

## Genotyping of KELL blood group in multiethnic populations of Quetta, Pakistan

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### ABSTRACT

KEL1 (Kell or K) is highly immunogenic with limited distribution while KEL2 (Cellano or k) antigen is more prevalent. The antigenic diversity in the Kell gene is a result of Single Nucleotide Polymorphisms (SNPs). Blood samples of 160 males and females from each ethnic group with no age restrictions were subjected to Tetra-ARMS PCR to genotype the SNP (C578T) on exon 6 of chromosome 7. Genotype and allele frequencies were estimated in correlation with gender, age, and ethnicity with p-value less than 0.05. In general, antigen KEL2 k was more prevalent than antigen KEL1 k.

**Keywords:** Kell blood group, ethnic population, polymorphism

### INTRODUCTION

Blood type refers to the blood group system that contains erythrocytes Red Blood Cells (RBC) antigens unique to agglutinin of a particular system. Numerous proteins with varying structures and functionalities are located on the outer layer of these erythrocytes,

expressing the blood group [1]. Several significant blood groups include ABO, Rhesus (Rh), MNS, P, Lutheran, Kell, Duffy, and Kidd, which are largely encoded by their respective autosomal genes [2]. The variety in these blood group systems is caused by vast genetic mutations, alterations, and polymorphisms.

The third most clinically significant and complex blood group system is Kell with several antigens that generate an immune response. It is a 19-exon gene located on 7q33 that occupies roughly 21 kbp of DNA and encodes 36 antigens. The KEL locus seems to be polymorphic, with antigens found on just one transmembrane protein [3]. KEL1 (K, Kell) and KEL2 (k, Cellano) are antithetical pairs; KEL3 (Kp<sup>a</sup>), KEL4 (Kp<sup>b</sup>), and KEL21 (Kp<sup>c</sup>) are antithetical sets; and KEL6 (Js<sup>a</sup>) and KEL7 (Js<sup>b</sup>) are antithetical sets while the residual is individualistically expressed.[4] Those deficient in the XK protein exhibit weak Kell antigens, manifesting as the McLeod phenotype or some Gerbich-negative phenotypes.[5] Due to the lack of XK protein and the absence of Kell antigens and protein, an uncommon phenotype known as Kell null exists (K<sup>0</sup>).[6] The two most prevalent codominant alleles, k, and K vary by a single amino acid (T193M) due to an exon 6 SNP (C578T). A single amino acid difference between KEL3 and KEL4 antigens (T281A) is caused by a point mutation in exon 8 (C841T). A single nucleotide polymorphism (T1790C) in exon 17, which codes for proline in KEL6

and leucine in KEL7, is associated with the antigens KEL6 and KEL7 [7].

The Kell gene polymorphism is caused by SNPs; a Threonine in exon 1 was replaced by Methionine 193 amino acids in exon 6 by SNP (C578T), resulting in an antithetical pair of KEL1 and KEL2 antigens.[8] Anti-KEL1 is of critical clinical relevance; about 10 % of documented occurrences of severe anemia in children are caused by anti-KEL1. Additionally, this antibody has been shown to induce myelosuppression, resulting in anemia [9] while anti-K, anti-k, anti-Kp<sup>a</sup>, and anti-Js<sup>b</sup> may produce transfusion responses ranging from moderate to severe.[10] Because the number of SNPs in blood group genes varies according to ethnic origin, Kell antigens exhibit population diversity, K-antigen is reported to be less prevalent than 'k' antigen worldwide, with a prevalence of 5.6 percent among Asians, 8 to 9 percent in the European population, and roughly 1.5 to 2 percent in the African population [11]. Two distinct investigations in Pakistan reported a Kell antigen prevalence of 0.4 (4 %) in Islamabad [12] and a K-k+ phenotype of 100 % in the Karachi community.[13]

Accurate blood group phenotypic identification may be accomplished by obtaining the genomic DNA molecular information. It now has two primary applications in transfusion medicine: determining the risk of hemolytic illness in pregnancy by the identification of the fetal blood group; and checking blood group characteristics in many transfused, transfusion-dependent patients [14]. To the best of our knowledge, this paper examines Kell blood group genotyping in the ethnic population of the Balochistan province for the first time. A large number of diverse ethnic populations with genetic ties to their original populations congregate in Balochistan. It is inevitable that there will be significant genotypic variability in this pool of racial diversity. This may also introduce Kell blood group genotype diversity, and various genotypes result in the production of various antigens in the body. It is a neglected blood type and is never examined before any blood transfusion in a population where there is obvious ethnic diversity. This could indicate a Kell blood group mismatch and present clinical issues in emergency transfusion situations. The paper investigates the frequency of genotypic variability among the province

existing ethnic diversity, which may aid in identifying the issues that may endanger innocent lives. Based on the overall results, clinicians can design strategies to emphasize the identification of the variety of kell genotypes in order to prevent further issues.

## **MATERIALS AND METHODS**

### *Study Sample and data collection*

A total of 160 blood samples and demographic data were randomly collected from people in Quetta city who belonged to various ethnic groups, with an equal number of men and women. The participants, whose ages ranged from 20 to 59, were chosen at random from several age groups. Questionnaires were used to obtain demographic data with informed consent. Additionally, all people residing in different sections of Quetta had their blood obtained by venipuncture using sterile, 5cc disposable syringes. Blood samples were kept at -20 °C in falcon tubes with 20 ul of EDTA. The review committee of Sardar Bahadur Khan Women's University, Quetta, accorded ethical approval. Genomic DNA was isolated from blood samples using an organic DNA extraction technique. The extracted DNA was

homogenized overnight in a water bath. In order to inactivate the nucleases, the samples were heated for an hour at 60 °C the following day. The extracted DNA samples were tested through 0.8 % agarose gel electrophoresis to test for the presence of nuclear material. The presence and approximate composition of the nuclear material were estimated by the width, size, and brightness of fragments. The prepared DNA stock samples were stored at -20 °C for long-term storage. Stock DNA was diluted to make working DNA, and a dissolving buffer was used for the working dilution.

#### ***DNA amplification***

To amplify specified KEL gene sequences, Tetra primers-ARMS (amplification refractory mutation system) PCR (Polymerase Chain Reaction) was performed, which is a more convenient and cost-effective approach for genotyping SNPs. In a thermocycler, the SNP (C578T) responsible for KEL1, KEL2 was investigated using tetra primer-ARMS PCR (Prime thermal cycler: SPRIMEG/02) using four specific primers (Macrogen Korea): two primers for amplification of the SNP's outside area (Forward outer primer, Reverse outer

primer) and two primers for amplification of the SNP's inner region (Forward inner primer, Reverse inner primer) (Table 1). The master mix of 25 µl per amplification with a master mix of 25 µl per amplification reaction, where 5 pmol of two primers (forward inner primer and forward outer primer), 4 pmol of two primers (reverse inner primer and reverse outer primer), (nmol) 2µl or 100 mM of each dNTP, (nmol) 2.5µl or 100 mM of MgCl<sub>2</sub>, 0.5 µl or 2.5U of Taq DNA polymerase, 10X or 2.5µl buffer and PCR water were used. The PCR cycling conditions were as follows: Initial denaturation at 95 °C for 5 min, three stages of 10 cycles for 30 seconds each at 94, 59 and 72 °C, again at 94, 58, 72 °C, and again at 94, 57 and 72 °C, followed by 72 °C for 1 min for a final extension [15].

#### ***Gel electrophoresis analysis***

10 ul of PCR product and 2 ul of 6X DNA loading dye (gene DireX) were mixed and ran at 125 volts for about 50 minutes for the separation of desired fragments on a 3 % agarose gel stained with 7 ul of ethidium bromide (Figure 1). A 50-bp DNA ladder (GeneDireX) was used to analyze the size of the PCR fragments under the UV Transilluminator (UVi

Tech). The electropherogram data was statistically analyzed using IBM SPSS version 20 software

## **RESULTS**

The C/C homozygote was the most frequent genotype in 96.2 % (154) of the participants, followed by the T/C heterozygote in 3.75 % [6] of the participants. The T/T homozygote was not detected in our community, indicating a total absence of homozygous KEL1 (Kell, K) antigen in the local population. The wild-type allele (C) has a current dispersion of 98.1 percent, but the mutant allele has a proportion of 1.9 percent. This demonstrates the existence of the C allele, which is associated with the antithetically high prevalence of the KEL2 (Cellano, k) antigen in the local community (Table 2).

For this study, 3.8 percent of individuals had heterozygous T/C genotypes, 96.2 percent of individuals had homozygous C

genotypes, and nobody had homozygous T genotypes. 82 individuals in the age group 20-29 had heterozygous T/C genotypes, with 1 (1.2 percent) individual having heterozygous T/C and 81 (98.8 percent) individuals having homozygous C genotypes. 29 people in the age range 30-39 were found to be heterozygous T/C, while 27 (93.1 %) were found to be homozygous C. 29 people in the age range 40-49 were found to be heterozygous T/C, whereas 27 (93.1 %) were found to be homozygous C. Twenty individuals between 50 and 59 were heterozygous T/C, whereas twenty (100 %) were homozygous C. However, no age group had homozygous T alleles. When age was taken into account, no significant correlation between the Kell blood group genotype and age was seen ( $p=0.089$ ) (Table 3).

**Table 1** Primer Sequence for the SNP (C578T) and their Product Size

| Allele                                 | Primer Sequence  | Product Size (bp)  |
|--|--|--|
| <i>KEL1/KEL2</i><br><br>SNP<br>(C578T) | Forward inner primer (T allele):<br>272-TAAATGGACTTCCTTAAACTTTAACCGCAT-301<br><br>Reverse inner primer (C allele):<br>328-CCATACTGACTCATCAGAAGTCTCATCG-301<br><br>Forward outer primer (5'-3'): 53-<br>ATGGAAATGGTTATGCCCTATGAAAGTATC-82<br><br>Reverse outer primer (5'-3'):<br>512-TTCCTATATCACACAGGTGTCCTCTCTTC-483 | T allele: 242<br><br>C allele: 276<br><br>Outer primers: 460 |

**Table 2.** The genotype and allele frequencies of the K1 and K2 antigens of the Kell blood group classification in the Quetta community

| Genotype SNP (C578T)         |            |                           | Allele Frequency |       | Total |
|------------------------------|------------|---------------------------|------------------|-------|-------|
| C/C k/k<br>KEL2 (Cellano, k) | T/C<br>K/k | T/T K/K<br>KEL1 (Kell, K) | T (K)            | C (k) |       |
| 154                          | 6          | 0                         | 6                | 314   | 160   |
| 96.25                        | 3.75       | 0                         | 1.9              | 98.1  | 100   |

**Table 3.** The genetic distribution of the K and k antigens of the Kell blood group system in Quetta city residents of various ages

| Age         | Total<br>(n) | Genotype n (%) K(Kell) k (Cellano) |            |              | Chi-square | p-value |
|-------------|--------------|------------------------------------|------------|--------------|------------|---------|
|             |              | T/C K/k                            | T/T<br>K/K | C/C k/k      |            |         |
| 20-29       | 82           | 1 (1.2%)                           | 0 (0%)     | 81 (98.8 %)  | 6.52       | 0.08    |
| 30-39       | 29           | 2 (6.9 %)                          | 0 (0 %)    | 27 (93.1 %)  |            |         |
| 40-49       | 29           | 3 (10.3 %)                         | 0 (0 %)    | 26 (89.7 %)  |            |         |
| 50-59       | 20           | 0 (0 %)                            | 0 (0 %)    | 20 (100 %)   |            |         |
| Gross Total | 160          | 6 (3.8 %)                          | 0 (0 %)    | 154 (92.2 %) |            |         |

The present study included different ethnic populations living in Quetta, such as Baloch, Pathan, Persian, and Punjabi. 3.8 percent of individuals in each ethnic group had T/C heterozygous genotypes, 154 (96.2 percent) had C homozygous genotypes, and homozygous T genotypes were not reported at all. Two (5 %) individuals had heterozygous T/C genotypes, whereas 38 (95.0 percent) had

homozygous C genotypes. Individuals of Persian ancestry lacked heterozygous T/C genotypes and all have homozygous C genotypes.

160 individuals contributed, with half (80) being male donors and the other half (80) being female donors. Six (3.8 %) individuals had heterozygous T/C genotypes, whereas 77 (96.2 %) had

individuals had homozygous C genotypes, as each gender had an equal number of heterozygous T/C genotypes among three (3.8 %) individuals and homozygous C genotypes among 77 (96.2 %) individuals. There were no

homozygous T genotypes identified in people of either sex (Tables 4, 5).

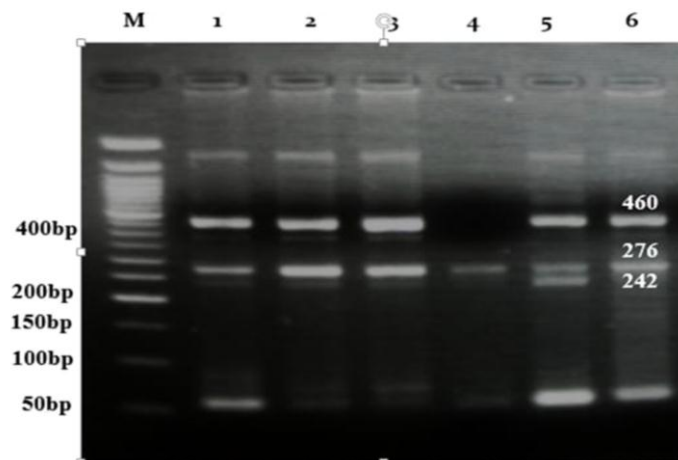
**Table 4.** Prevalence of the Kell blood group system genotype K and k antigens in participants of various ethnic groups

| Ethnic Groups | Total (n) | Genotype n (%) K(Kell) |         |              | Chi-square | p-value |
|---------------|-----------|------------------------|---------|--------------|------------|---------|
|               |           | T/C K/k                | T/T K/K | C/C k/k      |            |         |
| Baloch        | 40        | 2 (5.0%)               | 0 (0%)  | 38 (95.0 %)  | 2.07       | 0.55    |
| Pathan        | 40        | 2 (5.0%)               | 0 (0%)  | 38 (95.0 %)  |            |         |
| Persian       | 40        | 0 (0%)                 | 0 (0%)  | 40 (100 %)   |            |         |
| Panjabi       | 40        | 2 (5.0%)               | 0 (0%)  | 38 (95.0 %)  |            |         |
| Gross Total   | 160       | 6 (3.8%)               | 0 (0%)  | 154 (96.2 %) |            |         |



**Table 5.** Genotype distribution in both genders

| Gender      | Total<br>(n) | Genotype n (%) K(Kell) k(Cellano) |         |              | Chi-square | p-Value           |
|-------------|--------------|-----------------------------------|---------|--------------|------------|-------------------|
|             |              | T/C K/k                           | T/T K/K | C/C<br>k/k   |            |                   |
| Male        | 80           | 3 (3.8 %)                         | 0 (0 %) | 77 (96.2 %)  | 0.00       | 1.0 <sup>ns</sup> |
| Female      | 80           | 3 (3.8 %)                         | 0 (0 %) | 77 (96.5 %)  |            |                   |
| Gross Total | 160          | 6 (3.8 %)                         | 0 (0 %) | 154 (96.2 %) |            |                   |

**Figure 1.** Agarose gel stained with Ethidium Bromide showing PCR products of Kel Gene, Lane M=50bp DNA ladder, Lane 1 to 4 and 6= C/C genotype, Lane 5= C/T genotype.

## DISCUSSION

The antigens that correspond to various blood types are distributed differently among different populations and ethnic groups. Blood group antigens accumulate uniquely in each person, triggering a variety of different immunological reactions. It is critical to research the prevalence of both major and minor blood-type antigens to facilitate blood transfusion and organ transplantation.[16] The study found that while the majority of people in both sexes are homozygous for the C allele (KEL2 antigen), the prevalence of the K and K antigens is similar in males and females. Additionally, the study discovered that genotype allelic incidence was nearly the same among the city's four major ethnic groups. The ethnic groups of Baloch, Pathan, and Punjabi were quite similar; each group had a high prevalence of the k antigen, indicated by fewer than 5 % of individuals having heterozygous alleles (T/C) and 95 % having homozygous alleles (C/C). However, individuals of Persian ethnic heritage lacked heterozygous alleles and were completely homozygous for the C/C genotype (k antigen). The individuals sampled for this

## *Thermal stability of extract of Ciplukan*

study did not contain any individuals with the homozygous allele (T/T) [17].

A cross-sectional observational study was conducted at the Aga Khan University Hospital in Karachi, Pakistan, on 100 randomly selected blood donors (ages 19-60 years). Blood has been typed for ABO, Rh, and K blood types, as well as for the k antigens of the Kell blood group system. Antisera testing revealed 100 % positive findings for the k phenotype and 100% negative results for the K allele. Since K antigen is regarded to be less prevalent than k antigen everywhere.[13] The phenotypes of 625 people from Islamabad, Pakistan, were determined. There were 425 men and 200 females between the ages of 25 and 29. Both major and minor blood groups, including Kell, were examined and were detected in just 25 individuals (11 men and 14 females), a rate of 4 %. [12] The current data indicated that the frequency of the K type was greater than that of the k type, suggesting that the T allele genotype was present only in heterozygous form (T/C), while the T/T genotype was completely missing.

The distribution of Kell blood types varies considerably across the globe. In India, studies done on 500 samples revealed a 4.4 percent Kell positivity rate.[18] Kell

positivity was found to be 1.92 percent [19], 3.5 percent [20], 2.8 percent [21], and 5.56 percent in "O" blood group donors.[22] The population of South Gujarat, India, was typed for extended antigens, and no Kell phenotype was detected.[23] The current findings indicate a lower prevalence of K antigen (1.9 percent), heterozygous T/C (K+k+) which is lower than the majority of the studies cited above from India. In genotypic and phenotypic research conducted in the Chinese province of Jiangsu, 100% of donors possessed the (k) antigen, whereas no individuals possessed the K antigen.[24] Another research conducted in Bangladesh discovered a 0.8 percent prevalence of the Kell phenotype.[25] Nevertheless, research done in several districts of Iran discovered that the Kell blood type was present in 1.89 percent of 5522 out of 291857 samples.[26] The current study showed a larger percentage of K antigen than previous studies done in surrounding countries, with a lower incidence of k. The K allele is prevalent in the Caucasian population at 9 % but not in other ethnic groups [27].

Another research discovered that 9 percent of Caucasians had (K), although

its inverse, (k), is prevalent more than 99 %. [4] The K-k+ phenotype was the most prevalent (98.03 %) in the Thai population.[28] Sudanese populations have 5.6 percent of the K antigen, while Sokoto, Nigeria has 2.0 percent.[29,30,31] In the Saudi population, the Kell system revealed an 18.2 percent prevalence of the K antigen and a 97.0 percent prevalence of the k antigen. The most frequently encountered Kell phenotype was K-k+ at 81.5 percent, followed by K+k+ at 15.5 percent, K+k- at 3 percent, and K-k- at zero percent [30]. Following the ABO and Rh blood group systems, this is necessary to ascertain the genotype frequencies of significant clinical secondary blood types such as the Kidd, Duffy, and Kell blood group systems. It may be important to regulate pregnancies, avoid transfusion-related diseases such as erythroblastosis fetalis, fetal hemolytic sickness, and neonatal HDFN, and to give antigen-negative matching blood to a large number of transfused patients (thalassemic and anemic). In resource-constrained nations, blood transfusion facilities only test for ABO and RhD antigens during cross-matching. On the other hand, alloimmunization may occur

in well-matched ABO-RhD transfusions. As a result, blood banks should routinely undertake extended antigen typing on minor blood groups. In general, it is anticipated that phenotypic patterns and related research will aid in the understanding and illuminating of several relevant biological pathways in the future [32]. The study determined the frequency of the primary Kell antigens KEL1 (Kell or K) and KEL2 (Cellano or K) as well as the most frequent genotype across various ethnic groups in the Quetta urban areas to manage pregnancies and blood transfusion techniques, despite the absence of a relatively uncommon genotype (K) in our population. However, a larger population study may be necessary to determine the precise genotype of the respective ethnic group in the population of our city. This study's future objectives should include the registration of donors with unusual blood groups and the building of data banks on numerous uncommon antigens in all of Pakistan blood banks. When an antigen-negative donor is necessary, this may be advantageous to avoid a transfusion response. It will contribute to reducing the danger of vaccination while preserving resources and time [33].

The KEL 1 and KEL 2 antigens associated with the Kell blood group genotyping have not been documented in Pakistan's different ethnic groupings. The study investigated the prevalence of the uncommon blood types K (Kell) and k (Cellano) antigens in Quetta, Balochistan's multiethnic population. SNP frequencies in the blood group gene are used to determine the impact of blood group gene polymorphisms. Randomized population-based research was conducted to assess the gene pool in the Quetta, Balochistan and population. The current work is significant clinically for blood transfusions, immunohematology laboratories, and blood-related disorders since it will aid in the identification of better antigen matches for transfusion patients.

## **CONCLUSION**

The Kell blood group has many antigens capable of eliciting an immunological response and should be monitored. It produces higher immunogenic antibodies and is used extensively in blood transfusion, hemolysis responses, and obstetric medicine. Genotyping is an advantageous technique for identifying uncommon blood-type antigens. This is the first study to provide genotyping

information on the Kell blood group system among the diverse ethnic communities of Quetta. This study examined the allele frequencies of KEL1 (K) and KEL2 (k) within inhabitants of the Balochistan provincial capital (Quetta) using Tetra ARM PCR for rapid DNA sample screening and discovered that the antigen KEL2 (k) is significantly more prevalent in the population than KEL1 (K), regardless of age, race, or gender. This technology can be used frequently to expedite screening because it is quicker and less expensive, and for laboratories with limited resources, it might be better than PCR-RFLP.

## REFERENCES

- [1]. Ameen R, Al Shemmari S, Harris S, Teramura G, Delaney M. Classification of major and minor blood group antigens in the Kuwaiti Arab population. *TAS*, 2020; 59(4): 102748.
- [2]. Lamba DS, Kaur R, Basu S. Clinically significant minor blood group antigens amongst North Indian donor population. *Adv Hematol*, 2013; 215454.

- [3]. Davison GM, Hendrickse HL, Matsha TE. Do blood group antigens and the red cell membrane influence human immunodeficiency virus infection? *Cells*, 2020 31; 9(4): 845.
- [4]. Quirino MG, Colli CM, Macedo LC, Sell AM, Visentainer JE. Methods for blood group antigens detection: cost-effectiveness analysis of phenotyping and genotyping. *Hematol Transfus Cell Ther*, 2019; 15(41): 44-49.
- [5]. Sullivan JK, Gleadall N, Lane WJ. Blood Group Genotyping. *Adv Mol Pathol*, 2021; 4: 127-43.
- [6]. Prinja N, Narain R. ABO, Rh, and kell blood group antigen frequencies in blood donors at the tertiary care hospital of Northwestern India. *Asian J Transfus Sci*, 2020; 14(2): 179.
- [7]. Arnoni CP, Muniz JG, Paula TA, Person RD, Gazito D, Baleotti Jr W, Barreto JA, Castilho L, Latini FR. An easy and efficient strategy for KEL genotyping in a multiethnic population. *Rev. Bras.*

*Hematol. Hemoter*, 2013; 35: 99-102.

[8]. Prinja N, Narain R. ABO, Rh, and kell blood group antigen frequencies in blood donors at the tertiary care hospital of Northwestern India. *Asian J Transfus Sci*, 2020; 14(2): 179.

[9]. Storry, J. R., Clausen, F. B., Castilho, L., Chen, Q., Daniels, G., Denomme, G. Yahalom, V. International society of blood transfusion working party on red cell immunogenetics and blood group terminology: report of the Dubai, Copenhagen and Toronto meetings. *Vox Sang*, 2019; 114(1): 95-102.

[10]. Pahuja, Sangeeta, Sonal Jain, Manupriya Nain, Ruchika Goel, Shivali Sehgal, and Manjula Jain. Assessment of rhesus and kell blood group antigens, phenotypes, and their allelic frequencies in North Indian blood donors. *Asian J Transfus Sci*, 2020; 14(2): 137-39.

[11]. Jabin F, Waheed U, Ahmed S, Arshad M, Arshad A, Zaheer HA. Red blood cell phenotyping of blood donors in Islamabad,

Pakistan. *Global J Transfus Med*, 2018; 3(1): 26.

[12]. Karim F, Moiz B, Muhammad FJ, Ausat F, Khurshid M. Rhesus and Kell phenotyping of voluntary blood donors: foundation of a donor data bank. *J Coll Physicians Surg Pak*, 2015; 25(10): 757.

[13]. Rosse WF, Telen MJ. The biochemistry of the antigens of the red blood cell membrane. *In Red Blood Cell Membranes CRC Press*, 2020; 7: 299-323.

[14]. Denomme GA. Molecular basis of blood group expression. *Transfus Apher Sci*, 2011; 44(1): 53-63.

[15]. Manoharan S, Kaur AP, Imanina CW. Distribution of ABO blood group and Rhesus factor among students in ASIA Metropolitan University, Malaysia. *Int J Biol Med Res*. 2013; 4(1): 2962-65.

[16]. Reyhaneh S, Naser A, Arezoo O. Genomic analyses of KEL alleles in alloimmunized thalassemia patients from Iran. *Transfus Apher Sci*, 2020; 59(5): 102840.

- [17]. Rani R, Sharma N, Bedi D, Singh A, Sharma R. Incidence of Kell blood group in blood donors: A population-based study. *Asian J Transfus Sci*, 2015; 9(1): 107.
- [18]. Bhardwaj P, Kumari M, Mishra S, Agrawal R. A retrospective cross-sectional study to analyze ABO & Rh blood group distribution among blood donors, issued units and seasonal trends of blood donations at a tertiary care hospital of Northern India. *J Family Med Prim Care*, 2018; 7(1): 34-38.
- [19]. Makroo RN, Bhatia A, Gupta R, Phillip J. Prevalence of Rh, Duffy, Kell, Kidd & MNSs blood group antigens in the Indian blood donor population. *Indian J. Med. Res*, 2013; 137(3): 521.
- [20]. Lamba DS, Kaur R, Basu S. Clinically significant minor blood group antigens amongst North Indian donor population. *Adv Hematol*, 2013; 9(1): 215454.
- [21]. Thakral B, Saluja K, Sharma RR, Marwaha N. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in

- north Indian blood donors. *Transfus Apher Sci*, 2010; 43(1): 17-22.
- [22]. Kahar MA, Patel RD. Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in blood donors of south Gujarat. *Asian J Transfus Sci*, 2014; 8(1): 51.
- [23]. Zhong LI, Rong ZE, Qing CH, Min LI, Shi GY, Peng WE, Huang CY, Tang RC, Jun SU, Zhang XG. Genotyping for Kidd, Kell, Duffy, Scianna, and RHCE blood group antigens polymorphisms in Jiangsu Chinese Han. *Chin. Med. J*, 2012; 125(6): 1076-81.
- [24]. Rahman M. Incidence of important blood groups in Bangladesh. *Bangladesh Med. Res. Counc. Bull*, 1975; 1(1): 60-63.
- [25]. Shahverdi E, Moghaddam M, Talebian A, Abolghasemi H. Distribution of blood groups in the Iranian general population. *Immunohematol*, 2016; 32(4): 135-39.

- [26]. Thornton NM, Grimsley SP. Clinical significance of antibodies to antigens in the ABO, MNS, P1PK, Rh, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Yt, and Xg blood group systems. *Immunohematol*, 2019; 35(3): 95-101.
- [27]. Nathalang O. Red cell genotyping in Thailand: trends for blood transfusion in Asian populations. *ISBT Sci. Ser*, 2020;15(3):310-4.
- [28]. Akasha AS. The frequency of Kell red cell antigens (K, k) among the major sudanese tribes. *Recent Res. Sci. Technol*, 2012; 4(1): 44-45.
- [29]. Adewoyin AS, Lee GM, Adeyemo TA, Awodu OA. Rh and Kell blood group antigen prevalence in a multi-ethnic cohort in Nigeria: implications for local transfusion service. *Immunohematol*, 2018; 34(2): 61-65.
- [30]. Felimban RI, Sumeda SM. Distribution of Kell antigens K, k, Kpa, and Kpb among blood donors in Jeddah city of Western Saudi

- Arabia. *Asian J Transfus Sci*, 2021; 15(1): 75.
- [31]. Khan A, Kulsoom U, Nawab SN, Fatima S, Saleem S, Azhar A, Zehra S. Association of non-synonymous+326A>T and synonymous+258G>A polymorphisms of omentin-1 gene (ITLN-1) among type 2 diabetes patients of Pakistan. *Health Biotechnol Biopharm*. 2021; 5(2): 46-60.
- [32]. Afzal M, Sardari S, Sikandar S, Afzal I. A novel approach for analysis of shared genetic architecture of drug response based on summary statistics from genome-wide association studies. *Health Biotechnol Biopharm*. 2019; 3(3): 12-49.
- [33]. Kavousi S, Ahmadifar M, Fard TM, Lavasani NS, Jalili A, Sadrabadi AE, Branch I. Specific signaling pathways and drugs play key role in regulation of angiogenesis. *Health Biotechnol Biopharm*. 2020; 4(1): 7-37.