DOI: https://doi.org/10.53350/pjmhs2023171698

ORIGINAL ARTICLE

Kell Blood Group System Antigen Genotypic Frequencies in Northern Pakistani Healthy Blood Donors a Multi Center Study

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ABSTRACT

Objective: To investigate the genotypic frequencies of the K and K blood group system antigens in Northern Pakistan's healthy blood donors.

Material and Method: this Multi center study conducted in department of medicine Hmc Hospital Peshawar From February 2021 to February 2022, The study comprised 59 blood donors in total. The traditional PCR method was used for genotyping, and the amplified products were then run over polyacrylamide gel electrophoresis. Version 26 of SPSS was utilised for the analysis.

Results: In this study, 59 blood donors with a mean age of 31.47.07 years were totaled. Male to female ratio was 29.22 to 1. KEL*2/KEL*2 was the most prevalent genotype, found in 53 (91%) donors, followed by KEL*1/KEL*2 in 3 (06%) and KEL*1/KEL*1 in 03 (03%) donors.

Conclusion: Identify the antigens in our nation and contrast the findings with those of other populations. The results of this research may be utilised to create a database of genotypic frequencies.

Keywords: genotyping, Kell blood group, polymerase chain reaction, and blood donors (PCR).

INTRODUCTION

After the ABO and Rh blood group systems, the Kell blood group system is the third most immunogenic blood group system in terms of inducing immunological responses. The Kell gene encodes all of the system's recognised antigens, which number at least 34 and make up the complicated antigenic system[1]. The 2 KL gene is located at 7q33 on chromosome One triplet and four pairs of allelic antigens make up the Kell system: Kpa, Kpb, and Kpc; K and k; Jsa and Jsb; K11 and K17; and K14 and K2[2]. The Kell blood group's most prevalent clinically relevant antigens are K, K, Kpa, Kpb, Jsa, and Jsb. Among them, K antigen is crucial for transfusion therapy and foetal and neonatal hemolytic diseases[3]. Blood transfusion responses are frequent, hence particular studies are carried out to guarantee secure blood banking and to provide patients antigen-free blood. The Kell blood type. Antigens are important in transfusion because of their immunogenicity and polymorphism[4]. They produce alloantibodies that target the Kell antigens, leading to severe transfusion responses and alloimmunization in mismatched blood transfusions. Studies have shown a link between a small number of uncommon occurrences of haemolytic illness of the pregnancy and newborn and the Kell blood type. Haemolytic disease of the foetus and newborn is mostly related with ABO and Rh incompatibility Kell vaccination causes foetal anaemia, a severe hemolytic condition of the foetus and baby. Fetal red blood cell synthesis is suppressed as a consequence of maternal anti-Kell antibodies that are directed towards foetal red cell precursors[5].Genotyping of blood groups is now a commonly used procedure in the transfusion medicine community[6]. Although several worldwide research being done in this area, this procedure is not yet a part of the standard genetic testing done in our nation. This research, which was the first to be conducted in Pakistan, helped create a database of the genotypic frequencies of Kell blood type antigens in our nation and allow for comparisons with data from other populations[7].

MATERIAL AND METHOD

the department of medicine of HMC Hospital Peshawar did this Multi center research. After permission from the ethical review board, the research included 59 blood donors in total from February 2021 to February 2022. After receiving informed permission, 59 blood donors in total were included in the research using a non-probability purposive sampling approach. Each patient had two 3.5 ml samples of venous blood placed in EDTA tubes for genotyping.

Genotyping: Using the Chelex technique, DNA was extracted from EDTA-ant coagulated venous blood samples. Using an ABI 2720 thermal cycler, the isolated DNA was amplified using a standard PCR procedure (Applied Biosystems 2720 USATM). First-step cycle conditions comprised 950C temperature holding for 2 minutes, 30 seconds of denaturation at 940C, 40 seconds of annealing at 650C, and 45 seconds of extension at 720C. During five cycles, these temperatures were held constant. The second stage consisted of 25 cycles, during which the following temperatures were maintained: denaturation for 30 seconds at 940C, annealing for 40 seconds at 650C, and extension for 45 seconds at 720C. The final extension was accomplished by maintaining 720C for two minutes. After the last extension, the temperature was held at 250C for 30 seconds. HGH was employed as an internal control to monitor the response. The following are the primers used and their sequences:

RESULTS

A total of 59 blood donors with a mean age of 31.47.07 years were included in the study. Male to female ratio was 29.26 to 1. KEL*2/KEL*2 was the most prevalent genotype, found in 53 (90%) donors, followed by KEL*1/KEL*2 in 3 (6.0%) and KEL*1/KEL*1 in 3 (4%) donors.. Distribution of study subjects according to gender is shown in Figure 2.

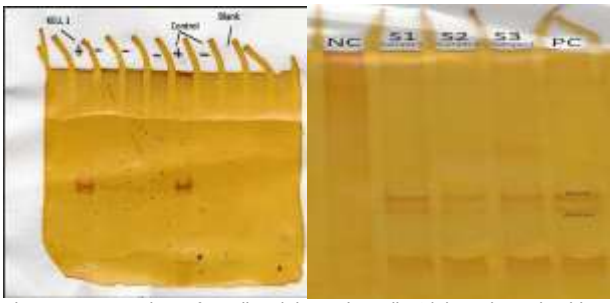

Figure 1: Results of Kell 1(K) and Kell 2(K) polyacrylamide gel electrophoresis with positive and negative controls

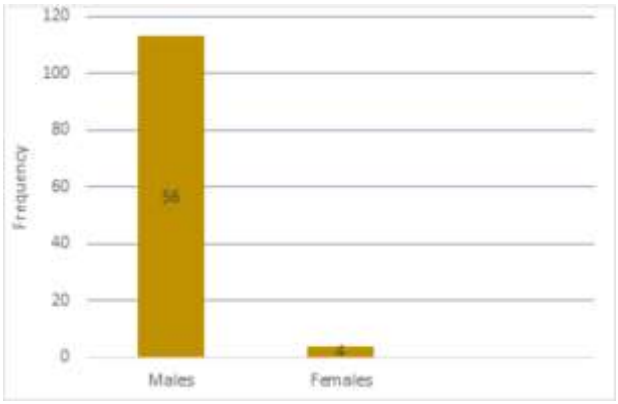

Figure 2: Gender Distribution n-59

individuals were genotyped for Kell (K and k) blood group system. Among these only 4 (6%) were found having Kell 1(K) antigen while 56 (97%) individuals had Kell 2 (k) antigen as shown in Table 2.

Statistical analysis: IBM SPSS Statistics 26 was used to analyse the data. For quantitative data, mean and standard deviation were calculated. For qualitative variables, frequency and percentages were determined.

Table 1: Primers and their sequences

Internal control	Forward primer (5´-TCCCTTCCCAACCATTCCCTTA-3´) Reverse primer(3´-CCACTCACGGATTTCTGTTGTGTTTC-5´)
Kell 1 (K)	Forward primer (5´-ACTCATCAGAAGTCTTTGCA-3´) Reverse primer (3´-GCTCCCCAGCCCCCTCCG-5´)
Kell 2 (k)	Forward primer (5´-CTCATCAGAAGTCTCAGCG-3´) Reverse primer (3´-GTGTCTTCGCCAGTGCATC-5´)

The amplified products were subjected to 6% polyacrylamide gel electrophoresis (Invitrogen) at 200 volts for 30 mins. Afterwards, the gel was stained with 0.1% silver nitrate (sigma) followed by counterstaining with 1.5% NaOH and 37% Formalin. Results were recorded after drying.

Table 2: Antigen frequency of Kell (K and k) antigens (n=59).

Antigen	Frequency (n)	Percentage (%)
Kell 1 (K)	04	3%
Kell 2 (k)	56	97%

PCR was used to determine the genotypic frequencies of the Kell blood group system. KEL*2/KEL*2 was the genotype that was most often found (91%). As revealed in Table 3, other genotypes included KEL*1/KEL*2 (06%) and KEL*1/KEL*1 (03%).

Table 3: Kell blood group system genotypic frequencies (n=59).

Genotype	Frequency (n)	Percentage (%)
KEL*1/KEL*1	02	03
KEL*1/KEL*2	04	06
KEL*2/KEL*2	53	91

DISCUSSION

Blood banks and transfusion labs utilise serological testing to detect the main blood antigens, including A, B, AB, O, and Rh D. Regular screenings avoid haemolytic transfusion reactions, which may occur during or after a blood transfusion[8]. A patient who has one or two transfusions in their lifetime does not have an issue with mismatched small blood antigens. One in 11000 transfusions has an adverse response, and one in 500,000 dies, according to the Canadian Public Health Agency Delays occur in 1 in 4000 transfusions but cause considerable morbidity. Nevertheless, growing reagent costs, variable serological findings, and a lack of testing for specific antigens prevent small blood antigen identification on blood donors and patients[8].

Researchers want to use molecular analysis to find additional minor blood kinds after discovering the ABO blood group's molecular foundation[9]. Virtually all human blood group genes have been cloned, and the molecular basis for all clinically relevant blood group genotypes has been uncovered utilising a rapidly growing variety of methods, from low throughput to high throughput. The molecular technique is designed to provide more precise blood genotypes for multiply-transfused patients[10].

Transfusion services may employ community blood type antigen prevalence to provide safe blood and evidence-based haemolytic disease of pregnancy and newborn care. Individuals with thalassemia, refractory anaemia, or multiparity who have been transfused several times are more prone to acquire antibodies against non-ABO blood type antigens[11]. Grouping red cell antigens before transfer prevents transfusion responses. Such patients are hard to place. Considering Kell antigens, K and k antigens are crucial to immunological responses. Several studies show their global frequencies.The Kell blood group system's most common genotype was KEL*2/KEL*2. KEL*2/KEL*2 was more common in Brazil than in our community[11].100% KEL*2/KEL*2 genotype was found in China. 11 KEL*1/KEL*1 and KEL*1/KEL*2 were other genotypes.

Molecular testing can accurately predict phenotype from genotype. Molecular approaches offer numerous benefits over haemaglutination-based procedures[12]. Several antisera are unavailable and serological methods are expensive[13].Many considerations are considered while choosing a molecular approach, including cost, time per test, sensitivity and specificity, throughput, and equipment availability. PCR-SSP was chosen for this investigation. Sequence specific priming PCR (PCR-SSP) is easy, specific, and economical. Red blood cell blood group genotyping may be done routinely using it in underdeveloped nations[14].

CONCLUSION

The K and K antigens are important in transfusion medicine and haemolytic diseases. This study provides information on the genotypic frequencies of the K and K antigens in Northern Pakistan's healthy blood donors, which can be used to identify the antigens in our nation and contrast the findings with those of other populations. The results of this research may be utilised to create a database of genotypic frequencies.

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