

Open Access

Original Article

# Frequency of Common Rh Antigens among Volunteer Blood Donors; A Single Centre Study

Imran Ahmed Khan<sup>1</sup>Mavra Fatima<sup>2</sup>Ayisha Imran<sup>3</sup>Nauman Aslam Malik<sup>4</sup>Akhtar Sohail Chughtai<sup>5</sup>

## Abstract

**Objective:** This study aims to ascertain the prevalence of common Rh antigens among willing blood donors.

**Methodology:** A cross-sectional study of 275 subjects was conducted at the department of Hematology Chughtai Institute of Pathology, Lahore region of Punjab, Pakistan from January 2019 to June 2019. Rh antigen expression was performed on the selected blood samples using specific monoclonal antisera (BIO-RAD) by standard tube technique. Agglutination reactions were graded from 0 to 4+, and results were recorded using Fisher-Race terminology.

**Results:** Among Rh antigens, Rh-e was the most common antigen (98.54%), followed by out of 275 subjects, 248(94.20%) were Rh-D positive while the remaining 27(5.8%) were Rh-D negative. Out of 275 topics, 193 (70.20%) were Rh-C positive, 131(47.70%) were Rh-c positive, 74(26.90 %) were Rh-E positive, and 271(98.54%) were Rh-e positive.

**Conclusion:** For the blood banks to effectively provide antigen-negative blood to patients who have acquired alloantibodies, more efforts must be concentrated on expanded Rh and other blood group antigen phenotyping and creating organizational databases.

**Keywords:** Red blood cells, Rhesus blood group system, Rh antigens, Rh phenotyping.

Department of Hematology, Chughtai  
Institute of Pathology, Lahore, Pakistan

## Address for Correspondence

Dr. Imran Ahmed Khan

Department of Hematology, Chughtai  
Institute of Pathology, Lahore, Pakistan  
ibaloch777@gmail.com

## Introduction

Red blood cell (RBC) antigens, which are controlled by several genes, make up the overall blood group system, which is referred to as a "blood group." Over time, our understanding of blood types has expanded to include issues with transfusions and specific diseases linked to RBC surface antigens. The Rh system is the largest blood group system, alongside the ABO system. Alloimmunization and antibody formation in the recipients against the "Rh or minor blood group antigens like Kell, MNSs, Duffy, etc." is still potential even after adequate blood grouping and cross-matching. The general blood group system, which consists of genes that regulate the RBC antigens, is referred to as a "blood group".<sup>1</sup> Over time, our understanding of blood types has expanded to include particular illnesses related to RBC surface proteins and issues with donations.<sup>2</sup> The ABO blood group system was reportedly discovered in 1900 by "Karl Landsteiner".<sup>3</sup> The "ABO, RH, MNS, Duffy, Kell, Kidd,"

and Lewis groups are among the approximately 43 blood group systems with a total of about 349 antigens that are now known.<sup>4</sup> On rare occasions, "the Kell, Kidd, and Duffy groups" are responsible for the newborn hemolytic illness. ABO and Rhesus (Rh) systems are the most popular blood groups.<sup>5</sup> The most immunogenic red cell antigens are the ABO blood group antigens, which are also the first to be identified. Rh antigens are next in immunogenicity. Every blood bank or transfusion service routinely types for both.<sup>6</sup> The advancement of safer blood transfusions was greatly aided by identifying the ABO blood group system antigens.<sup>7</sup> While Landsteiner and Weiner identified the "Rhesus (Rh) blood group in 1940, Alfred von Decastello and Adriano Sturli found the fourth type, AB, in 1902".<sup>8</sup> People could be categorized into 4 main groups based on RBC agglutination patterns: A, B, AB, and O.<sup>9</sup> Hereditary characteristics such as "ABO and Rhesus (Rh) blood group" antigens are useful in population genetic studies, analyzing population migration trends, and resolving medico-legal problems, notably paternity issues. But testing in blood donation procedures is more important.<sup>10</sup> Although the involved antigens remain stable throughout life, there are significant differences between ethnic groups, races, and geographical limits in the "ABO and Rh genes and phenotypes".<sup>11</sup> As with ABO frequencies, Rh frequencies

Authorship Contribution: <sup>1,2</sup>Conceived and planned the idea of the study, drafting the work or revising it critically for important intellectual content, <sup>4</sup>Collecting the data, Data Analysis, <sup>5</sup>Final approval of the version to be published

Funding Source: none

Received: Aug 21, 2022

Conflict of Interest: none

Accepted: Dec 17, 2022

vary amongst ethnic groups. Nearly 100% of persons from the "Far East, 95% of black Africans, and 82% to 88% of Caucasians are D-positive".<sup>12</sup> The immunogenic Rh blood group system is mostly to blame for HDFN. However, the same illness process can also be caused by the ABO blood group. Red blood cells from the foetus or newborn suffer immunological hemolysis in HDFN because of maternal antibodies that cross the placenta. The implicated antibodies may be naturally occurring or immunological antibodies that develop following a sensitizing event, such as a transfusion or pregnancy.<sup>13</sup> A prevalent newborn issue thirty years ago, HDFN was virtually always associated with Rh D alloimmunization. The prevalence of "maternal D alloimmunization" decreased from 14% to 1-2% after postnatal "immunoprophylaxis" was introduced in 1970. Antenatal immunoprophylaxis was initiated, further lowering Rh-D alloimmunization to 0.1%.<sup>14</sup> Over the past ten years, moderate-severe HDFN cases linked to various alloantibodies besides the anti-D alloantibody have been reported in Asian nations.<sup>15</sup>

The study's goal was to count the amount of common Rh antigens present in willing blood donors. When a patient needs a certain type of antigen-negative blood, the information from this study will be useful in identifying potential donors.

## Methodology

A cross-sectional study of 275 subjects was conducted at the Department of Hematology of the Chughtai Institute of Pathology, Lahore region of Punjab, from 1st January 2019-30th June 2019. After collecting patient data on the questionnaire using the Nexus pro, consented participants were assigned a unique identification patient and case number and then directed to a designated trained phlebotomist at the blood sample collection point. In this study, consecutive male and female blood donors between 18 and 60 who had not bled in the previous three months were included. Donors who weighed less than 50 kg, tested positive for viral markers (HIV and hepatitis) on an ELISA, had typhoid fever within the previous six months, were on medication for HCV, or had received blood transfusions within the last year were excluded from this study.

Within 24 hours of sample collection by the manual standard tube method by manufacturer's instructions, Rh

antigen expression was carried out on the chosen blood samples using particular monoclonal antisera (BIO-RAD).<sup>16</sup> Agglutination reactions were graded from 0 - 4+. Any response of 1+ or more was considered positive. All "D" negative samples were tested for weak D. All negative results were confirmed by microscopy. Results were recorded using Fisher-Race terminology. All the Rh antigens data was recorded per the operational definition on the pre-designed Proforma.

The process to determine the presence of different Rh antigens on the RBC surface included cross-reacting them with commercially available antisera containing known anti-Rh antibodies. Visible clumping after 1 minute was considered positive for the corresponding antigen.<sup>17</sup> The participants of the study belonged to different parts of the country. Using a 6% margin of error and taking a 95% confidence level, the sample size was 275. All data were recorded in the table, stratified by age and Rh results, and analyzed in terms of gender, mean and standard deviations. Rh antigen frequency was determined.<sup>18</sup> All data were analyzed using SPSS version 21.0; comparisons between categorical variables age and gender were computed using the post-stratification Chi-Square test. A p-value < 0.05 was considered statistically significant.

## Results

Of the 275 study participants, 200 (72.73%) were males, and 75 (27.27 %) were females, with ages ranging from 18 years to 41 years. Most donors were aged between 26 to 33 years, i.e. 54.5%. The highest percentage of donors belonged to the Lahore region of Punjab, i.e., 147 (53.4%), followed by other areas of Punjab, 101 (36.7%), 14(5.09%) from Khyber Pakhtunkhwa (KP) and 13 donors (4.7%) from Sindh (Figure 1). The characteristics of the study population defined by various demographic variables are (Figure 2)

Post-stratification Chi-Square test with a confidence level of 95% showed that the antigen positivity distribution was significantly different according to gender. There was a significant association between the Rh-D, Rh-c and Rh-E results and gender ( $\chi^2(273) = 144.85, p < 0.05, \chi^2(273) = 23.46, p < 0.05, \chi^2(273) = 16.27, p < 0.05$ ). There was no significant association between the Rh-c and Rh-e result and gender ( $\chi^2(273) = .14, p > 0.05, \chi^2(273) = 1.073, p > 0.05$ ). So there was a significant relationship between Rh

D, Rh c and Rh E results and gender, and there was no important relationship between Rh C, Rh e and gender. (Table I) Figure 3 explains the frequency of Rh Antigen in various Pakistani provinces.

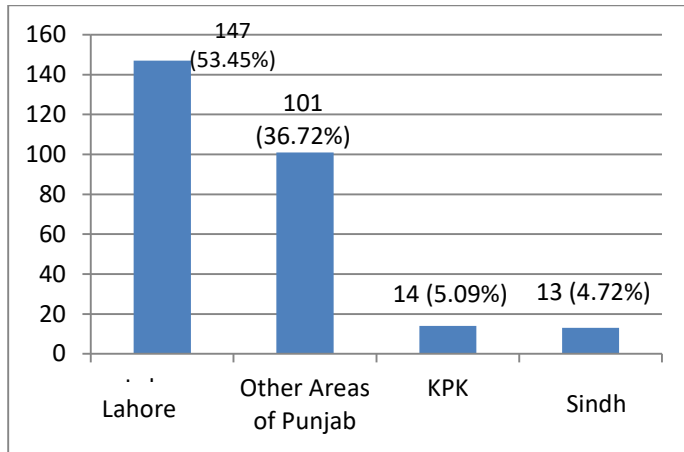


Figure 1. Distribution pattern according to the area.

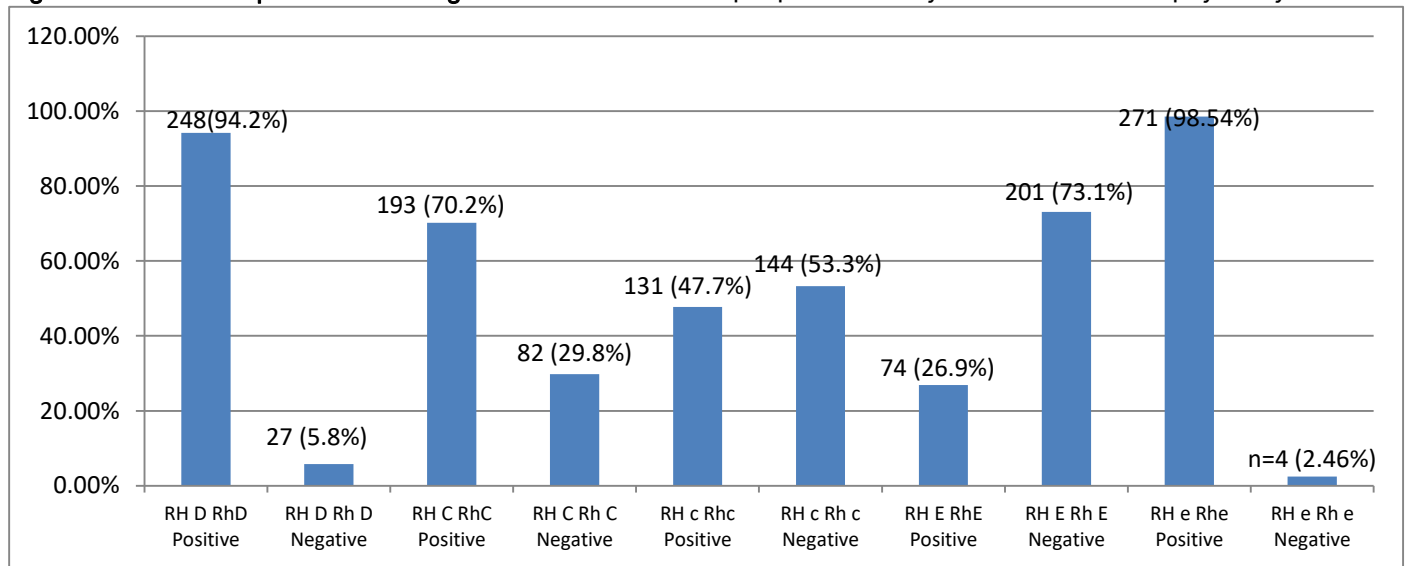


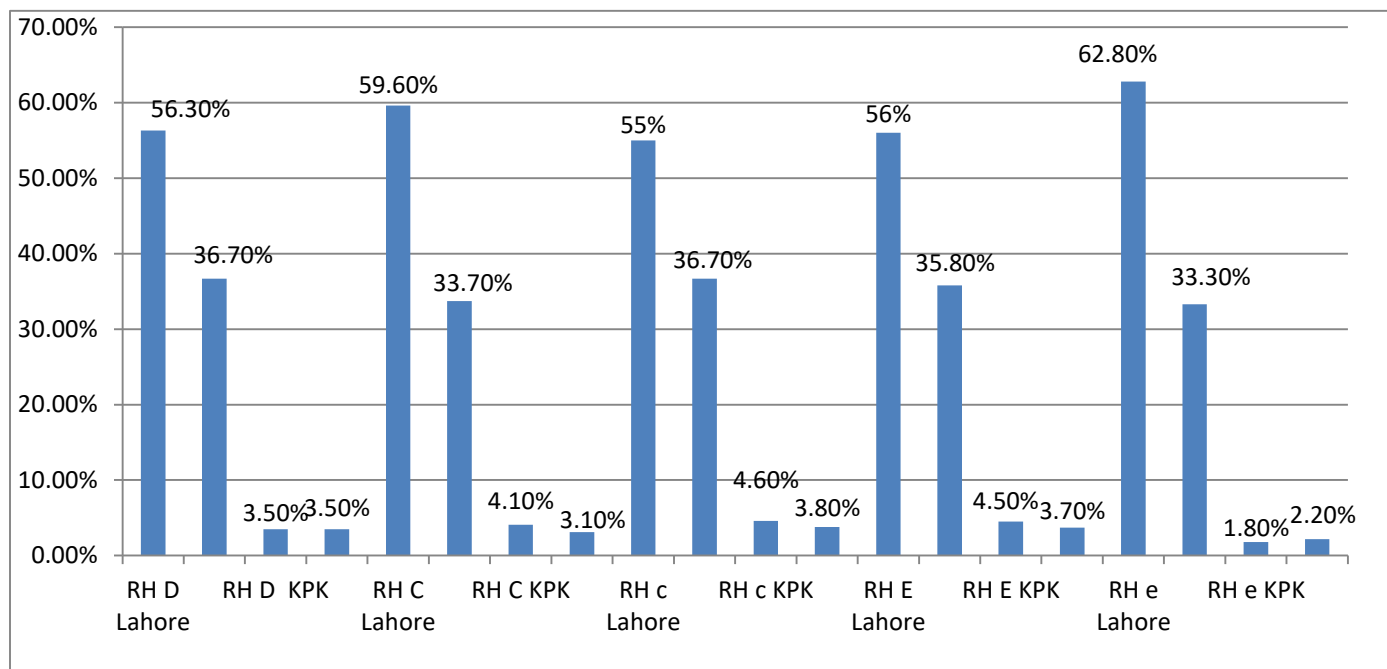
Figure 2. Frequency and Distribution pattern of Rh antigens among Donors.

Table I: Distribution pattern of Rh antigen-positive cases according to Gender.

A pattern of Rh antigen		Gender		Total	P value
		Male	Female		
Rh D	Positive	173 (69.8)	75 (31.2)	248 (94.2)	<0.05
	Negative				
Rh C	Positive	141 (73.1%)	52 (26.9%)	193 (70.2%)	
	Negative				
Rh c	Positive	104 (79.4%)	27 (20.6%)	131 (47.7%)	
	Negative				
Rh E	Positive	50 (67.6%)	24 (32.4%)	74 (26.9%)	
	Negative				
Rh e	Positive	198 (73.1%)	73 (26.9%)	271 (98.54%)	
	Negative				

## Discussion

The Rh system is regarded as the second most important blood group system after the ABO system because of its relationship to hemolytic transfusion reactions and infant hemolytic disorder. The bulk of blood donors in the study, or about 58.54% of all donors, were between the ages of 26 and 33. It was followed by 30.18% of group 1 donor recipients between the ages of 18 and 25. 11.28% of donors fall into group 3 and are between the ages of 34 and 41. About 51.9% (2764/5316) of blood donors in research by Gupta et al. were between the ages of 31 and 40.<sup>19</sup> According to Kotwal et al., the mean age of donors was 29.87 years, and 89.09% were under the age of 40. In research conducted in Pakistan by Karim et al., the median age of donors was 35 years. This results from our society's core strength being its younger age groups. Furthermore, there are fewer deferrals among younger people since they are more alert and physically active.



**Figure 3. Distribution pattern of Rh antigen to area**

Therefore, they are the most frequently encountered age group when giving blood.<sup>20</sup>

Additionally, demographic differences are significant. For example, the majority of the population in Japan is older than that of Japan, whereas the majority of the people in India are younger.<sup>21</sup> The highest frequency of Rh antigen was found in the current investigation. The antigen was found similarly frequently, according to Gupta et al.<sup>19</sup> Sarkar et al. reported RhD positives of 94.36%, 84.76%, and 91.6%, roughly identical to our data.<sup>22</sup> Worldwide, the prevalence of the RhD antigen varies greatly, ranging from 60% to 99%. Rh D is most prevalent in people from India, where it affects 99–100% of the population, while it is least prevalent in persons from Southern France and Northern Spain, where it affects 60–80% of the population.<sup>23</sup> In Pakistan, "Karim et al. reported 97% RhD positive donors while in Bangladesh it was 94.6% as reported by Shil et al."<sup>24, 25</sup> The least prevalent Rhesus antigen globally is RhE. The incidence of RhE antigen was found by Sharma et al. and Thakral et al. to be 17.9%, 25.6%, and 26.55%, respectively.<sup>26, 27</sup>

Our study's data have revealed details on the genetic diversity and polymorphism of the rhesus antigens and blood types among Pakistan's diverse groups. Our research results show that the Rh blood groups distribution pattern in Pakistan conforms to the general way across India and many parts of the world. The

outcome for the Rh blood group is consistent with the globally documented trend, with Rh positives being the most prevalent. The managers of blood banks and clinicians would find this information very helpful when organizing blood transfusion operations. The prevalence of RHD blood types among Pakistan's blood donors was reported in an important thorough study. Large blood banks in Pakistan may need to use specific blood types more frequently than others in emergencies due to this study.

## Conclusion

Results of the study show that in the population studied, "e" is the most prevalent antigen while "E" is the least pervasive antigen. Consequently, transfusion recipients who are antigen "e" negative and acquire an "anti e" allo antibody will face the most hardships in getting a cross-match compatible blood product. At the same time, antigen "E" negative recipients will be in a relatively better situation to get a cross-match compatible product(s). Hence there is a need to develop registries of phenotyped volunteer blood donors to be established on a large scale to facilitate these transfusion-dependent patients in getting a safe and compatible product. Data from the current study is being organized and used to help patients with Rh antibodies get Rh-compatible donors.

## References

- Chakraborty S. Genetic analysis on frequency of alleles for Rh and ABO blood group systems in the Barak Valley populations of Assam. *Not. Sci. Biol.* 2010 Jun 13;2(2):31-4.
- 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 Jul;14(2):179.
- Abdul Z. An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services (Doctoral dissertation, Cape Peninsula University of Technology).
- Owen R. Karl Landsteiner and the first human marker locus. *Genetics.* 2000 Jul 1;155(3):995-8.
- Bakare AA, Azeez MA, Agbolade JO. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South–West Nigeria. *Afr. J. Biotechnol.* 2006;5(3):224-9.
- Bakare AA, Azeez MA, Agbolade JO. Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South–West Nigeria. *African Journal of Biotechnology.* 2006;5(3):224-9.
- Zaoui S, Fingold J, Meguenni K, Chabane Sari D. ABO and rhesus blood groups system in Tlemcen population, West Algerian. *Biologie et Santé.* 2007;7(1):62-71.
- DİLEK İ, DEMİR C, Ali BA, AKDENİZ H. ABO and Rh blood groups frequency in men and women living in eastern Turkey. *Int J Hematol Oncol Stem Cell Res.* 2006;32(3):023-6.
- Fagherazzi G, Gusto G, Clavel-Chapelon F, Balkau B, Bonnet F. ABO and Rhesus blood groups and risk of type 2 diabetes: evidence from the large E3N cohort study. *Diabetologia.* 2015 Mar;58:519-22.
- Loua A, Lamah MR, Haba NY, Camara M. Frequency of blood groups ABO and rhesus D in the Guinean population. *Transfusion clinique et biologique: journal de la Societe francaise de transfusion sanguine.* 2007 Nov 1;14(5):435-9.
- Alimba CG, Adekoya KO, Oboh BO. Prevalence and gene frequencies of phenylthiocarbamide (PTC) taste sensitivity, ABO and Rhesus factor (Rh) blood groups, and haemoglobin variants among a Nigerian population. *Egyptian Journal of Medical Human Genetics.* 2010 Nov 1;11(2):153-8.
- Dali SM, Aour MA, Belmokhtar F, Belmokhtar R, Boazza F. The relationship between ABO/rhesus blood groups and type 2 diabetes mellitus in Maghnia, western Algeria. *South African Family Practice.* 2011 Nov 1;53(6):568-72.
- Suresh B, Sreedhar Babu KV, Chandra Mouli P, Arun R, Jothibai DS. Distribution of ABO and rhesus (D) blood group antigens among blood donors at a tertiary care teaching hospital blood bank in south India. *J Clin Sci Res.* 2015;4(2):129.
- Hé B, Kabobo KI, Mukena TS, Ngiele MD, Kabingie NG, Kasolva TC, Joë K, Kasendue EP. Frequency of erythrocyte phenotypes in blood group systems ABO and Rhesus at Moba, Province of Tanganyika, Democratic Republic of Congo. *Open Access Library Journal.* 2017 Mar 2;4(3):1-2.
- Erhabor O, Adias TC, Jeremiah ZA, Hart ML. Abnormal hemoglobin variants, ABO, and Rhesus blood group distribution among students in the Niger Delta of Nigeria. *Pathology and Laboratory Medicine International.* 2010 Apr 30;2(7):41-6.
- Hassawi DS. Allele frequency and molecular genotypes of ABO blood group system in a Jordanian population. *J Med Sci.* 2007 Jan 1;7(1):51-8.
- Pennap GR, Envoh E, Igbawua I. Frequency distribution of hemoglobin variants, ABO and Rhesus blood groups among students of African descent. *Br. Microbiol. Res. J.* 2011;1(2):33-40.
- Yadav B, Raina A, Kumar A, Bansal SK, Dogr TD. Allelic and phenotypic diversity of abo and rhesus (d) blood groups in the medical and dental apprentices of a university of Haryana, India. *Int. Res. J of Nat. & Appl Sci.* 2014;1(6):57-67.
- Gupta I, Kumar R, Bhardwaj A, Raturi G. Prevalence of Rh phenotype in voluntary blood donors of Uttrakhand. *IOSR-JDMS.* 2018;17:37-41.
- Fufa AW, Debelo DG. Distribution of ABO and Rh (D) blood groups among students attending secondary and preparatory schools in Bote town, Oromia national regional state, Ethiopia. *nt. J. Sci. Technol. Educ. Res.* 2019 Mar 31;10(1):1-8.
- Pabreja K, Bhasin A. A Predictive Analytics Framework for Blood Donor Classification. *International Journal of Big Data and Analytics in Healthcare (IJBDAH).* 2021 Jul 1;6(2):1-4.
- Sarkar RS, Philip J, Mallhi RS, Yadav P. Proportion of Rh phenotypes in voluntary blood donors. *medical journal armed forces india.* 2013 Oct 1;69(4):330-4.
- N. Garg, D. Singh, R. Tomar, and B. Singh, "Phenotype Prevalence of Blood Group Systems (ABO, Rh, Kell) in Voluntary, Healthy Donors-Experience of a Tertiary Care Hospital in Delhi, North India. *J Blood Disord Transfus* 6: 297.

## Frequency of Common Rh Antigens Among Volunteer Blood Donors; A Single Centre Study

24. 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.
25. Shil N, Sultana N, Sormin S. Study of rhesus genotype and phenotype in Bangladeshi population attended in a tertiary care hospital transfusion medicine. *Anwer Khan Mod. Med. Coll. J..* 2016;7(2):25-8.
26. 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. *Transfusion and Apheresis Science.* 2010 Aug 1;43(1):17-22.
27. Sharma DC, Singhal S, Rai S, Iyenger S, Sao S, Jain B. Incidence of Rh antigens, phenotype & probable genotype in the population of Gwalior and Chambal region, Central India. *Methodology.* 2010 Sep.