

First Report of Phenotype Prevalence of Five Major Rh Blood Group Antigens in the Blood Donor Population of Peshawar, Pakistan

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ABSTRACT

Objective: The current study investigated the prevalence of five major Rh blood group antigens in the Peshawar region of Khyber Pakhtunkhwa province, Pakistan.

Methodology: This was a cross-sectional study, conducted at the Peshawar Regional Blood Centre from February to April 2023. A total of 13,148 blood donor samples were analyzed for Rh D, C, c, E, and e antigens using monoclonal reagents. The frequencies were calculated by direct counting on a spreadsheet programme and the Statistical Package for Social Sciences was used to conduct a meta-analysis for comparison with national and international studies.

Results: The findings of the current study revealed that 92.17% of donors were RhD positive. The e antigen showed the highest frequency of 98.31%, followed by D antigen and the C antigen (92.17% and 78.37%, respectively). Comparative analyses with earlier Pakistani studies and international data indicated significant variations in antigen frequencies, potentially attributed to the diverse ethnic population and geography of the region.

Conclusion: The findings emphasize the importance of local antigen frequency data for efficient blood transfusion strategies, especially for thalassaemia patients requiring frequent transfusions.

Key words: Rhesus, Blood Group, Phenotype

Authors' Contribution:

^{1,2}Conception; ^{1,2}Literature research; ^{1,2}manuscript design and drafting; ^{1,4}Critical analysis and manuscript review; ^{2,3}Data analysis; Manuscript Editing.

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Article info:

Received: October 29, 2023
Accepted: December 15, 2023

Cite this article. Saba N, Waheed U, Khan MN, Saeed M, Khan TH, Wazeer A, Ahmed S. First Report of Phenotype Prevalence of Five Major Rh Blood Group Antigens in the Blood Donor Population of Peshawar, Pakistan. *J Islamabad Med Dental Coll.* 2023; 12(4): 286-291.
DOI: <https://doi.org/10.35787/jimdc.v12i4.1093>

Funding Source: Nil
Conflict of interest: Nil

Introduction

The phrase "blood group" often describes the unique combination of surface antigens on red blood cells (RBCs) in an individual. Blood group antigens on red blood cells (RBCs) are hereditary, polymorphic structures made of proteins or carbohydrates that are found on the extracellular

layer of the RBC membrane. They contribute to the architecture of the RBC membrane, and their specific function(s) include: membrane transporters (Diego, Kidd), receptor and adhesion molecules (Duffy, Lutheran), complement regulatory glycoproteins (Cromer, Knops), enzymes (Yt, Kell, Dombrock), structural components (Diego, Gerbich) or components of the glycocalyx (MNS).^{1,2} Our

knowledge of blood groups has expanded over time to include specific disease associations with RBC surface antigens as well as transfusion-related issues.³⁻⁶

Technically, blood group systems are described as complexes of two or more closely related homologous genes or systems of one or more antigens controlled by a single gene. Every blood group system is genetically distinct from the others. To identify a blood group system and its antigens, the underlying genetic variation that influences phenotype needs to be found, sequenced, and verified.^{7,8}

The International Society of Blood Transfusion (ISBT) has identified 45 blood group systems containing 360 red cell antigens (a.o. July 2023).⁹ Rh is one of the most clinically significant and complex blood group systems, second only to the ABO blood group system. It's warm-reacting antibodies can result in mild to severe haemolytic transfusion reactions as well as haemolytic diseases in foetus and newborn.¹⁰ The frequency and distribution of Rh antigens vary greatly across the globe. Less research has been done in Pakistan on the frequencies and immunogenicity of C, c, E, and e. Besides, there are no reports available from the Khyber Pakhtunkhwa province regarding the distribution of the major Rh blood group phenotypes.

Pakistan is the fifth most populated country in the world, with a wide range of ethnic backgrounds, especially in the province of Khyber Pakhtunkhwa, which has a long history of immigrants due to its proximity to the Afghan border. Due to consanguineous marriages¹¹ and the lack of a national policy on thalassaemia prevention,¹² the province of Khyber Pakhtunkhwa has a higher frequency of thalassaemia than other parts of the country.¹³

Consequently, the mainstay of treatment for people with thalassaemia major is multiple transfusions. In these patients, alloimmunization occurs frequently and the likelihood of alloimmunization rises with the frequency of transfusions.¹⁴ The majority of blood

banks in Pakistan currently solely use random cross-matching of available inventory units to provide patients with appropriate blood. Therefore, in the absence of a local donor database, providing packed red blood cells that are phenotypically matched could be difficult. In order to identify antigen-negative blood in blood centres, data for the major Rh blood group phenotypes in various ethnicities have been thoroughly examined and utilized as a reference.

There have only been five studies in Pakistan to date that have assessed the frequency of Rh major antigens; however, no data from the north-western part of the country has ever been reported. This encouraged us to carry out this study in order to determine the prevalence of Rh D, C, c, E, and e antigen frequencies in the Peshawar region of Khyber Pakhtunkhwa province, the north-western part of Pakistan. The objective is to enable the provision of antigen-negative blood to patients with alloantibodies and will help to make cost-effective use of blood centre resources and shorten turnaround times for phenotyping and crossmatching.

Methodology

The Regional Blood Centre Peshawar, attached to six tertiary teaching hospitals, performed this prospective and cross-sectional study from February to April 2023. Ethical approval was granted by the Institutional Review Board of Regional Blood Centre Peshawar. The sample size was calculated by using WHO sample size calculator by using prevalence of principal Rh blood group antigen - E 18.8%, confidence level 95%, and absolute precision of 3% and sample size turned out to be 652. However, additional testing of samples was performed to include all blood donors during the study period. Hence, a total of 13,148 blood samples were randomly collected from healthy male and female blood donors for Rh antigen typing. A 5% red cell suspension was prepared in 0.9% normal saline. The

Rh D, C, c, E, and e antigens phenotyping was performed by standard tube technique using monoclonal reagents (Bio-Rad Laboratories) and tested against known positive (heterozygous antigens from antibody screening cells) and negative antigens according to manufacturer instructions. The frequencies were calculated by direct counting on a spreadsheet programme and the Statistical Package for Social Sciences (SPSS for Windows version 25.0. Armonk, NY, USA) was used to conduct a meta-analysis for comparison of results with national and international studies. All results were standardized to percentages.

Results

A total of 13,148 blood donor samples were typed for five major antigens of Rh blood group system including D, C, E, c, and e. Of these, 92.17% of the donors were RhD positive and 7.83% were RhD negative. Table 1 shows the antigen frequency of the Rh antigens in our study. The e antigen showed the highest frequency of 98.31%, followed by D antigen and C antigen (92.17% and 78.37%, respectively). The results were compared with five earlier studies conducted in Pakistan and those conducted in neighbouring countries (Table 2 & 3).

Table 1: Rh antigens frequency in current study

Rh Antigens	Prevalence (n)	Prevalence (%)
D	12,118/13,148	92.16%
C	10,304/13,148	78.36%
c	6,008/13,148	45.69%
E	3,306/13,148	25.14%
e	12,925/13,148	98.30%

Discussion

The results of current study indicated a significant difference in the frequency of antigens E, C, and c between our population and those reported from Pakistan¹⁵⁻¹⁹ (Table 2). This may be attributed to diverse ethnic population and geography of our region. It is pertinent to mention that the combined sample size of these five studies was 1,694, much less than our sample size of 13,148. When compared with studies from other countries, our findings indicated a statistically significant difference in the frequency of D, C, c, and E antigens. In the Türkiye²⁵ population, the D antigen frequency was 75% much less than our study (92.16%). The C antigen frequency showed a wide variation in the distribution; 59% in Saudi Arabia²⁴ to 91.0% in India²² while our study reported a frequency of 78.36%. Antigen c showed the highest frequency (86%) in Saudi Arabian²⁴ population whereas lowest frequency was seen in our study 45.69%. Likewise, the E antigen's frequency varied between 14% in Türkiye²⁵ to 50.78% in China²⁰ (Table 3).

The data from current study may be used to help blood centres prepare in-house antibody identification extended panels to detect unexpected multiple antibodies when commercial antibody identification kits are not conclusive. The comparison of the study findings demonstrated that various regions of Pakistan have different frequencies of Rh blood group phenotypes. Given the high prevalence of the thalassaemia in the Peshawar region of Khyber Pakhtunkhwa province, the challenge for hospital blood banks and thalassaemia centres is to supply antigen-negative blood units to thalassaemia patients who need frequent transfusions as part of their treatment plan. In order to prevent alloimmunization, it is important to ascertain the antigen frequencies of the local donor population.²⁶

Table 2: Rh antigens frequency compared with earlier studies from Pakistan

Author and Year	Institute and City	Sample Size	D	C	c	E	e
Karim <i>et al.</i> , 2015 ¹⁵	Aga Khan University Hospital, Karachi	100	97%	87%	57%	19%	99%
Anwar <i>et al.</i> , 2016 ¹⁶	National Institute of Blood Disease and Bone Marrow Transplantation, Karachi	242	95%	89.6%	62.8%	22.6%	97%
Mahmood <i>et al.</i> , 2018 ¹⁷	Armed Forces Institute of Transfusion, Rawalpindi	850	94.94%	87.53%	61.18%	21.41%	98.59%
Tariq <i>et al.</i> , 2022 ¹⁸	National Institute of Blood Disease and Bone Marrow Transplantation, Karachi	227	90.3%	80.6%	66.5%	25.1%	97.8%
Khan <i>et al.</i> , 2022 ¹⁹	Chughtai Institute of Pathology, Lahore	275	94.20%	70.20%	47.70%	26.90%	98.54%
Saba <i>et al.</i> , 2023	Regional Blood Centre, Peshawar (current study)	13,148	92.16%	78.36%	45.69%	25.14%	98.30%

Table 3: Rh antigens frequency compared with other countries

Author and Year	Country	Sample Size	D	C	c	E	e
Yu <i>et al.</i> , 2016 ²⁰	China	1,412	98.94%	88.81%	58.43%	50.78%	92.28%
Shahverdi <i>et al.</i> , 2016 ²¹	Iran	3,475	90.71%	77.04%	73.58%	30.47%	96.66%
Shah <i>et al.</i> , 2018 ²²	India	200	91.0%	91.0%	50.5%	16.5%	100%
Al-Riyami <i>et al.</i> , 2019 ²³	Oman	337	89.3%	74.3%	77.3%	28.2%	98.3%
Owaidah <i>et al.</i> , 2020 ²⁴	Saudi Arabia	100	80%	59%	86%	21%	97%
Orhan <i>et al.</i> , 2022 ²⁵	Türkiye	100	75%	68%	76%	14%	86%
Saba <i>et al.</i> , 2023	Pakistan (current study)	13,148	92.16%	78.36%	45.69%	25.14%	98.30%

Utilizing a multiethnic cohort, this study represents a pioneering effort in delineating the prevalence of the primary Rh antigens within the specific geographic context of Pakistan. It stands as the inaugural investigation to provide comprehensive insights into the distribution of major Rh antigens in Peshawar region, contributing valuable data to the

existing body of knowledge. This research not only addresses a critical gap in understanding the immunogenetic landscape of Pakistan but also sets a precedent for future studies aiming to explore the diverse genetic variations within multiethnic populations in this part of the world.

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