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Genotypic Prevalence of KELL1/KELL2 and KELL3/ KELL4 Blood Group Antigens in Individuals from Four Cities of Punjab, Pakistan

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Abstract

Kell blood group system (KBGS) plays a vital role in transfusion medicine and bears a multidimensional character. Kell antigens are very reactive and vital responses can be observed in case of mismatched blood. This research is aimed to check the frequency and prevalence of different antibodies (KELL1, KELL2, KELL3, and KELL4) of the *KEL* gene in the local population of four different cities of Punjab, Pakistan. To this end, two hundred volunteers (from different ethnic groups) were enrolled for blood sampling. A questionnaire was designed to collect the required demographic information covering gender, age, cast, blood group, and city of origin of all the participants. Genomic DNA was extracted using the phenol-chloroform method and DNA was quantified using a UV spectrophotometer. Screening for the two known variants (C578T, T193M) in exon 6 of KELL1/KELL2 and for another two variants (C841T, R281W) in exon 8 of KELL3/KELL4 was performed by using tetra arms PCR. Data was entered in the SPSS software. The frequency of contributing factors was calculated, followed by cross-tabulation and chi-square analysis. The results showed that the incidence of KELL2 was 90%, while KELL1 was observed only in 7.5% of samples and the heterozygous allele KELL1/KELL2 was reported in only 2.5% of the total samples, respectively. Similarly, the frequency of wild-type allele KELL3 was 2.5% and of mutant allele KELL4 it was 96%, while the frequency of heterozygous allele KELL3/KELL4 was 1.5%, respectively. Chi-square values of cast and the city of origin showed non-significant results for both variants, although the type of blood group was found to have a significant association with KELL3/KELL4 genotype. Our results are in line with the previously reported results of prior studies.

1. Introduction

Blood group antigens are clinically important due to their ability to produce antibodies and the resultant proficiency of these antibodies to destroy red blood cells (RBCs). RBCs, in human beings, contain many glycoproteins and glycolipids on their surface which constitute the blood

group antigens. The International Society of Blood Transfusion (ISBT) recognizes 308 RBC antigens, constituting 30 blood group systems [1]. Following the ABO and Rh blood groups, Kell antigens are the third most potent due to the high immune response and the risk of alloantibody production [2], as well as severe hemolytic transfusion reactions [3]. KBGS was discovered in 1946 by Coomb et al. The subject was a child of Mrs. Kelleher who was suffering from hemolytic disease as a newborn [4]. KBGS is known to contain 36 antigens (http://www.isbtweb.org) constituting five antithetical groups of high or low frequency located on the red-cell transmembrane protein [5], along with independently expressed antigens that do not have antithetical partners [6, 7]. The antithetical groups consist of K/k (KELL1/ KELL2), Kpa/ Kpb (KELL3/ KELL4), and Jsa/ Jsb (KELL6/ KELL7).

The *KEL* gene located at chromosome 7q33 contains 19 exons and the mutations of this gene may result in kell antigens. Mutation (c.C578T; p. T193M) in exon 6 results in KELL1/KELL2 antigens, while KELL3/KELL4 antigens are due to point mutation (C841T) in exon 8 [8]. Kell antigens were once thought of as restricted to the blood cells (precursors of RBCs) of erythroid origin but they have been detected also in myeloid tissues, recently. They have expressions on multiple body systems including lymphatic muscles and the nervous system [9, 10]. Anti-Kell antibody is an immunoglobulin G antibody produced as a result of antigen exposure during pregnancy or blood transfusion. Antibodies against Kell antigens can cause severe blood transfusion reactions, in addition to the hemolytic disease of the fetus and newborn (HDFN) [11]. HDFN, due to Kell immunization, can cause severe anemia to the baby. This is due to the fact that maternal anti-Kell antibodies attack the red blood cell precursors of the baby, in addition to the suppression of RBC production.

Clinically, the most significant antibodies including anti-KELL1/KELL2 [12] and anti-KELL3/KELL4 [13] have been associated with moderate to severe HDFN. The purpose of the current research is to check the distribution, prevalence, and frequency of Kell antigens in the local people of four cities (Bahawalpur, Lodhran, Multan, Vehari) of Punjab, Pakistan.

2. Materials and Methods

The current study is based on a sample of 200 blood donors (between 15 to 60 years of age) of both sexes chosen via random sampling. All the male individuals had Hb >13g/dL and the females had >12g/dL. A questionnaire was designed to collect the required demographic information including gender, age, cast, blood group, and city name. The consent of all the donors was obtained before blood collection. A specific amount of blood (5 ml) was drawn from each individual. Moreover, genomic DNA was extracted using the phenol chloroform method [14] with minor changes, as described below.

Blood samples were thawed before DNA extraction. Falcon tubes were filled up to 12-14 ml with TE buffer (EDTA 2Mm and Tris-HCL 10 Mm). To re-suspend all the cells equally, these tubes were shaken gently and centrifuged for 20 min at 25^{o}C at 4000 rpm. After centrifugation, the supernatant was discarded and the pellet was broken down by gently beating the tubes. Again, TE washing buffer was added (up to 14 ml) and centrifugation was repeated at 4000 rpm at 25^{o}C for 20 min (second wash). The centrifugation and washing steps were repeated five to six

times to get the light pink/white color of the pellet. Then, the pellets were suspended in 1.8 ml of TNE buffer (10mM Tris HCl, 2mM EDTA, 400mM NaCl), 60 µl of 10% SDS, and 150µl of Proteinase K. The samples were kept at 37ºC for incubation in a water bath, overnight. On the second day, the protein in the samples was precipitated by adding 2 ml of phenol and shaking the falcon tubes for a few minutes, then keeping the samples in ice for 10 minutes. The centrifugation process was repeated at 4000 rpm for 10 minutes. The supernatant of all the samples was then transferred to other falcon tubes to extract protein and pellets. Then, solution D (in chilled form) was added in an equal volume and centrifuged for 10 minutes. The supernatant was transferred to new falcon tubes and the chilled isopropanol was added in equal volume in order to concentrate DNA and to obtain it in thread form. Afterwards, 70% of ethanol was used to wash the DNA pellet, thus eliminating all the contamination. Furthermore, 200µl low TE buffer was used and DNA was incubated at 65°C for 1 hour in a water bath to dissolve the DNA in TE buffer. After extraction, screening for the two known variants (C578T, T193M) of KELL1/KELL2 and KELL3/KELL3 was performed using tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR**)** [13], followed by gel electrophoresis. It is a simple and cost-effective method for SNP genotyping system since it needs just one PCR with gel electrophoresis using 4 primers (Microgen Korea). Two outer primers (forward and reverse) are needed for the amplification of the outer regions of the SNP and two inner primers (wild type and mutant) are needed for SNP detection. The primer sequence is as given as follows: KEL1/KEL2, (C578T), p.T193M Forward inner (T): 282 TCCTTAAACTTTAACCGCAT 301, Reverse inner (C): 321 GACTCATCAGAAGTCTCATCG 301, Forward outer (5' - 3'):, 31 AATTTCTAGGAGGGAGATGG 50, Reverse outer (5' - 3'):, 525 TAAGGTAGGGTTGTTTCCTATATC 502. KELL3/KELL4, (C841T, R281W): Forward inner (T): 280 GGCCTCAGAAACTGGAACATCT 301, Reverse inner (C): 321 GTCAATCTCCATCACTTCCCG 301, Forward outer (5' - 3'): 117 AATGAAAAGGTATTAAGGGCACTA GG 142, Reverse outer (5' - 3'): 533 CAAGCAAGATCAAGAACAGAAGAT CT 508. Gel electrophoresis results were documented as images. Data was entered in the SPSS software and p-value ≤ 0.05 was considered significant.

3. Results

The current study was designed to check the occurrence of KBGS in the local population of four cities (Bahawalpur, Lodhran, Multan and Vehari) of Punjab, its prevalence particularly in females, different age groups, and castes, as well as the frequency of its alleles for the various blood groups.Two hundred blood donors participated in the study due to limited wet lab resources. Out of the 200 volunteers, 76 (38%) were male and 124 (62%) were female. The age range of the participants was 15-60 years, although most of them were 15-30 years of age. They belonged to 8 different castes including Arian (19%), Joiya (14%), Baloch (13.5%), Jutt (12%), Syed (10.5%), Marha (14.5%), Sial (10.5%), and Rajput (6%). Blood group of the donors was determined via the kit method. The most frequent blood group was B (31%), followed by A (28%), O (21.5%), and AB (19.5%), respectively. Individuals were enrolled from four districts including 114 individuals from Bahawalpur (57%), 43 from Lodhran

(21.5%), 31 from Multan (15.5%), and 12 from Vehari (6%). Among the selected individuals the incidence of KELL1 was 7.5%, while KELL2 antigen was found at a higher percentage (90%), and the incidence of KELL1/KELL2 was 2.5%, respectively. Moreover, the prevalence of KELL3 was low (2.5%), while KELL4 was found at a higher frequency (96%), and the heterozygous allele KELL3/KELL4 was found to be the least frequent (1.5%) (see Table 1).

Table 1:Demographic Characteristics of the Participants

Characteristics		Frequency	Percent
Gender	Male	76	38
	Female	124	62
	Total	200	100
Age group	15-30	115	57.5
	31-45	39	19.5
	46-60	46	23
	Total	200	100
Caste	Arian	38	19
	Joiya	28	14
	Baloch	27	13.5
	Jutt	24	12
	Syed	21	10.5
	Marha	29	14.5
	Sial	21	10.5
	Rajput	12	6
	Total	200	100
Blood group	A	56	28
	B	62	31
	AB	39	19.5
	O	43	21.5
	Total	200	100
City name	Bahawalpur	114	57
	Lodhran	43	21.5
	Multan	31	15.5
	Vehari	12	6
	Total	200	100
Genotype	TT(K2)	180	90
	CC(K1)	15	7.5
	CT(K1/K2)	5	2.5
	TT(K4)	192	96
	CC(K3)	5	2.5
	CT(K3/K4)	3	1.5

Cross-tabulation of demographic factors such as caste, blood group, and city name with *Kell* gene polymorphisms was performed. KELL1/KELL2 and KELL3/KELL4 genotypes were observably more frequent in the Arian cast as compared to all other ethnic groups included in the study, with non-significant chi-square values 0.108 and 0.511, respectively. Similarly, KELL1/KELL2

and KELL3/KELL4 genotypes were determined to be more frequent in individuals with blood group B as compared to A, AB, and O blood groups, with a non-significant chi-square value of 0.177 for KELL1/KELL2 and a significant chi-square value of 0.028, respectively. When genotype KELL1/KELL2 and KELL3/KELL4 were cross-tabulated with the individuals' city of origin (Bahawalpur, Lodhran, Multan, and Vehari), non-significant chi-square values of 0.71 and 0.335 were obtained (Table 2).

Table 2:Cross-Tabulation of Demographic Factors with Kell Gene Polymorphisms
*significant chi-square value (<0.05)

Characteristics		Genotype			p-value	Genotype			p-value
		TT (K2)	CC (K1)	CT (K1/K2)		TT (K4)	CC (K3)	CT (K3/K4)	
Caste	Arain	38	0	0	0.108	37	0	1	0.511
	Joiya	24	3	1		28	0	0	
	Baloch	24	2	1		26	1	0	
	Jutt	21	3	0		23	1	0	
	Syed	16	2	3		18	2	1	
	Marha	26	3	0		29	0	0	
	Sial	20	1	0		19	1	1	
	Rajput	11	1	0		12	0	0	
	Total	180	15	5		192	5	3	
Blood group	A	54	2	0	0.177	53	0	3	0.028*
	B	55	6	1		58	4	0	
	AB	33	5	1		38	1	0	
	O	38	2	3		43	0	0	
	Total	180	15	5		192	5	3	
City name	Bahawalpur	102	8	4	0.71	109	3	2	0.335
	Lodhran	37	5	1		41	2	0	
	Multan	29	2	0		31	0	0	
	Vehari	12	0	0		11	0	1	
	Total	180	15	5		192	5	3	

4. Discussion

The genotype of KELL1/KELL2 and KELL3/KELL4 in 200 blood samples was analyzed by using tetra-arms PCR, a conventional molecular method. KELL2 antigen was present in 90% of samples, while KELL1 was observed in only 7.5% of samples. These findings are similar to the results of a study performed by Ashraf (2001), which reported the frequency of KELL1 as 4.6% and of KELL2 as 93.8% in the selected samples [15]. Similarly, in

Germany, the frequency of KELL1 was determined to be 4%, while the frequency of KELL2 was found to be 96% [10]. Finally, in Brazil, the frequency of KELL1 was recorded as 2.2% and the frequency of KELL2 antigen was found to be 97.8% [16].

KELL3/KELL4 antigen prevalence in the local population was not different from other populations around the world. It was determined that KELL3 was less frequent (2.5%) in the local population and KELL4 was the most frequent (96%) antigen, with almost similar frequencies of KELL3 (2%) and KELL4 (99.4%) in the Sudanese population [17]. Comparable results were recorded in a study of 19,000 Caucasian individuals from North America and Europe, with a lower percentage of KELL3 (2.28%) [16]. Saudi population screened for KELL3 and KELL4 antigens was found to have a similar frequency of 11% for KELL3 and 96% for KELL4 genotype [18, 19]. The current study proved that there exist no significant differences in the prevailing frequencies of Kell antigens KELL1/KELL2 and KELL3/KELL4 in different ethnic groups of Pakistan. This may be due to the fact of living in the same geographical area which increases the likelihood of interaction between them. There were no significant changes observed between the distribution of Kell antigens in the Pakistani population and the other populations of the world. In this regard, our results conform to the previously reported results of prior studies. This is purely a matter of coincidence.

KBGS is the third most common antigen system reported in the literature. Clinically, it is the most important for triggering immune response. In blood transfusion, genotyping of blood groups is essential to reduce complications in case of incompatible blood. The main constraint to this work was the absence of the registered data of KBGS, in addition to the limited number of samples. To reduce the risk of transfusion reaction, Kell antigens should be constantly screened, genotyped, and cross-matched, properly. This data may help in providing antigen-compatible blood for patients with multiple alloantibodies.

5. Conclusion

Our findings are in line with those reported by prior studies, which stated that the percentage of KELL1 is very low as compared to KELL2. Moreover, KELL3 genotype was observed in a very small number of individuals, while KELL4 was found to be very common. On the basis of the importance of Kell antigens in alloimmunization and keeping in view the limitations of serologic methods, this research advocates the use of a tetra-arms PCR assay for KELL1/KELL2 and KELL3/KELL4 genotyping.

Conflict of Interest

The authors declare no conflict of interest.

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