ORIGINAL REPORT

# Trends of ABO and Rh phenotypes in transfusion-dependent patients in Pakistan

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The objective of this study was to determine the prevalence of ABO and Rh phenotypes in the general Pakistan population. This information could be used to help reduce the rate of alloimmunization in patients with blood disorders, such as thalassemia major, who require frequent blood transfusions. A total of 242 patients with blood disorders requiring frequent blood transfusions were enrolled in the study. ABO and Rh typing was performed on samples from these patients using tube and gel methods. Of these 242 patients, 146 (60.4%) were male and 96 (39.6%) were female. The prevalence of ABO and D phenotypes was as follows: group O, D+ (38.8%), group O, D– (2.5%), group B, D+ (32.2%), group A, D+ (17.4%), group A, D– (1.7%), and group AB, D+ (7.4%). Of the 242 patients, 232 (95.8%) were D+ and 10 (4.2%) were D–. The most prevalent Rh antigen was found to be e (97%), followed by D (95%), C (89.6%), c (62.8%), and lastly, E (22.6%). The prevalence of Rh phenotypes was: $R_1R_1$ (37.7%), $R_1r$ (33.4%), $R_1R_2$ (19.4%), $R_2r$ (5.2%), and rr (4.3 %). All of the D– patients were rr. In our study, the highest prevalence of ABO phenotypes was group O and the most prevalent Rh antigen was e. Rh phenotyping, along with antibody screening and identification should be performed prior to transfusion of patients requiring multiple transfusions to reduce and possibly prevent the rate of alloimmunization. ***Immunohematology* 2016;32:170–173.**

**Key Words:** ABO, Rh phenotype, alloimmunization

Among the blood group systems discovered to date, the ABO and Rh systems are the most clinically significant in the field of transfusion medicine.[1–3] The ABO system was identified by Karl Landsteiner in 1901, and the Rh system was delineated in 1940 by Landsteiner and Weiner.[3,4] The ABO blood group system is critical because it is the only blood group system in which antibodies are constantly, predictably, and naturally present in the serum of people who lack the antigen.[2] Currently, more than 50 Rh antigens have been discovered in the Rh system, 5 of which are associated with commonly made clinically significant antibodies, namely, D, C, E, c, and e.[3,5,6]

Both systems are important because of the immunogenicity of their antigens and the potency of their antibodies; the diverse genetic polymorphism within the Rh system is particularly immunogenic, because Rh antigens have been implicated in hemolytic disease of the newborn and delayed hemolytic transfusion reactions.[3,6] Previous studies that focused on patients with thalassemia of predominantly Asian descent emphasized that transfusion of phenotypically matched blood for the four Rh antigens, compared with blood phenotypically matched for the standard ABO and D antigens, proves to be effective in preventing alloimmunization.[7,8]

In Pakistan, thalassemia major constitutes the major bulk of red blood cell (RBC) transfusion-dependent disorders where alloimmunization is frequently observed—with Rh antigens being implicated as the most common cause, occurring because of incompatibilities between patients and blood donors.[8] Furthermore, routine blood group typing of patients identifies ABO and D only.[7,8] Typing patients and donors to match for the other four common Rh antigens would significantly reduce RBC alloimmunization and reduce the frequency of transfusion in patients with thalassemia.[8,9] Moreover, it is important to know the prevalence of Rh phenotypes in the patient population receiving regular blood transfusions in order to prevent alloimmunization. ABO phenotypes have been observed and studied in various regions of the country, but limited data have been reported from Pakistan on Rh phenotype prevalence. Delineating Rh prevalence is necessary in finding compatible blood for patients with Rh alloantibodies requiring regular blood transfusion, as emphasized by a regional study done on blood donors.[9] Therefore, with this objective, this study was undertaken to determine the prevalence of ABO and Rh phenotypes in patients with transfusion-dependent blood disorders.

## Materials and Methods

The study was conducted at the blood bank department of the National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan, from August 2012 to October 2014 and was approved by the institutional ethics committee. All patients who presented with blood disorders requiring frequent blood transfusions during the study period and who had not yet received their first transfusion were included in the study. Informed consent was obtained in the local language before enrolling patients in the study.

A 5.0-mL blood sample was drawn from each patient: 2.0 mL in a tube containing ethylenediaminetetraacetic acid

(EDTA) and 3.0 mL in a plain vial. ABO and Rh were tested using the tube method for serologic testing.

Forward and reverse ABO grouping was performed by the tube method and by gel technology. For forward ABO grouping, commercially available monoclonal blood group antisera (anti-A; anti-B; anti-A,B; anti-H; anti-A1) were used, and for reverse grouping, 3–5 percent pooled RBC suspensions of group A, B, and O cells prepared in-house were used. Gel testing by IDcard LISS/Coombs was also used as required. D typing was done by the tube method using monoclonal/polyclonal anti-D (Rh0 and Rh1) and by using the gel method card. For detecting C, c, E, and e, specific monoclonal reagent antisera were used and testing was performed using the standard tube agglutination method. Samples showing no agglutination with anti-D were tested by the indirect antiglobulin test (IAT) for the presence of weak D.

Both Rh control and Coombs control cells were used to ensure diagnostic sensitivity and specificity for the detection of D. In addition, commercial RBCs (rr, $R_1R_1$, and $R_2R_2$) were used with negative antigenic expression of E, C, c, and e to serve as controls for the antisera testing; false-positive and false-negative results were avoided by performing quality control with each step.

All antisera, gel cards, and reagent red cells used in the ABO and Rh testing were obtained from Diamed (Cressier FR, Switzerland).

A statistical package (SPSS-17, IBM, Armonk, NY) was used to analyze the data. Prevalence percentages were computed for categorical variables, and mean and standard deviation (SD) were calculated for quantitative variables.

## Results

A total of 242 patients, having known blood disorders that required frequent blood transfusions and having no prior history of transfusion, were enrolled after obtaining informed consent; 146 (60%) were male and 96 (40%) were female. The patients included 222 with known cases of beta thalassemia major, 12 with aplastic anemia, 4 with pure RBC aplasia, 2 with Diamond-Blackfan anemia, and 2 with chronic lymphocytic leukemia. Thus, the majority were patients with thalassemia major. Their ages ranged from 1 month to 40 years, and their median age was 3 years. Prevalence of ABO and D phenotypes is shown in Figure 1. The overall prevalence of Rh phenotypes is shown in Figure 2. In this group, 232 (96%) were D+ and 10 (4%) were D–. All of the D– patients were observed to be rr; weak D was not found in any of the D– patients. The prevalence of Rh antigens in our study and their similarities with other Asian populations is shown in Table 1.[10,11]

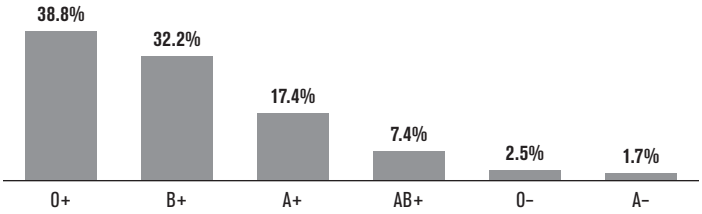


**Fig. 1** Prevalence of ABO and D phenotypes in the studied Pakistan patient population.

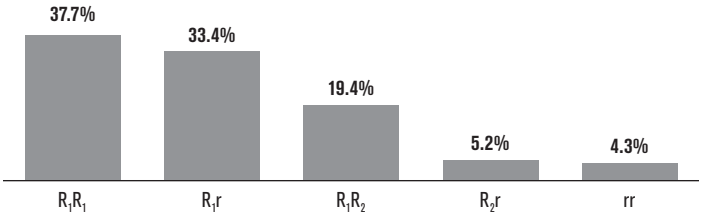


**Fig. 2** Prevalence of Rh phenotypes in the studied Pakistan patient population.

**Table 1.** Comparison of prevalence of Rh antigens in the studied Pakistan patient population with that in other Asian populations

| Rh antigen | Our study (*n* = 242) % | UAE study[10] (*n* = 661) % | India study[11] (*n* = 1240) % |
|---|---|---|---|
| e | 97 | 97.3 | 98.3 |
| D | 95 | 91.1 | 84.76 |
| C | 89.6 | 73.2 | 84.76 |
| c | 62.8 | 71 | 52.82 |
| E | 22.6 | 21 | 17.9 |

UAE = United Arab Emirates.

## Discussion

The prevalence of ABO and D phenotypes among the studied patients were as follows: group O, D+ (38.8%), group O, D– (2.5%), group B, D+ (32.2%), group A, D+ (17.4%), group A, D– (1.7%), and group AB, D+ (7.4%). Analysis from previous studies on the prevalence of ABO in the Pakistan population revealed that, in the provinces of Sindh and Baluchistan, the order of prevalence of ABO phenotypes is O > B > A > AB, which is similar to that of the present study; whereas in the regions of Punjab and Khyber Pakhtunkhwa, the order was B > O > A > AB, with B being the most prevalent

ABO blood group.[12–16] Studies done in the United States, Britain, Bangladesh, Sudan, India, and Saudi Arabia[6,17–21] also revealed that group O is the most prevalent ABO blood group. In Nepal, however, group A is the most prevalent ABO group.[22] Group AB is the least prevalent blood group throughout the world, and the same was found in our study.[18–22]

Our results show that none of the patients whose samples typed as D– had weak D; Sharma and colleagues in India[1] observed the same. The most and least prevalent Rh antigens in our study population were e (97%) and E (22.6%), respectively. This finding is in concordance with other Asian studies, as shown in Table 1.[10,11] Similar findings were observed in Palestine and Europe, where e was the most prevalent and E was the least prevalent Rh antigen.[6,23] In another Indian study, however, among Rh antigens, D was the most prevalent.[1] According to a study done in Sudan, the prevalence of Rh antigens was D (93%), e (79.5%), c (68.5%), C, (27%), and E (18.5%).[3]

In our study, $R_1R_1$ was found to be the most prevalent Rh phenotype, and all of our D– patients were observed to be rr. Similar results were observed by Sharma et al.[1] In another study, $R_1R_1$ was also found to be most prevalent.[24]

In Pakistan, limited studies are available on Rh phenotypes. A recent regional study identifying the ABO and Rh phenotypes in blood donors showed quite similar results as we found in our patients, which shows that the phenotypes of frequently transfused patients do not differ from the regional donor pool in Pakistan.[9] Nonetheless, studies on a larger scale are needed for us to identify the actual RBC phenotypes of our population. Because antibodies against Rh antigens are implicated as the most common cause of alloimmunization in patients in Pakistan requiring frequent blood transfusions, transfusion of Rh antigen–matched blood, especially in this patient population, may significantly reduce the rate of alloimmunization.[7–9]

Once an antibody develops in a patient, the only blood that can be transfused to that patient without harm is blood that is antigen-negative for the identified antibody. In addition to patient Rh typing, donor Rh typing must also be performed. Donor typing would help us build an inventory of various Rh-phenotyped units that could be matched to a patient's Rh phenotype in addition to providing the required antigen-negative blood if the patient has non-Rh antibodies. This inventory would save time and resources in times of need. The lack of information on the Rh phenotypes in our donor pool is one of the major limitations of our study.

In conclusion, our study showed the order of prevalence of ABO phenotypes in Pakistan patients was O > B > A > AB. In the Rh system, e was the most prevalent antigen and the least common was E. In Rh phenotypes identified in our study population, $R_1R_1$ was the most prevalent and rr was the least prevalent. Rh antigenic phenotyping, along with antibody screening and antibody identification prior to transfusion of patients requiring multiple transfusions, should be performed on all patients to reduce alloimmunization. Furthermore, complete Rh typing of blood donors and regional studies on larger donor populations are needed to help not only in finding compatible units of blood, but in building a donor database of common as well as rare phenotypes.

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