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ORIGINAL ARTICLE

Genotyping for Scianna blood group system polymorphism in Pakistan.

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ABSTRACT… Objective: To establish the occurrence of Scianna blood group alleles via genotyping in Pakistani blood donors. **Study Design:** Cross-sectional Analytical study. **Setting:** Department of Hematology, Army Medical College (AMC) in association with the Armed Forces Institute of Transfusion (AFIT) Rawalpindi. **Period:** January 2019 to January 2020. **Material & Methods:** In this study 300 blood donors were incorporated after taking informed consent; DNA was extracted from their EDTA anticoagulated blood samples and tested for SC*01 and SC*02 alleles by Sequence-Specific Primer Polymerase Chain Reaction (PCR-SSP), which was ensued by gel electrophoresis. The allele frequencies were established and Hardy Weinberg equilibrium was determined. Chi-square test was applied to compare the observed and expected genotype frequencies as well as for comparison with published allele frequencies of other populations. We used SPSS version 22 for the analysis of study data. **Results:** All donors were homozygous for SC*01, demonstrating allele frequency for SC*01 nearly 1.00. Genotype frequencies were congruent with the Hardy Weinberg equilibrium. The difference between the allele frequencies of SC*01 and SC*02 alleles of population of Pakistan was not statistically significant when compared to those of other populations. **Conclusion:** This maiden research illustrates Scianna blood group distribution in Pakistani community by PCR-SSP. This method is cheap and proficient and can be applied in developing nations. The findings of this study can aid in the production of local red cell panels, detection of uncommon blood types, and setting up of a domestic rare blood donor platform.

Key words: Genotyping, Hardy Weinberg Equilibrium, Red Blood Cell Antigens, Scianna, Sequence Specific Primers Polymerase Chain Reaction (PCR-SSP).

INTRODUCTION

The analysis of human blood group antigens has evolved as a robust area of research, principally due to the advancements in molecular testing procedures. The International Society of Blood Transfusion (ISBT) has accredited over 300 antigens[1,2], and most of them are arranged in 43 blood group systems. The blood group genes express polymorphism and immunogenicity.[3] Antibodies directed against erythrocyte antigens can reduce the lifetime of transfused RBCs and can also lead to hemolytic transfusion reactions and fetal and neonatal hemolytic disease.[4]

Red blood cell panels that are commercially available are usually typed for: ABO, RH1-5, FY1 and 2, KEL1-4, 6 and7, JK1 and 2, MNS1-4, LE1and 2, LU1 and 2, P1 and XG.[5] Serological reagents for comprehensive phenotyping are expensive, available in limited amounts, inadequately reacting and lacking for certain blood group systems like Scianna.[5,6] Thus, identification of red blood cell antigens at genetic level can help in mass-scale screening of blood donors and to solve complicated serologic problems.[7] All erythrocyte antigens with established gene polymorphisms may be genotyped since contrary to antisera, chemicals used for molecular techniques are available in surplus amounts.[8] Due to DNA-based procedures, it is now probable to determine way more erythrocyte antigens as compared to serology.[9,10]

The Scianna blood group system was assigned the symbol SC and digit 013.[11] It is known as the "lucky thirteenth" among blood group systems.[12]

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In the Scianna blood group system eight antigens have been identified up till now[13], including the counter antigens SC1 and SC2.[14] SC8 named SCAR has been recently recognized by Srivastava et al. in a Saudi Arabian patient.[13,15] The gene encoding the Scianna system is positioned on chromosome number one[16] and its antigens are braced on ERAMP; a red cell membrane-related protein.[14,15] These are well established in neonates and are expressed on cord RBCs.[17] The rising trend of molecular testing techniques has uncovered the importance and increased the awareness of the Scianna system.[12] SC*01, SC*03, SC*05 to SC*08 are globally highly prevalent whereas SC*02 and SC*04 are found in low frequency.[13,14] The incidence of SC*01 is around 99 percent around the world whereas only about 1 percent of the people of different regions express SC*02.[3]

Antibodies directed against Scianna antigens are infrequent and there is slight knowledge concerning their clinical importance.[16] Scianna antibodies are typically created due to RBC sensitization by pregnancy or transfusion, although there are some examples of naturally occurring anti-SC2 antibodies. These are generally IgG type, react efficiently in the antiglobulin phase and do not bind complement.[16]

Molecular testing is now being extensively used for genotyping red cell antigens and is considered an indispensable tool in the field of immune hematology.[18] Genotyping has made it possible to identify a far larger number of erythrocyte antigens than by serological techniques.[9] Awareness of the hereditary basis of these antigens allows us to ascertain blood group phenotype via genomic DNA.[19] Several transfusion centers are implementing a genomic approach for donor typing in an attempt to alleviate or prevent alloimmunization.[10] DNA-based methods can be used to determine the occurrence of numerous erythrocyte antigens[20] however, the distribution of Scianna blood group system polymorphisms remains unaddressed in Pakistan till to date. Keeping this in view, the current study was undertaken to describe the polymorphism of Scianna in Northern Pakistani population.

MATERIAL & METHODS

This is a cross sectional study was conducted at Department of Hematology, Army Medical College from January 2019 to January 2020. This study was approved by ethical committee (ERC/ID/21).

Sample size calculated by the WHO calculator was 214. Three hundred blood donors participated in our study after giving their informed consent. Donors' particulars were noted on a proforma and were kept confidential.

The DNA of the donors was obtained from their peripheral blood with the help of the QIA amp DNA blood kit (Qiagen, Germany). The donors were screened for SC*01 and SC*02 alleles using PCR-SSP. Each PCR reaction tube had 10μL of PCR buffer with 1.5mmol/L of $MgCl_2$, 1μL of allele specific primer, common primer and human growth hormone (hgh) primer (Gene Link, USA), as well as 0.1 U of 5U/μL of Taq polymerase (Thermo Scientific, USA). Two microliters of genomic DNA were put in each tube. Primers particulars are shown in Table-I.

Blood Group System or Gene	Allele	Orientation	Sequence	Tm (°C)	Final concentration (μmole/l)
Scianna	SC1	Forward	TCACCTCCTTGGGTACCGTACC	61.9	0.25
		Forward	TCACCTCCTTGGGTACCGTACT	60.0	0.25
	SC2	Common Rev	CTCCCAGTTGGCCTTGTCTC	59.5	0.25
HGH		Control	GCCTTCCCAACCATTCCCTTA	57.3	0.1
		Control	TCACGGATTTCTGTTGTGTTT	53.6	0.1

Table-I. Sequence and specifications of primers (Gene Link USA) used in PCR

The PCR was executed by means of Gene Amp thermo cycler PCR 2700 according to these settings: preliminary DNA denaturation at 94 °C for 120 seconds, then10 rounds of denaturation for 10s each at 94°C, 6 extension for 60 seconds at 65°C and lastly 25 rounds of denaturation at 94°C for 30s, 60 seconds annealing at 61°C, followed by 30s of extension at 72°C. Elongation for 5 minutes at 72°C concluded the protocol.

Internal control of Human Growth Hormone and a blank (negative control) was set up with every PCR. Whenever the results were unclear, the PCR was rerun. Every PCR result was assessed by skilled specialists. Later we performed polyacrylamide gel electrophoresis (PAGE) by mixing 0.4μl ethidium bromide with amplified DNA and filling it inside wells made up of 6 percent polyacrylamide gel. Then electric current at 200 volts is applied to the gels for 30 minutes, lastly staining of the gels was done with silver nitrate, sodium hydroxide and formaldehyde to observe the DNA bands.

LIMITATIONS

To carry out this molecular research we needed to ponder on various issues for instance funds, the time it takes for each test, the precision of techniques, accessibility of equipment etc. High-efficiency analyzers like, Human Erythrocyte Antigen (HEA) Bead Chip, Blood Chip, Luminex xMAP, etc. are pricey and therefore inconceivable. Consequently, we selected PCR-SSP for this study.

Calculation of allele frequencies:
SC*01 and SC*02 genotype frequencies were calculated with the help of Microsoft Excel worksheets by direct totalling while their allele frequencies were determined by the given equation:

Allele frequency= number of alleles/2xsample number.
The status of Hardy-Weinberg equilibrium was established through the Hardy-Weinberg equation which states that: $p^2+2pq+q^2=1$

'P' denotes the frequency of dominant allele, 'q' shows the frequency of latent allele, P^2 is the fraction of homozygous dominant persons; q^2 indicates the fraction of homozygous recessive persons whereas 2pq is for the fraction of heterozygotes.

Statistical Analysis

Observed and expected genotype frequencies were equated by the help of chi-square test that is, χ^2 = (observed value – expected value)^2/ expected value. Similarly comparisons of frequencies of Pakistanis with other populations were carried out via chi-square test. P-value was regarded significant where its value was smaller than 0.05. The data of our study was processed using SPSS software.

RESULTS

The donors' ages spanned between 18 and 63 years (mean 29.8 ± 7.6). Most of them were males (99.7%) and a single female (0.3%). Amongst the 300 donors, the blood type O was present in 92 (30.7%), type A in 83 (27.7%), type B in 90 (30%) while AB blood type was noted in 35 (11.6%) donors. As regards the Rh type, 263 (87.7%) were Rh positive and 37 (12.3%) were Rh negative.

They belonged to various ethnicities; 178(59.3%) were Punjabis, 69(23%) were Pathans, 12(4%) Sindhis, 2(0.7%) Balochis, 18(6%) Kashmiris while Hazarawals, Bultis, and Mohajirs were collectively 21(7%). The donors were genotyped for Scianna (SC1 and SC2) blood group system. The allele frequencies of the donors were computed on the whole (Table-II), as well as of the individual ethnic groups (Table-III).

The genotype frequencies of were in harmony with Hardy-Weinberg equilibrium and didn't have significant difference between observed and expected Scianna frequencies (p-value □ 0.05).

Monomorphism was found in the allele frequencies of Scianna, that is, all the 300 blood donors (100%) typed for the Scianna blood group system were SC*01/SC*01. The frequency of SC*01 allele was nearly 1.00 and that of SC*02 was 0.00.

Blood Group System	Genotypes	Observed Values	Expected Values	Allele Frequencies
Scianna	SC1/SC1 SC2/SC2 SC1/SC2	1.00 0.00 0.00	1.00 0.00 0.00	1.00 0.00

Table-II. Allele frequencies of Scianna blood group alleles in Pakistani blood donors

Blood Group	Allele	Frequency in Punjabis	Frequency in Pathans	Frequency in Sindhis	Frequency in Balochis	Frequency in Kashmiris	Frequency in Others
Scianna	SC1 SC2	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00

Table-III. Allele frequencies of Scianna blood group antigens among different ethnic groups in Pakistan

Ethnicity	SC1	SC2
Pakistani	1.00	0.00
Chinese	1.00	0.01
South Asia	0.99	0.02
Southeast Asia	0.99	0.00
Korean	1.00	0.04
Filipino	0.99	0.02
Japanese	1.00	0.03
American	0.99	0.04
European/ Caucasian	0.99	0.01

Table-IV. Scianna blood group allele frequencies in different world races

DISCUSSION

We performed genotyping to establish the gene frequency of the Scianna system in our population. Blood banks need to have the data of erythrocyte antigen frequencies and polymorphisms to tackle serological difficulties and to issue blood and blood products in special situations. Numerous molecular studies on blood group antigen frequencies in Caucasians and Blacks have been performed but only minute data exists regarding Pakistanis. As far as we know, this study is the first to declare the occurrence of SC*01 and SC*02 alleles in our country.

Pakistan is a land of several civilizations and cultures. Among these are Punjabis, Pakhtoons, Sindhis, Balochis, Kashmiri, as well as others such as Hazarawalls, Mohajirs, and Baltis. SC*01 is prevalent in the Pakistani population; however, SC*02 was not detected at all. The SC gene frequencies of major ethnic groups of Pakistan were not found to be significantly dissimilar from one another.

Comparison of SC*01 and SC*02 frequencies of the local people and other populations was done. Even though Pakistanis have different frequencies of the Scianna blood group system as compared to other races, these differences turned out as statistically insignificant (p-values > 0.05).

DNA-based methods have unveiled the medical importance of the Scianna classification system.[12] SC1 and SC2 are immunogenic. The prevalence of auto and alloantibodies against Scianna antigens and their clinical implications have been recently revised.[11] Anti-SC1 and anti-SC2 have been implicated in mild HDFN.[3,13] Whereas one case of severe HDFN, which required exchange transfusion, has been attributed to anti-SC4.[16] In 2018 anti-SC2 was reported to cause acute hemolytic transfusion reaction.[3] Autoantibodies against SC1 and SC3 have been associated with autoimmune hemolytic anemia.[21] One case of mild delayed HTR has been attributed to anti-Sc7.[3]

SC1is ubiquitous and its frequency is almost 100 percent around the world. SC2 is rare all over the globe (less than 1%)[3,20] except in some people of European descent, mainly Mennonites.[3] In our study all participants were positive for SC1, displaying 100% frequency in the Pakistani population. Studies from China reported a 100% prevalence of SC1 in their population.[19,22] According to an Italian study SC1 is 99.6% whereas SC2 is 0.4% in healthy Italian blood donors.[23] Yun Ji Hong et al studied the prevalence of Scianna antigens in Korea and reported that the frequency of SC1 and SC2 in Koreans is 100% and 0% respectively.[24]

The awareness of the dispersal of different blood group antigens within the population is crucial

for transfusion practice since the donor versus recipient disparity results in the production of allo-antibodies[1] which can lead to fatal consequences. Geographically and anthropologically different populations have marked dissimilarities in the distribution of red cell antigens.[25]

Molecular methods have several benefits as compared to the conventional serological approach.[19] Serological reagents are expensive and several antisera are commercially lacking.[2] While, PCR-based techniques are faster, more accurate and require less manpower. Genotyping can upgrade donor blood screening for prevalent as well as uncommon erythrocyte antigens[9] thus enhancing the plausibility of issuing antigen-precise blood components for the recipients.

CONCLUSION

This innovative research in Pakistani blood donors gives frequencies of Scianna blood group alleles via PCR-SSP technique. This methodology is proficient as well as economical and is applicable in limited means countries such as ours. Our results may aid in the identification of rare blood groups, manufacture of internal RBC antibodies screening tools and initiation of Pakistan's rare blood donor platform.

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2	Mariam Riaz	Project administration writing reviewing and editing.	
3	Sidra Aslam	Methodology, Investigations.	
4	Shakila Khadim	Data collection.	
5	Hasan Ikram	Writing reviewing and editing.	
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