RESEARCH ARTICLE

Consanguinity, the driving force behind inheritance of HbS-β thalassemia in Southern Districts of KP

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Abstract

Objective: To determine the clinical, haematological and genetic factors responsible for variable phenotypes of sickle haemoglobin, sickle haemoglobin-beta, and beta-thalassemia patients.

Methods: The study was conducted in Bannu, Lakki, Tank and Dera Ismail Khan districts of Khyber Pakhtunkhwa province of Pakistan from September 2016 to November 2017, and comprised sickle haemoglobin, sickle haemoglobin-beta, and beta-thalassemia patients. Clinical, haematological and genetic determinants were evaluated using haemoglobin electrophoresis and allele-specific primers through polymerase chain reaction to determine alpha and beta thalassemia, and C→T substitution at position -158 (referred to as Xmn-I polymorphism) in gamma-globin gene. Data was analysed using SPSS 20.

Results: Eight β-thalassemia mutations were identified that included IVS I-5(G C), codon 8/9 (+G), codon 30 (G C), -88 (C T), Cap+1(A G), codon 41/42 (-TCTT), IVS I-1(G T) and codon 16(-C). Codon 30 (G C) and -88 (C T) were found only in Pashtoon subjects, Cap+1(A G) and IVS I-1(G T) in Balochi subjects, while 75% of IVS I-5(G C) mutation cases were found in Punjabi ethnic group. In the Pashtoon group, 13 sickle haemoglobin homozygous patients were identified for the first time. Both alpha thalassemia and Xmn-I polymorphism in homozygous condition were common among those with mild phenotype.

Conclusion: Phenotypic expression of sickle haemoglobin beta thalassemia was found to be extremely variable and alpha thalassemia and Xmn-I polymorphism in homozygous condition were found to be additional genetic modifiers of the disease.

Keywords: HbS-β thalassemia, Consanguinity, Haematology, β-globin gene, Haplotype. (JPMA 70: 978; 2020)
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Introduction

Beta (β) thalassemia is caused by a variety of mutations that result in a quantitative reduction of structurally normal β-globin chains,[1] in contrast to sickle cell disease (SCD) which is caused by sickle hameglobin (HbS), an abnormal variantin which glutamate is replaced by valine at position 6 of β-globin chain (βGlu6Val), caused by point mutation in the gene β-globin gene.[2,3] This change predisposes HbS to polymerisation when deoxygenated.[3-5] Sickle cell beta-thalassemia (HbS-β-thalassemia) is a condition that results from co-inheritance of a sickle cell gene with β thalassemia gene. The polymerisation of deoxygenated HbS leads to the formation of long fibres inside the red blood cells (RBCs), the sickle shape RBCs that ultimately cause increased haemolysis and vaso-occlusion of sickle red cells.[4-6] The clinical and haematological characteristics of HbS-β-thalassemia are very heterogeneous and varied, ranging from a phenotypically asymptomatic carrier state to a regular transfusion-dependent state.[7-9] This heterogeneous nature of thalassemia patients depends not only upon the type of mutation in β-globin gene, but is also closely linked to certain other genetic modifiers like the coinheritance of alpha (β) thalassemia, polymorphism in the promoter region of gamma (β)-globin gene, and some other unknown factors.[10-12] The patients become anaemic with mild to severe condition that can be felt early in life.[12-14] β-thalassemia in Pakistan is one of the commonest Hb disorders.[15-17] Its carrier frequency is 5.4%.[16,18] The incidence of β-thalassemia ranges from 1.5% to 7.5%.[15-17] The disease is highly prevalent in the region along the Arabian Sea coast, in the south of the country, and in the Khyber Pakhtunkhwa (KP) province situated near the border with Afghanistan. One of the main reasons of its prevalence in these regions is the invasion and settling of people from the Middle East, Central Asia and Mediterranean regions during various periods of history. Additionally, factors like consanguineous marriages or preference to have marriage within one's own ethnic group contributed to increased incidence of the disease in Pakistani population.[16-18] SCD in Pakistan is characterised neither haematologically nor at the molecular level, and is

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grouped together with patients of thalassemia intermedia. Lack of public awareness about the disease and its inheritance pattern, and non-availability of good quality care has resulted in low quality of life for patients of β-thalassemia in Pakistan. Furthermore, the facilities for bone marrow transplantation are either very expensive or are rarely available in the country, thus adding to the miserable life of thalassemia patients.

The current study was planned to determine the clinical, haematological and genetic factors responsible for variable phenotypes of HbS, HbS-β-thalassemia and β-thalassemia patients in parts of KP.

Materials and Methods

The study was conducted in Bannu, Lakki, Tank and Dera Ismail (D.I.) Khan districts of KP province of Pakistan from September 2016 to November 2017. After getting approval from the ethics review committee of Gomal University, D.I. Khan, and the review boards of Bannu and Gomal medical colleges, the sample size was calculated while keeping the level of significance at 0.1% and accordingly for the reliability coefficient Gaussian standardised normal distribution value was 3.08 which was used for assessing the precision with reference[19] while executing the formula: ((3.08)^2*0.02*0.98)/(0.055)^2). A total of 63 blood samples (41 patients of thalassemia intermedia and 22 patients of thalassemia major were collected. Of the 63 total samples, 69.84% (36 males, 16 female), 15.87% (6 males, 2 female) and 14.28% (3 males, 2 female) were Pashtoon, Punjabi and Balochi respectively.

After taking written consent, age of diagnosis and age at presentation, Hb level at the time of diagnosis, frequency of blood transfusion and relationship between parents were recorded for all the subjects who belonged to Pashtoon, Punjabi and Balochi ethnic groups. Subsequently, blood samples were collected for haematological characterisation at least one month after the last transfusion. Hb electrophoresis and quantitative measurement of HbA2 was done using alkaline cellulose acetate electrophoresis at potential of hydrogen (pH) 8.8, while HbF level was quantified by the alkali denaturation method.[20] Hb electrophoresis was the only facility available as the facility of high-performance liquid chromatography (HPLC) was not available in the study region. Genetic determinations were carried out in all HbS-β-thalassemia cases. Deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes by standard salting out method[21] and stored at -20°C.

For the detection of HbS-β-thalassemia by allele-specific polymerase chain reaction (PCR), all the samples were screened through tetra primer-based allele-specific PCR.[21] Two PCR reactions were performed, containing either of the allele-specific primer and control primers. For PCR reaction to perform, 200-250ng genomic DNA, 260µM of each deoxynucleoside triphosphate (dNTP), 1 unit of Taq.

Polymerase, 5 pmol of each primer i.e. two primers for control fragment and two primers for each of the mutant or control allele and 1x Taq reaction buffer were used in 25µ reaction volume. The reaction was done through 27 cycles that consisted of 1-minute denaturation at 94°C, 1-minute annealing at 65°C and 90-second extension at 72°C. During the first cycle, denaturation was done at 95°C for 5 minutes, while the final extension was done at 72°C for 10 minutes. Gel electrophoresis of the PCR product was done on 2% agarose gel that contained ethidium bromide for visualisation. Hind III digest was used as a marker. β-globin genotypes were assigned on the basis of presence or absence of allele-specific bands. The positive controls used were previously characterised sample and distilled water (dH2O) was used as a negative control.

For the detection of $-\alpha^{3.7}$ kb deletions in β-globin genes, the forward primer used was C10; 5/-GATGCACCCACTG GACTTCCT-3/ located in the homologous Y regions of both α1 and α2 genes and reverse primers used were C2; 5/-CCATGCTGGCACGTTTCTGA-3/ and C3; 5/-CCATTGTTGGCACATTCCGG-3/ located in the non-homologous 3/ non-coding regions of α1 and α2 genes in separate reactions. The reaction consisted of 30 cycles with 1-minute denaturation at 94°C, 1-minute annealing at 52°C and 90-second extension at 72°C. During the first cycle, denaturation was done at 95°C for 5 minutes, while extension in the last cycle was done for 10 minutes at 72°C. Electrophoresis of PCR products was done on 1.5% agarose gel containing ethidium bromide. A normal α1 gene was detected as a 2.1 kb fragment with C10 and C2 primers, while the $-\alpha^{3.7}$ mutations gave rise to 1.9 kb product with the same primers. A normal α2 gene and reciprocal event [$\alpha\alpha\alpha^{anti\ 3.7}$] were detected as 1.9 kb fragment and 2.1 kb respectively with C10 and C3 primers.

Haplotype analysis for β-globin gene region and a Gγ-158T polymorphism in the β-globin gene was done by PCR with specific primers.[22] A 25µl standard PCR reaction was carried out through 30 cycles that consisted of 30-second denaturation at 94°C, 30-second annealing at 55°C or 62°C, and 90-second extension at 72°C. During the first cycle, denaturation was done at 95°C for 5 minutes and the final extension at 72°C for 3 minutes after the last cycle. Amplified product was digested with appropriate restriction enzyme under the conditions recommended by the manufacturer (New England Bio Labs, Inc. USA). Electrophoresis of the digested product was carried out

on 3% agarose gel. β-globin gene haplotypes were assigned by the presence or absence of specific restriction site. The restriction enzymes with their restriction sites used to construct haplotypes were Hinc II 5/ to ε, Xmn-I 5' to Gγ, Hind III within εψ, Hind III within Gγ and Aγ, Hind II 3/ to ψβ, Ava II within β and Hinf-I 3/ to β.

Data was analysed using SPSS 20, and results were presented as mean ± standard deviation (SD) where applicable.

Results

Of the 63 patients [43(68.25%) male and 20(31.75%)

Table-1: Haematology and Genetics of sickle haemoglobin (HbS) Homozygous patients of Khyber Pakhtunkhwa (KP), Pakistan.

S. No	Ethnic Group/Age/Sex	Transf/Y	Hb (g/dl)	HbA2(%)	HbS (%)	HbF (%)	Xmn-1	β-thal/α- Genotype	Haplotype
1	Pashtoon/16/F	1	9.3	6.0	97.8	22.5	+/+	HbS homo/-α/αα	Saudi/Saudi
2	Pashtoon/39/M	0	8.9	5.6	97.7	20.8	+/+	HbS homo/-α/αα	VII/Saudi
3	Pashtoon/26/M	1	9.4	4.8	98.0	20.7	+/+	HbS homo/ αα/αα	VII/Saudi
4	Pashtoon/14/M	1	8.6	5.7	97.8	19.7	+/+	HbS homo/ -α/αα	Saudi/Saudi
5	Pashtoon/24/M	0	8.4	6.0	98.5	21.0	+/-	HbS homo/ -α/αα	VII/Saudi
6	Pashtoon/16/F	1	8.2	4.9	98.0	20.4	+/+	HbS homo/ αα/αα	Saudi/Saudi
7	Pashtoon/23/M	1	9.3	5.9	97.0	23.6	+/+	HbS homo/ -α/αα	VII/Saudi
8	Pashtoon/19/M	1	8.8	5.8	99.0	22.0	+/+	HbS homo/ -α/αα	Saudi/Saudi
9	Pashtoon/15/M	0	8.6	5.6	97.3	20.7	+/+	HbS homo/ -α/αα	VII/Saudi
10	Pashtoon/12/F	0	8.5	4.9	97.0	22.7	+/+	HbS homo/ -α/αα	Saudi/Saudi
11	Pashtoon/14/F	0	9.0	4.8	98.5	21.9	+/+	HbS homo/ -α/αα	VII/Saudi
12	Pashtoon/8/F	1	8.5	5.2	98.7	22.3	+/+	HbS homo/ -α/αα	VII/Saudi
13	Pashtoon/17/M		8.3	5.5	97.9	23.0	+/+	HbS homo/ -α/-α	VII/Saudi

Table-2: Haematological and Genetic Determination of sickle haemoglobin-beta (HbS-β) thalassemia Heterozygous Patients of Khyber Pakhtunkhwa (KP), Pakistan.

S. No	Ethnic Group/Age/Sex	Transf/Y	Hb (g/dl)	HbA2(%)	HbS (%)	HbF (%)	Xmn-1	β-thal/α- Genotype	Haplotype
1	Pashtoon/15/M	0	9.0	5.3	87.0	23.0	+/+	88 (C T)/HbS/ αα/αα	IX/Saudi
2	Pashtoon/7/F	0	9.2	6.1	77.2	22.0	+/+	88 (C T)/HbS/ -α/αα	IX/Saudi
3	Pashtoon/24/M	0	8.3	5.5	75.0	16.7	+/-	88 (C T)/HbS/ -α/-α	IX/Saudi
4	Pashtoon/22/M	0	9.0	6.4	78.0	18.0	+/-	88 (C T)/HbS/ -α/-α	IX/Saudi
5	Pashtoon/13/F	0	8.6	5.1	77.7	17.8	+/-	88 (C T)/HbS/ -α/-α	IX/Saudi
6	Pashtoon/18/M	5	7.7	4.7	70.1	13.6	+/-	Cd 8/9 (G)/HbS/ -α/αα	IX/III
7	Pashtoon/16/M	3	8.0	4.3	65.0	11.0	+/-	Cd 8/9 (G)/HbS/ αα/αα	IX/III
8	Pashtoon/6/M	8	7.2	4.0	62.0	6.0	+/-	Cd 8/9 (G)/HbS/ -α/αα	IX/I
9	Pashtoon/5/F	11	7.5	4.0	54.0	9.8	+/-	Cd 8/9 (G)/HbS/ αα/αα	IX/III
10	Pashtoon/14/M	7	7.1	4.0	52.0	11.3	+/-	Cd 8/9 (G)/HbS/ αα/αα	IX/I
11	Pashtoon/17/M	6	8.0	4.1	53.0	11.2	+/-	Cd 41/42(TCTT)/ HbS/ αα/αα	IX/I
12	Pashtoon/8/M	6	8.8	4.3	52.6	11.4	-/-	Cd 41/42(TCTT)/ HbS/ αα/αα	Saudi/IX
13	Pashtoon/7/F	5	8.4	4.3	51.8	10.8	-/-	Cd 41/42(TCTT)/ HbS/ αα/αα	Saudi/III
14	Pashtoon/11/M	2	7.2	5.0	65.0	14.0	+/-	Cd 30 (G C)/HbS/ -α/αα	VII/I
15	Pashtoon/17/F	2	8.6	6.1	63.5	12.6	+/-	Cd 30 (G C)/HbS/ αα/ααα$^{3.7}$	VII/I
16	Pashtoon/18/M	2	8.5	5.6	61.5	13.3	+/-	Cd 30 (G C)/HbS/ αα/αα	Saudi/I
17	Pashtoon/25/M	2	8.0	5.8	64.3	12.8	+/-	Cd 30 (G C)/HbS/ -α/αα	Saudi/I
18	Punjabi/21/M	1	9.0	5.5	66.2	26.4	+/+	IVSI 5(G C)/HbS/ -α/αα	Saudi/I
19	Punjabi/5/F	0	9.8	4.7	65.5	32.6	+/+	IVSI 5(G C)/HbS/-α/αα	Saudi/I
20	Punjabi/21/M	1	10.0	4.8	69.0	27.8	+/+	IVSI 5(G C)/HbS/ αα/-α$^{3.7}$	Saudi/I
21	Punjabi/23/F	1	8.7	5.5	40.1	19.2	+/-	IVSI 5(G C)/HbS/ αα/-α$^{3.7}$	IX/I
22	Punjabi/22/M	0	9.3	5.4	66.8.	23.5	+/-	IVSI 5(G C)/HbS/-α/αα	Saudi/I
23	Punjabi/13/M	4	7.6	4.1	64.0	12.4	+/-	Cd 8/9 (G)/HbS/ αα/αα	IX/III
24	Balochi/7/F	1	8.5	4.7	77.0		+/+	IVSI 5(G C)/HbS/ -α/-α	VII/Saudi
25	Balochi/24/M	0	8.8	4.9	78.7	18.7	+/+	Cap 1(A G)/HbS/ -α/αα	VII/VII
26	Balochi/6/F	0	9.3	4.8	76.4	13.3	+/+	Cap 1(A G)/HbS/-α$^{3.7}$/-α$^{3.7}$	Saudi/VII
27	Balochi/13/M	0	9.4	4.7	75.3	17.8	+/+	Cap 1(A G)/HbS/ -α/αα	VII/Saudi
28	Balochi/15/M	1	9.1	4.9	75.4		+/+	IVS I 5(G C/HbS/-α3.7/-α$^{3.7}$	Saudi/I

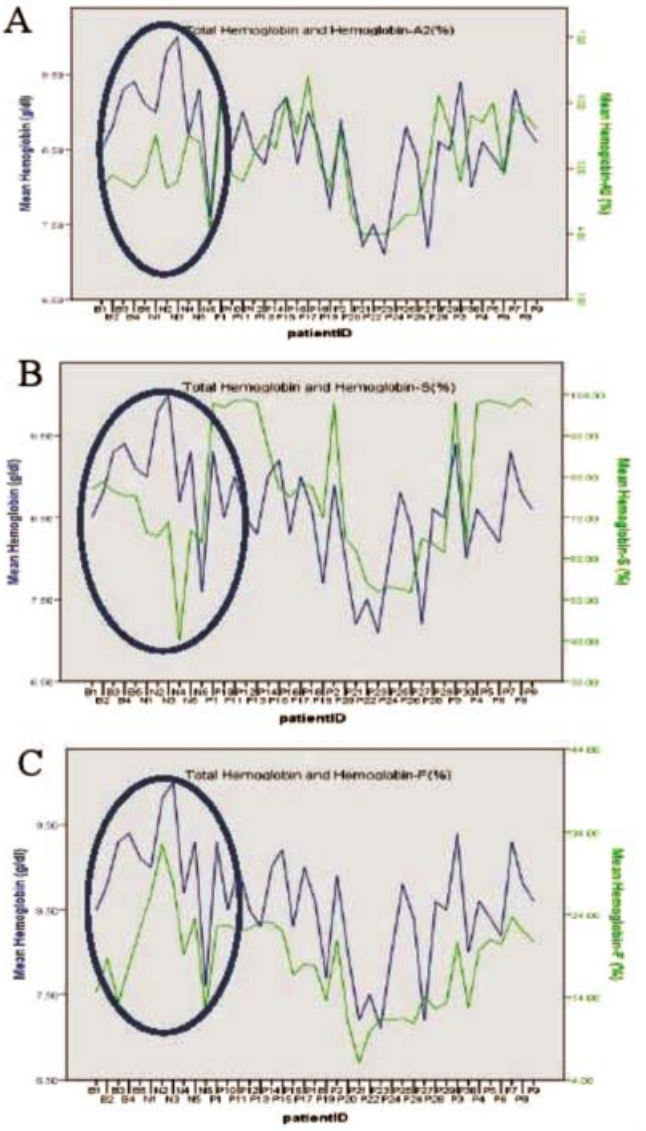

Figure: Comparative account of mean haemoglobin with hemoglobin-A2, haemoglobin-S and haemoglobin-F. The encircled section shows significant variations in patients of Pashtoon ethnic group.

female)], 41(65%) had HbS-β-thalassemia and 22(35%) had β-thalassemia. Further, 47(75%) showed mild clinical presentations and 16(25%) had severe clinical manifestations. Parents of 70% of patients were first cousins. The age at presentation ranged from 5 years to 39 years. Of the HbS-β-thalassemia patients, 36(87.8%) had mild clinical presentation, and 5(12.20%) had severe clinical manifestations in the form of acute pain in the joints, abdomen, bones and chest. Although hepatosplenomegaly was observed in both mild and severe cases, it was more common in severe cases as 85% (14 patients) and 36% (17 patients) of the cases had liver and spleen enlargement in severe and mild cases respectively. None of the patients was regularly transfused every month. All β-thalassemia major patients were regularly transfused and had splenectomy in 85%(9 out of 10) of the cases. Mean Hb levels in the severe cases was 7.8±0.70) compared to 9.1 ± 2.02 in patients with mild clinical manifestations. Total Hb was non-significant ($p>0.05$) for gender classification. Difference in terms of ethnicity was significant with respect to total Hb ($p<0.05$) as Pashtoons had mild haematological problems compared to Balochi and Punjabi groups.HbA2 level was non-significant ($p>0.05$), but HbF level was statistically significant, revealing over-expression of HbF in certain cases (Figure). HbA2 level ranged from 4% to 6.4% while HbF levels varied from 6% to 32.6%. HbF levels among the patients with mild clinical presentations was 19.0±3.10 compared to 10.1±4.20 in severe cases. Among the HbS-β-thalassemia patients, 22(53.65%) were homozygous (+/+), 17(41.46%) were heterozygous (+/-) Xmn-I polymorphism, and 2(4.87%), both with Cd 41/42(TCTT) β-thalassemia mutation, were lacking this mutation. HbF levels were significantly higher in patients homozygous for Xmn-I polymorphism compared to those heterozygous for the said mutation ($p<0.001$). Seventy-eight percent of the patients possessing severe β-thalassemia mutations [Cd 8/9 (G), Cd 30 (G C), and Cd 41/42 (TCTT)] were either heterozygous for Xmn-I or were lacking this mutation. In β-thalassemia cases, 2(9%) patients, both of thalassemia intermedia, were homozygous for Xmn-I polymorphism while 20(91%) patients were either heterozygous or lacking the said polymorphism.

Eight different mutations were identified including IVS I 5(G C), codon 8/9 (G), codon 30 (G C), 88 (C T), Cap 1(A G), IVS I-1(G T), codon 41/42 (TCTT) and codon 16(-C). Codon 30 G>C and 88 (C T) were found only in Pashtoon group, and Cap 1(A G), IVS I-1(G T) and codon 16(-C) were observed only in Balochi group. All the 13 HbS homozygous cases found for the first time were of Pashtoon ethnicity. In case of β-thalassemia, 7(32%) patients were identified as thalassemia major, 12(54.5%) as thalassemia intermedia, and 3(13.5%) remained unidentified for 1 allele each.

Among the HbS-β-thalassemia patients, 10(24.4%) were found with αα/αα genotype regarding α-thalassemia re-arrangements, 21(51.2%) with -α/αα genotypes, 5(12.2%)with -α/-α genotype, 2(4.9%) each with $-\alpha^{3.7}/-\alpha^{3.7}$ and $\alpha\alpha/-\alpha^{3.7}$ genotype, and 1(2.4%) patient possessed $\alpha\alpha/\alpha\alpha\alpha^{3.7}$ genotype. Among β-thalassemia patients, 4(18.2%) and 18(81.8%) patients had -α/αα and αα/αα genotypes respectively.

Haplotype analysis revealed 5 different haplotypes where the Saudi haplotype was associated with HbS only, and haplotype III was associated with 75% of the patients

Table-3: Genetic determination of the patients of thalassemia major and thalassemia intermedia.

S.No	β-Genotype/Phenotype	α-Genotype	Haplotype	Xmn-1 Polymorphism	Ethnic Group
1	Cd 8/9(+G)/Homo/ β0/β0	αα/αα	I/I	--	Pashtoon
2	Cd 41/42(-TCTT)/homo/ β0/β0	αα/αα	I/I	--	Pashtoon
3	Cd 8/9(+G)/ 88(C T)/ β0/β+	αα/αα	I/IX	--	Pashtoon
4	Cd 30 (G C)/ Homo/ β0/β0	αα/αα	I/IX	--	Pashtoon
5	Cd 30 (G C)/ 88(C T)/ β0/β+	αα/αα	I/IX	--	Pashtoon
6	Cd30(G C)/ 88(C T)/ β0/?+	-α/αα	I/IX	++	Pashtoon
7	Cd 8/9(+G) / Unidentified? β0/-	αα/αα	I/II	--	Pashtoon
8	Cd 41/42(-TCTT) /Unidentified/ β0/-	αα/αα	I/VII	--	Pashtoon
9	IVSI 5(G C)/ Cd 8/9(+G)/ β+/β0	αα/αα	I/VII	+-	Pashtoon
10	Cd 8/9(+G)/Homo/ β0/β0	αα/αα	I/III	--	Pashtoon
11	Cd 41/42(-TCTT)/homo/ β0/β0	αα/αα	I/IX	--	Pashtoon
12	Cd 30 (G C)/ Homo/ β0/β0	αα/αα	I/IX	+-	Pashtoon
13	Cd 30 (G C)/ 88(C T)/ β0/β+	αα/αα	IX/I	+-	Pashtoon
14	Cd 30 (G C)/ Unidentified/ β0/-	αα/αα	IX/I	--	Pashtoon
15	IVSI 5(G C)/ Homo/ β+/β+	-α/αα	VII/VII	++	Punjabi
16	IVSI 5(G C)/ Cd 8/9(+G)/ β+/β0	αα/αα	I/VII	+-	Punjabi
17	IVSI 5(G C)/ Cd 8/9(+G)/ β+/β0	αα/αα	I/I	+-	Punjabi
18	IVSI 5(G C)/ Cd 8/9(+G)/ β+/β0	αα/αα	I/VII	--	Punjabi
19	IVSI-1(G T)/ Cap 1(A G)/ β0/β+	-α/αα	VII/IX	+-	Balochi
20	Cap 1(A G)/ Cd16(-C)/ β+/β0	αα/αα	VII/III	+-	Balochi
21	IVSI-1(G T)/ Cap 1(A G)/ β0/β+	-α/αα	VII/IX	--	Balochi
22	IVSI-1(G T)/ Homo/ β0/β0	αα/αα	IX/IX	+-	Balochi

possessing codon 8/9(+G) of Pashtoon ethnicity (Tables 1-3).

Discussion

The current study is the first ever to observe the clinical, haematological and molecular determination of HbS, HbS-β-thalassemia and β-thalassemia patients in southern KP. The study has a long-term plan of screening the entire KP population for both β and α thalassemia. In, Pakistan, genetic characterisation of thalassemia patients is rarely done, and all the patients are therefore regularly transfused, becoming transfusion-dependent when the fact is many of them have mild mutations that do not require regular blood transfusion.[9,12,23] Due to strong cultural preference for consanguineous marriages, there is high prevalence of recessively inherited disorders in Pakistan. The five most common β thalassemia mutations are IVS-I-5 (G>C) (37.7%), codon 8/9 (+G) (21.1%), 619bp del (12.4%), IVS1-1 (G>T) (9.5%) and codon 5 (-CT) (9.1%).[15-18] The current study found that the prevalence of HbS-β mutation was not only in the dominant Pashtoon ethnic group, but also in Balochi and Punjabi groups. Interestingly, all the homozygous HbS cases were found only in Pashtoon. This mutation was previously reported in Sindhi ethnic group only in the Sindh province of Pakistan.[16,18] Moreover, codon 30 G>C and -88 C>T, IVSI 5(G C), and Cap+1 mutations were found confined to Pashtoon, Punjabi and Balochi ethnic groups respectively, showing the strong role of consanguinity in the inheritance of thalassemia. Thus, HbS is a generalised phenomenon in the region studied, inherited in all the three major ethnic groups, which is in contrast to what has previously been reported.[23-26] On comparing the mean Hb with HbA2, HbS and HbF, a significant natural ethnic discrimination was noted as it was not found in Punjabi and Balochi people of the region. Regarding the clinical severity, the coinheritance of α-thalassemia with β-thalassemia reduces the severity of the disease.[12,23,26] In the current study, the same coinheritance of α-thalassemia with β-thalassemia was found. Interestingly, most of the patients possessing milder β-thalassemia mutations had deleted α-globin gene, either one or both, but, most of the patients with severe β-thalassemia mutations possessed normal αα/αα genotype except the one with triplicated α-globin genes and 2 with -α/αα genotype. All the thalassemia patients were further characterised for haplotype analysis to see whether or not there was any close association between a specific β-globin gene mutation and a haplotype the patient possessed. It was seen that codon 8/9 (+G), HbE, codon 41/42 (-TCTT) and IVSI-1 (G>A) were found with different haplotypes, but haplotype VII was found only with 619bp deletions.[16,18,23] Haplotype analysis showed that the Saudi haplotype was the most frequent (27.78%), followed by haplotype I (26.98%), haplotype IX (21.42%), haplotype VII (18.25%), haplotype III (4.76%) and haplotype II (0.80%). The Saudi haplotype was associated with HbS only, while haplotype III was mostly associated with codon 8/9 (+G). Keeping in view the general clinical and haematological

situation in Pakistan, all the HbS-β thalassemia patients were rarely transfused as possibly they were rarely consulting medical practitioners since most of the samples were collected from the remote areas. Thus, the origin of β-thalassemia mutation in this region, according to the current study, was found to be unicentric and the haplotype association was typical Arab-Indian.

In terms of limitations, the study was conducted only in four southern districts and comprised patients of thalassemia intermedia rather than thalassemia. Also, collection of blood samples from clinically diagnosed thalassemia intermedia patients were brought to medical colleges for haematological studies, extraction of DNA and molecular characterisation through PCR. The facility of HPLC was not available in the study region, and, hence, to avoid any delay in processing, Hb electrophoresis was done which is one of the recommended methods globally[27,28] for quantitative determination of HbA, HbA2 and HbF.

Conclusion

Clinical and haematological manifestations of Pakistani HbS-β-thalassemia patients were not only influenced by β-thalassemia mutations, but also by associated α-thalassemia and Xmn-I polymorphism.

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Conflict of Interest: None.

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