verma *et al.*, 1997; Vaz *et al.*, 2000).

In Pakistan, thalassemia is one of the most common blood disorders. Although comprehensive epidemiological studies are lacking and the disease allele frequencies remained to be elucidated, yet it is estimated that 5-7% the population are carriers of this diseases. Several molecular studies conducted basically in larger cities, are available for Pakistan and 20 different mutations have been reported (Ahmad *et al.*, 1996; Khan and Riazuddin, 1998; Khateeb *et al.*, 2000; El-Kalla and Mathews, 1997; Baig *et al.*, 2005, 2006a,b). Among these, five most common mutations, IVS-I-5 (G-C), FSC-8/9 (+G), 619bp del, FSC-41/42 (-TTCT) and IVSI-1 (G-T), were detected in population through out the country. The IVS-I-5 (G-C) mutation is more prevalent in Sindh and Balochistan while the FSC-8/9 (+G) is found more common in Punjab and NWFP.

In the present work, we have studied the prevalence, distribution and mutation spectrum of thalassemia in various population strata of Dera Ghazi Khan District.

Subjects Studied

During the study, 112 unrelated families having one or more family member affected with beta thalassemia major were ascertained (Table 6.1). Among these, eighty two families gave their consent to participate in the study. The diagnosis was made through clinical data, hematological indices and hemoglobin electrophoresis. β -thalassemia trait was diagnosed when the percentage of hemoglobin A2 was $\geq 3.5\%$

(Steinberg and Adams, 1991). Collectively, 392 individuals (82 patients, 310 relatives) were included in this study.

Molecular Analysis

In this study 164 β -thalassemia chromosomes were analyzed in 392 blood samples (82 transfusion dependent patients, 310 carriers /normal).

Our analysis showed a spectrum of β -thalassemia mutations in the Dera Ghazi Khan population. By applying ARMS-PCR and Multiplex ARMS-PCR techniques, 164/164 (100%) β -globin alleles were characterized for the nine β -thalassemia mutations i.e. IVS- I-5 (G-C), FSC- 8/9 (+G), FSC- 41/42 (-TTCT), IVS- I-1(G-T), IVS-II- 848 (C-A), CD 15 (G-A), CD 16 (-C), CD 30 (G-C), and FSC-5 (-CT). The relative frequencies of mutant chromosomes are listed in Table 6.2. Two mutations IVS-I-5 (G-C; 59.15%) and FSC 8-9 (+G; 33.54%) accounted for 92.68 % of the β -thalassemia chromosomes.

Figure 6.1 presents the ARMS-PCR results for most common β -thalassemia mutation IVS-1-5 (G-C), while Figure 6.2 shows Multiplex ARMS-PCR results for the mutations IVS-1-5 (G-C), FSC-8/9 (+G) and FSC- 41/42 (-TTCT).

Among patients, true homozygote and compound heterozygous mutations were observed in 65 (79.27%) and 17 (20.73%) patients, respectively (Table 6.3). These mutations included homozygous mutations of IVS -1-5 (G-C) in 38 (46.34%), FSC 8/9 (+G) in 25 (30.49%) and FSC-5 (-CT) in 2 (2.44%) patient while compound heterozygous, a combination of IVS -1-5 (G-C) with FS- 8/9 (+G) , CD30 (G-C) and CD 15 (G-A) were detected in 12 (14.63%), 01 (1.22%), and 01 (1.22%) patients respectively. In addition, two combinations of FSC- 8/9 (+G) with FSC- 41/42 (-TTCT) and IVS 11- 848 (C-A) with CD 16 (-C) were found in 01 (1.22%) and 01 (1.22%) patient, respectively.

Ethnic Distribution of β -Thalassemia Mutations

The frequency and distribution of β -thalassemia mutations by the various ethnic groups are presented in Table 6.4. Among ethnic groups Baloch and Migrants were found to be relatively more homogeneous than Native in their β -globin mutations distribution.

Fifty four β -thalassemia chromosomes were analyzed in the Baloch subjects. Three different β -globin mutations were detected in Baloch subjects. Two mutations IVS- I-5 (G-C; 81.48%) and FSC-8/9 (+G; 16.67%) accounted for 98.15% of the β -thalassemia

chromosomes. The CD 15 (G-A) mutation was rare (1.85%) but exclusive to the Baloch ethnic group.

In the Native subjects, 105 β -thalassemia chromosomes were analyzed. This group was found most heterogeneous with seven different mutations. The IVS-I-5 (G-C) mutation (50.98%) followed by FSC- 8/9 (+G; 39.22%) were the most common. Five mutations IVS-I-1 (G-T; 1.96 %), IVS-II-848 (C-A; 0.98%), CD16 (-C; 0.98%), CD30 (G-C; 1.96%), and FSC- 5 (-CT; 3.92%) were found to be exclusive to Native ethnic group.

For the Migrants, three mutations were found in twenty β -thalassemia chromosomes. Two mutations IVS-I-5 (G-C; 12.50%) and FSC-8/9 (+G; 75.0 %) accounted for 87.50% of the β -thalassemia chromosomes. The FSC- 41/42 (-TTCT) mutation was also 12.50% but exclusive to the Migrant ethnic group.

Distribution of β -Thalassemia mutations in various castes

Being part of Indian subcontinent, population is divided in to a number of endogamous groups called castes/sub tribes. Different castes were found with different types of molecular defects. The presence of various β -thalassemia mutations in different castes and tribes is presented in Table 6.5. In all the endogamous groups, only one or two mutations were found. However in some castes, the detected mutation was not found in any other group. Such as FSC-41/42 (-TTCT), FSC-5 (-CT) and CD15 (G-A) were reported in Sherwanii (Migrant caste), Jaskani (Native caste) and Jarwar (Khosha Baloch tribe) respectively. In two Native castes two mutations IVS-1-1 (G-T) and CD30 (G-C) in Sontra, and CD16 (-C), and IVS-II-848 (C-A) in Dasti families were reported.

Regional Distribution of β - Thalassemia Mutations

Table 6.6 presents the distribution of β -thalassemia mutations in administratively and geographically different areas of the District i.e. Tribal area, Taunsa, and DG Khan Rural and Urban areas. The families from Tribal area and Taunsa were found to be relatively more homogeneous than that of DG Khan in their β -globin mutations distribution.

Thirty six β -thalassemia chromosomes were found in the subjects belonging to Tribal area. Only two β -globin mutations, IVS-I-5 (G-C; 86.11%) and FSC-8/9 (+G; 13.84%), were present in tribal subjects. Similarly eighteen β -thalassemia chromosomes

were found in subjects belonging to Taunsa and only two β -globin mutations, IVS-I-5 (G-C; 77.78 %) and FSC-8/9 (+G; 22.22%), were found. However, the subjects belonging to rural and urban areas of DG Khan were found more heterogeneous with five and six different mutations, respectively. The IVS-I-5 (G-C) mutation and FSC -8/9 (+G) were the most common in both areas. Other mutations like IVS-I-1 (G-T), CD15 (G-A), and CD30 (G-C) in rural areas, and IVS-II-848 (C-A), CD16 (-C), FSC- 5 (-CT) and FSC-41/42 (-CTTT) in urban areas were found.

Discussion

B-thalassemia is characterized by its genetic heterogeneity at the molecular level. More than 300 mutations of the β -globin gene have been detected all over the world, though each population seems to harbour only a few of these mutations (Bandyopadhyay *et al.*, 1999; <http://globin.cse.psu.edu>). Therefore, molecular characterization of β -thalassemia is absolutely necessary for premarital counseling, prenatal diagnosis, as well as epidemiological study in the region (Balgir, 2002; Baig *et al.*, 2005; Samara *et al.*, 2007).

In this regard, the advent of the polymerase chain reaction (PCR), the subsequent development of more convenient DNA analysis methods, and the continuous accumulation of knowledge on the β -globin gene mutations, gave a great impetus to the rapid screening of large numbers of individuals (Tadmouri and Gulen, 2003). Among various PCR based molecular available methods, Amplification refractory mutation system-PCR (ARMS-PCR) and Multiplex ARMS-PCR are the most convenient and cost effective methods for the detection of known β -thalassemia mutations. Several groups have already used the ARMS-PCR technique successfully for population screening (Ahmed *et al.*, 2000; Panyasai, *et al.*, 2004; Makhoul *et al.*, 2005; Baig *et al.*, 2006a, b).

By using ARMS-PCR method in present study, we analyzed 164 β -thalassemia chromosomes obtained from 82 different families from Dera Ghazi Khan and detected nine different mutations in the β -globin gene. The mutations found were IVS-I-5 (G-C), FSC-8/9 (+G), FSC-5 (-CT), IVS-I-1(G-T), CD41/42 (-TTCT), IVS-II-848 (C-A) and CD15 (G-A), CD16 (-C) and CD30 (G-C). The spectrum of β -globin gene mutations revealed by our study is in agreement with previous reports from different areas of Pakistan (Ahmad *et al.*, 1996; Khan and Riazuddin, 1998; Khateeb *et al.*, 2000; Baig *et al.*, 1999, 2005, 2006a, b).

The distribution and frequency of mutations in the population under studied have several striking characteristics and provide important demographic insights.

The IVS-I-5 (G-C) is the most common mutation, found in 59% of the subjects and was represented in all the ethnic groups as well as most of the castes (Table 6.6). The frequency of IVS-1-5 in this study is one of the highest as compared to the previous studies conducted in various regions of Pakistan (Baig *et al.*, 2005, 2006a, b; Table 6.8).

The second most common mutation identified in the present study is FSC- 8/9 (33.54%), which is in agreement with the earlier reports from Northern areas of the country and Bahawalpur, Faisalabad and other cities of Punjab province (Rabbi *et al.*, 1999; Ahmed *et al.*, 2000; Baig *et al.*, 2005, 2006a, b).

Other mutations FSC-5 (-CT), IVS-I-1(G-T), CD41/42 (-TTCT), IVS-II-848 (C-A) and CD15 (G-A), CD16 (-C) and CD30 (G-C) are in low frequencies and detected in one or two families belonging to same caste. Interestingly, the 619 bp deletion mutation reported in Sindh and DG Khan Region (Muzaffar Garh and Liah Districts) by various previous studies like Khan and Riazuddin (1998) and Baig *et al.* (2006a, b) was not detected in any family in present study.

In the present study, 79.27% affected children were true homozygotes. In these true homozygotes, 46.34% had IVS-I-5 (G-C)/IVS-I-5 (G-C), 30.49% had FSC8-9 (+G)/FSC8-9 (+G), and 2.44% had FSC-5 (-CT)/FSC-5(-CT) genotypes. The diagnosis of these homozygotes confirms our data, that majority of the couples were close relatives, first cousins or belonging to the same tribe/caste. The present study also confirms the association between consanguinity and high rate of true homozygosity of these mutations in Pakistani population (Ahmed and Saleem, 2002).

In this scenario, it became clear that the population is mainly divided in to two main groups: IVS-1-5 (G-C) and FSC-8/9(+G). The very specific pattern of distribution of these two mutations in population seems to be very old as previously described for the Indian subcontinent (Varawalla *et al.*, 1992; Agarwal *et al.*, 2000). This indicates that groups have different origin or they may be bifurcated before the appearance of these mutations. The group having IVS-1-5 (G-C) seems to have acquired this mutation from Dravidians or people of south India where the incidence is very high (Ambekar *et al.*, 2001). Other group having FSC-8/9 (+G) mutation may come and settled during the

various historic migrations and invasions from northern areas where high prevalence of FSC-8/9 (+G) mutation was reported by Khateeb *et al.* (2000).

Low frequency of other mutations i.e. FSC-5 (-CT), IVS-I-1(G-T), CD41/42 (-TTCT), IVS-II-848 (C-A) and CD15 (G-A), CD16 (-C) and CD30 (G-C), in the families concludes that these mutations dispersed through population migration and gene flow. However, surprisingly despite the claim of certain tribes/castes having origin from outside Indian continent, only mutation/s exclusively found in India and Pakistan were detected among these in this study.

We believe that these mutations represent most, if not all, of the mutations in the population because our study is not hospital based like most of the previous studies conducted in Pakistan. In this study, families were collected by random survey so the results can be used to extrapolate prevalence of thalassemia in the general population and can allow us to establish a prenatal diagnosis program for the population of DG Khan District.

Table 6.1: Ethnic distribution of families affected with thalassemia disorder in Dera Ghazi Khan

<i>Ethnic Group</i>				
		Natives	Baloch	Migrants
Caste/ Tribe	1	Angari = 1	Ahmadani = 3	Pathan = 1
	2	Arain = 3	Bindwani = 1	Sadiquee = 1
	3	Bhati = 5	Buglani = 1	Sh.queshi = 1
	4	Birmani = 1	Buzdar = 6	Sherwani
	5	Bubur = 1	Gulyani = 4	
	6	Chatani = 1	Habtani = 2	
	7	Chinah = 1	Hadyani = 1	
	8	Chugtai = 1	Hajbani = 2	
	9	Chunar = 1	Hatwani = 1	
	10	Dasti = 2	Hijrani = 1	
	11	Dhainga = 1	Jarwar = 1	
	12	Jafar = 1	Jhingal = 2	
	13	Jaskani = 2	Jindani = 1	
	14	Jat arabi = 1	Jiyani = 1	
	15	Kharpal = 1	Jogiani = 1	
	16	Khokher = 2	Khosa = 2	
	17	Malik = 6	Kungrani = 1	
	18	Mastoi = 1	Lashari = 5	
	19	Mehowna = 1	Laskani = 2	
	20	Mulghani = 1	Leghari = 2	
	21	Mundae = 1	Lund = 1	
	22	Munjhotha = 1	Muridani = 1	
	23	Native = 1	Nutkani = 2	
	24	Native = 2	Qaisrani = 5	
	25	Patafi = 2	Saisrani = 1	
	26	Qureshi = 1	Sikhani = 1	
	27	Saial = 2	Wadani = 1	
	28	Sandhela = 1		
	29	Sanghi = 2		
	30	Sayed = 1		
	31	Seraii = 1		
	32	Shadi khail = 1		
	33	Shamsi = 1		
	34	Sipal = 3		
	35	Somroo = 1		
	36	Sontra = 1		
	37	Uttra = 1		
Total		56	52	04

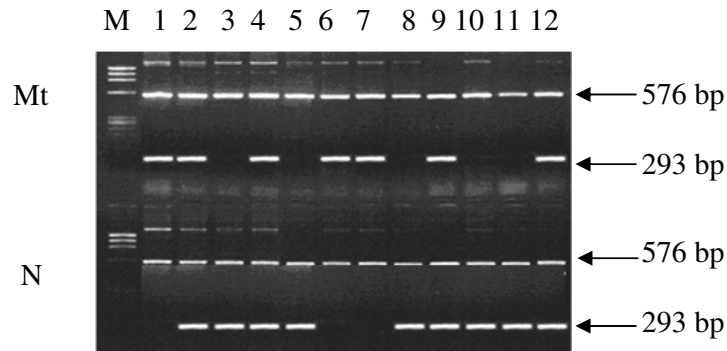


Figure 6.1: Photograph of agarose gel showing the analysis for IVS-I-5 (G-C) mutation. Lane 1: control homozygote; lane 2: heterozygote; lane 3: control normal for this mutation; lanes 4, 9, and 12 samples are heterozygote; lanes 6 and 7 samples are homozygote while lanes 5, 10, and 11 are negative for IVS-I-5 mutation.

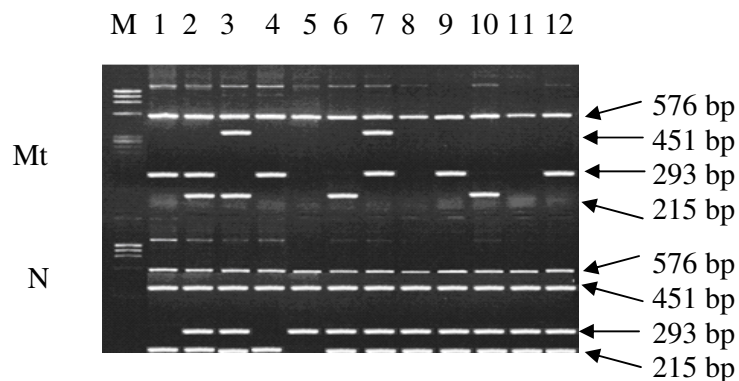


Figure 6.2: Photograph showing Multiplex ARMS-PCR and gel electrophoresis analysis for the most common mutations IVS -I -5, FSC-8/9 (+G) and FSC-41/42 mutations. lane 1: control Homozygous for IVS I-5, Lane 2: compound heterozygote for IVS I-5 and FSC-8/9; lane 3: control compound heterozygote for FSC-8/9 and FSC-41/42 ; lanes 4: homozygous for IVS I-5; 6 & 10 are heterozygous for FSC-8/9 ; 7 is compound heterozygous sample for FSC-8/9 and FSC-41/42; 9 & 12 are heterozygous for IVS- I-5; while 5, 8 & 11 samples are negative for these mutations.

Table 6.2: Frequency of β -thalassemia mutations in the population (164 Alleles)

<i>Mutations</i>	<i>Number of alleles</i>	<i>Frequency</i>
1.IVS-1 -5 (G-C)	97	59.15%
2. FSC-8-9 (+G)	55	33.54%
3 IVS-II-848 (C-A)	01	0.61%
4. CD 16 (-C)	01	0.61%
5. FSC-41-42 (-TTCT)	01	0.61%
6.CD 15 (G-A)	01	0.61%
7.IVS-1-1 (G-T)	02	1.22%
8.FSC-5 (-CT)	04	2.44%
9.CD 30(G-C)	02	1.22%
	164	100.01

Table 6.3: Frequency of β -thalassemia mutations in the patients

<i>Status</i>	<i>S.No</i>	<i>Mutation/s</i>	<i>Number of patients</i>	<i>Frequency</i>
Homozygous 79.27%	1	IVS 1-5/ IVS 1-5	38	46.34%
	2	FSC 8-9/ FSC 8-9	25	30.49%
	3	FSC-5/FSC-5	02	2.44%
Compound heterozygous 20.73%	4	IVS 1-5/ FSC 8-9	12	14.63%
	5	IVS 1-5/ Cd-15	1	1.22%
	6	FSC 8-9/FSC-41-42	1	1.22%
	7	IVS 1-1/ Cd-30	2	2.44%
	8	IVS 11-848/ Cd-16	1	1.22%
Total			82	100

Table 6.4: Frequency of β -thalassemia Mutations in ethnic groups

<i>Mutation</i>	<i>Baloch</i>	<i>Migrant</i>	<i>Native</i>	<i>Total</i>
IVS-I-5 (G-C)	44 (81.48%)	01 (12.50%)	52 (50.98%)	97
FSC-8/9 (+G)	09 (16.67%)	06 (75.00%)	40 (39.22%)	55
FSC-41/42 (-TTCT)	-	01 (12.50%)	-	01
IVS-I-1 (G-T)	-	-	02 (1.96%)	02
IVS-II-848 (C-A)	-	-	01 (0.98%)	01
CD 15 (G-A)	01 (1.85%)	-	-	01
CD16 (-C)	-	-	01 (0.98%)	01
CD30 (G-C)	-	-	02 (1.96%)	02
FSC- 5(-CT)	-	-	04 (3.92%)	04
Total	54	08	102	164

Table 6.5: Representation of various mutation/s in different castes and sub-tribes

<i>Ethnicity/ caste</i>		<i>Mutations</i>									<i>Total</i>
		1	2	3	4	5	6	7	8	9	
Migrant	Pathan	-	+	-	-	-	-	-	-	-	01
	Siddique	-	+	-	-	-	-	-	-	-	01
	Sheikh			-	-	-	-	-	-	-	
	Qureshi	+	+		-	-	-	-	-	-	02
	Sherwani	+	-	+	-	-	-	-	-	-	02
Baloch	Leghari	+	+	-	-	-	-	-	-	-	02
	Gulyani	+	-	-	-	-	-	-	-	-	01
	Lashari	+	-	-	-	-	-	-	-	-	01
	Khosa	+	+	-	-	-	+	-	-	-	02
	Kasrani	+	+	-	-	-	-	-	-	-	02
	Buzdar	-	+	-	-	-	-	-	-	-	01
	Laskani	+	-	-	-	-	-	-	-	-	01
Native	Bhati	+	-	-	-	-	-	-	-	-	01
	Arain	-	+	-	-	-	-	-	-	-	01
	Sipal	+	+	-	-	-	-	-	-	-	02
	Chughtai	-	+	-	-	-	-	-	-	-	01
	Chunar	+	-	-	-	-	-	-	-	-	01
	Dasti	-	-	-	+	+	-	-	-	-	02
	Sontra	-	-	-	-	-	-	+	-	+	02
	Jafar	+	+	-	-	-	-	-	-	-	02
	Malik	+	-	-	-	-	-	-	-	-	01
	Khoja	+	-	-	-	-	-	-	-	-	01
	Pathan	+	+	-	-	-	-	-	-	-	02
	Sanghi	+	-	-	-	-	-	-	-	-	01
	Saial	+	+	-	-	-	-	-	-	-	02
	Jaskani	-	-	-	-	-	-	-	+	-	01
	Khokhar	-	+	-	-	-	-	-	-	-	01
	Mujawar	-	+	-	-	-	-	-	-	-	01
	Mastoi	-	+	-	-	-	-	-	-	-	01

1. IVS- 1-5 (G-C), 2. FSC- 8/9 (+G), 3. FSC- 41/42 (-TTCT), 4. Cd-16 (-C), 5. IVS-II-848(C-A), 6.Cd-15(G-A), 7.IVS-1-1(G-T), 8.FSC-5 (-CT), 9.Cd-30(G-C)

Table 6.6: Geographical distribution of β -thalassemia Mutations

<i>Mutation</i>	<i>Tribal area</i>	<i>Taunsa</i>	<i>DGK Rural</i>	<i>DGK Urban</i>	<i>Total</i>
IVS-I-5 (G-C)	31 (86.11%)	14 (77.78%)	25 (62.25%)	27 (38.57%)	97
FSC-8/9 (+G)	05 (13.89%)	04 (22.22%)	10 (25.00%)	36 (51.43%)	55
FSC-41/42 (-TTCT)	-		-	01 (01.43%)	01
IVS-I-1 (G-T)	-	-	02 (05.00%)		02
IVS-II-848 (C- A)	-	-	-	01 (1.43%)	01
CD 15 (G-A)		-	01 (2.50%)	-	01
CD16 (-C)	-	-	-	01 (1.43%)	01
CD30 (G-C)	-	-	02 (05.00%)	-	02
FSC- 5(-CT)	-	-	-	04 (5.71%)	04
Total	36	18	40	70	164

Table 6.7: Regional comparison of Frequency of β -thalassemia Mutations

<i>Mutation</i>	<i>Pakistan*</i>	<i>RWP/IBD*</i>	<i>FAD*</i>	<i>LH, MN,*</i> <i>KHI</i>	<i>DGK**</i>
IVS-I-5 (G-C)	38.31	30.51	47.93	31.25	59.15%
FSC-8/9 (+G)	25.20	28.81	17.36	11.25	33.54%
CD41/42 (-TTCT)	7.46	9.61	9.1	2.50	0.61%
IVS-I-1 (G-T)	2.82	2.26	1.65	-	1.22%
IVS-II-I (G-T)	2.42	3.96	4.13	-	-
619bp del	0.40	00	1.65	-	-
IVS-II-848 (C-A)	4.23	2.82	5.79	8.75	0.61%
CD -15 (G-A)	3.23	2.26	3.31	7.50	0.61%
CD-16 (-C)	2.22	4.52	-	2.50	0.61%
IVSI-I (G-A)	3.43	3.39	4.96	6.25	-
CD-30 (G-C)	1.01	2.26	-	-	1.22%
CD-26 (G-A)	0.81	1.70	-	1.25	-
CD-39 (C-T)	0.40	1.13	-	-	-
CD-30 (G-A)	0.61	0.56	-	2.50	-
Initiation CD (T-C)	0.40	1.13	-	-	-
Cap+1	0.40	0.56	0.83	-	-
-88	0.40	0.56	-	1.25	-
FSC-5 (-CT)	-	-	-	-	2.44%
	96.01		96.70	75.00	100.01

* Khan and Riazuddin, 1998; Khateeb *et al.*, 2000; Baig *et al.*, 2006a,b

** Present study