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Genotypic Frequency of RHC Antigen in Women of Reproductive Age in Pakistan

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ABSTRACT

Objective: To determine the genotypic frequency of RHC antigen in females of reproductive age.
Study Design: Cross-sectional study.
Place and Duration of Study: Armed Forces Institute of Transfusion (AFIT) and Pak Emirates Military Hospital, (PEMH) in collaboration with NUMS Pakistan, from Dec 2020 to Dec 2021.
Methodology: Females aged 16 to 45 years were recruited in the study. Demographic data, including age, parity, ethnicity etc., was recorded on a proforma. Venous blood samples were collected in EDTA tubes, and DNA was extracted using 5% Chelex TM. A conventional Polymerase chain reaction and amplified products were subjected to Polyacrylamide Gel electrophoresis.
Results: Amongst 200 females, 172(86%) had the expression of RHC antigen, while 28(14%) were negative for the respective antigen. These results were valuable in predicting the risk of hemolytic disease in fetuses and newborns and alloimmunization in our population.
Conclusion: Females in the reproductive age group had RHC positivity of 86%. This data will help predict the risk of HDFN in future pregnancies and individuals at risk of alloimmunization in RHC-negative women.

Keywords: Alloimmunization, Genotyping, Hemolytic disease of the fetus and newborn (HDFN), Polymerase chain reaction (PCR).

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INTRODUCTION

Rh is clinically the most significant protein-based blood group system. Out of 36 blood group systems described to date, it is the second largest after ABO. It is one of the most complex and diverse blood group systems encompassing more than fifty antigens.[1] RhD and RhCE antigens are located on rhesus protein produced by differences in their protein sequence with nomenclature CD240D and CD240CE.[2] The RhD and RhCE genes that encode D, C, c, E and e antigens are located on the short arm of chromosome. The Rh proteins, designated as RhD and RhCE, 417-amino acid, non-glycosylated proteins; one carries the D antigen, and the other carries various combinations of the CE antigens (CE, Ce, cE or ce). RhD differs from RhCE by 32-35 amino acids depending on which form of RhCE is present, and both are predicted to traverse the membrane 12 times.[3] Antibodies to c antigen are IgG which is highly immunogenic and can easily cross the placenta.[4] These antibodies are frequently associated with developing moderate to severe hemolytic disease of the fetus and newborn (HDFN) after anti-D, followed by antibodies to Kell, Kidd and Duffy antigens.[5,6] Moreover, red cell alloantibodies to c antigen with resultant alloimmunization have been reported in c antigen-negative individuals who have received blood transfusions.[7] However, there is variability in alloimmunization rates in these transfused patients. This could be due to geographic disparities in blood group antigen expression, national blood transfusion policies, higher order birth and abortion rates in the community, and the sensitivity of laboratory tests utilized in prenatal antibody screening.[8] At present, Rh typing of fetuses is being done on fetal blood obtained through cordocentesis to determine the risk of fetal loss and estimate feto-maternal haemorrhage.[9] There is a need for more sensitive techniques to assess the risk of HDFN in young females. At present numerous PCR-based methods are being used worldwide. These include PCR–Restriction fragment length polymorphism (PCR-RFLP), real-time PCR, and sequence-specific priming PCR (PCR-SSP). These DNA-based msethods are faster, more accurate, require less manpower and are considered the need of the hour for international standard immune haematology laboratories.

No data has been available in our region regarding the genotypic distribution of Rhc in women of

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childbearing age. Therefore, our study aimed to determine the genotypic frequency of Rhc antigen in females of the reproductive age group in our popula-tion using a PCR-based methodology.

METHODOLOGY

This cross-sectional study was conducted at the Armed Forces Institute of Transfusion (AFIT) and Pak Emirates Military Hospital, (PEMH) Pakistan, from January to December 2021 after approval from the ethical review board (ERC/ ID/135).

Inclusion criteria: Females aged 16-45 years coming to Outpatient Departments for routine checkups were included in this study after obtaining informed consent.

Exclusion Criteria: Post-menopausal women were excluded from the study.

A total of 200 females were included in the study. The sample size was calculated using the WHO calculator by taking the prevalence of HDFN in Southern Pakistan of 2.2% as a reference, keeping a confidence level of 95% and required absolute precision of 5% and an anticipated population of 2.6%.6 Non-probability consecutive sampling technique was employed**.**

Demographic details like age, ethnicity, parity and marital status were recorded on proforma. Three ml of venous blood samples were collected in EDTA tubes and then transported to the molecular Laboratory (AFIT) within an hour of collection. DNA was extracted from blood samples by the ChelexTM method. The extracted DNA was amplified using ABI 2700 thermal cycler (Applied Biosystems). The cycling conditions in the first step included holding temperature at 94 0C for 120 seconds, denaturation at 94 0C for 60 seconds, annealing at 60 0C for 30 seconds and extension at 72 0C for 90 seconds. These temperatures were maintained for five cycles. The second step carried 25 cycles, and the following temperature conditions were maintained; denaturation at 94 0C for 30 seconds, annealing at 60 0C for 60 seconds and extension at 72 0C for 90 seconds. The final extension occurred by keeping the temperature at 720C for 3 minutes. The temperature was maintained at 250C for 30 seconds after the final extension was complete. Human growth hormone (HGH) was used as the internal control. The primers used along with their sequences were shown in the Table-I.

The amplified products were subjected to polyacrylamide gel electrophoresis at 220 volts for 25 minutes. The gel was then stained with 0.1% Silver nitrate, followed by counterstaining with 1.5% Sodium Hydroxide and 37% formalin. The results were interpreted after drying.

Table-I: Primers and their Sequences

Primers	Sequence
RHc RHc	
Forward	CTTGGGCTTCCTCACCTCAAA
Reverse	AAGCCGTCCAGCAGGATTGC
HGH	
Forward	TGCCTTCCCAACCATTCCCTTA
Reverse	CCACTCACGGATTTCTGTTGTGTTTC

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 25.00. For quantitative data, mean and (standard deviation) were determined. In addition, the frequency and percentages of qualitative variables were calculated. The chi-square test was applied to assess frequency differences. The *p*-value less than 0.05 was considered significant.

RESULTS

A total of 200 females were recruited for the study. Their mean age was 29.65+7.03 years, ranging from 16 to 45 years. Most of them were between 21 to 30 years of age, married and Punjabi. These general characteristics of our studied group were summarized in Table-II.

Table-II: General Characteristics of Study Participants (n=200

Characteristics		Frequency(%)
Total Participants		200(100)
Age	<20	16(8)
	21-30	106(53)
	31-40	63(31.5)
	>41	15(7.5)
Marital Status	Married	173(86.5)
	Unmarried	27(13.5)
Ethnicity	Punjabi	160(80.0)
	Pathan	27(13.5)
	Kashmiri	11(5.5)
	Balochi	2(1.0)
Duration of Marriage	0-5 years	6(3.0)
	6-10 years	58(29.0)
	11-15 years	28(14.0)
	16-20 years	14(7.0)
	>21 years	5(2.5)

Among these females, 172 had the genotypic expression of Rhc antigen, while the remaining 28 females were negative for the respective antigen. The results of Rhc expression were given in Table-III.

Among these, 82 females (41%) had blood Group-B was the most common ABO-Group, followed by O in

60(30%), A in 42(21%) and AB in 16(8%) females. These findings in relation to Rhc expression were shown in Table-IV.

Table-III: Genotypic Expression of Rhc in Study Population (n=200)

Rhc Factor	n (%)
Rhc +ve	172(86.0)
Rhc -ve	28(14.0)
Total	200(100.0)

Table-IV: Relation of ABO and RhD Phenotypes with RHC Genotypic Expression (n=200)

Phenotypic Expression	Genotypic Expression n (%)	
	Rhc positive	Rhc negative
B positive	60(30.0)	16(8.0)
B negative	6(3.0)	0(0)
O positive	53(26.5)	4(2.0)
O negative	2(1.0)	1(0.5)
A positive	36(18.0)	6(3.0)
AB positive	13(6.5)	1(0.5)
AB negative	2(1.0)	0(0)
Total	172(86.0)	28(14.0)

DISCUSSION

The current study was conducted to determine the frequency of Rhc antigen at the molecular level. It is crucial for the blood banks to know blood group antigen frequency distribution in their population to carry out routine transfusion services and resolve blood group discrepancies and compatibility of blood to the patient. Moreover, the number of units required to be cross-matched to find antigen-negative blood in patients with alloimmunization can also be determined. Many studies have been conducted worldwide to determine the genotypic blood group antigenic frequencies. However, in Pakistan, very little data is present concerning the distribution pattern of various blood group agglutinogens. Therefore, the current study can be considered one of the pioneer studies which reported the frequency of Rhc antigen in the Pakistani population.

HDFN, also characterized as erythroblastosis fetalis, is caused mainly by Rh immune antibody IgG crossing trans-placentally and binding to fetal antigen-positive RBC, causing hemolysis and, thus, anaemia. In Pakistan, maternal alloimmunization during pregnancy is reported as 1.8%, with devastating consequences.[10] Studies have reported that D-antigen is responsible for about 50% of cases of maternal alloimmunization while the remaining are mainly due to incompatibility to c, K, C, e and E antigens and less commonly due to antibodies against MNS and Diego blood group system. Moreover, Asian alloantibodies against Rh (E, c) blood groups are the most commonly reported antibodies.[10,11] Therefore, feto-maternal Rh incompatibility still represents the major cause of HDFN.7,[12] ABO distribution analysis in this study demonstrated that 'B' is the most common blood group, followed by O, A and AB, respectively. This finding is in complete agreement with the research conducted by Mahmood et al. in the year 2016.[1] These findings were also in accordance with the data obtained from the Northern region of Saudi Arabia.[13] However, the probable genotypic frequency of Rhc reported by Mahmood *et al*. was 58.49%1 which is contrary to the frequency calculated in our study, where the genotypic frequency of Rhc is 86%. Our findings are also in contrast to the studies carried out in Ankara, Turkey, on multi-transfused patients and healthy blood donors by Bakanay *et al*.14 in which the most frequent genotype of the Rh system was RhD+ (98.4%), RHCECc (51%) as well as to the research conducted in Brazil on Japanese Brazilian (JB) and mixed Japanese Brazilian descendants where RHCECc genotypic frequency was nearly same as the Turkish study.[15] Furthermore, Costa *et al*. reported a genotypic frequency of Rhc of 56% in the European population in the states of Austria, Naples, and Zurich, which does not agree with our results.[16] Two studies conducted in Nigeria on pregnant women reported a notable difference in Rhc antigenic frequencies of 100% and 60.7%, respectively.[17,18] However, these results were based on serological testing and not confirmed by molecular typing. This signifies the importance of molecular testing for the exact characterization of Rhc antigen to resolve these contrasting serological results.

Therefore, our study revealed that the Rhc positivity confirmed on genotyping is far more in our population than in the European and South Asian populations. A notable finding in our study was a higher incidence of Rhc-negative cases in females having blood group B positive. However, as the association was statistically insignificant, further studies should be carried out with a larger sample size to validate results.

LIMITATIONS OF THE STUDY

This was a pioneer study for detecting the genotypic frequency of Rhc in females. However, there was a limited representation of the community, and more sample size is required for further analysis and validation of results.

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CONCLUSION

Females in the reproductive age group had Rhc positivity of 86%. This data will help predict the risk of HDFN in future pregnancies and individuals at risk of alloimmunization in Rhc-negative women.

Conflict of Intrest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SB & FA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MAN & MK: Conception, Study design, drafting the manuscript, approval of the final version to be published.

ST & SAK: Drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Mahmood R, Alam M, Altaf C, Abbasi A, Malik H. Phenotypic profile of Rh blood group systems among females of child-bearing age in Pakistan. Hematol Transfus Int J 2018; 6(1): 00148. doi:10.15406/htij.2018.06.00148
2. Padoan A. Laboratory tests to monitoring physiological pregnancy. J Lab Precis Med 2020; 5(1): 7-7. doi: 10.21037/jlpm.2019.12.02.
3. Zhong LI, Rong ZE, Qing CH, Min LI, Shi GY, Peng WE, et al. Genotyping for Kidd, Kell, Duffy, Scianna, and RHCE blood group antigens polymorphisms in Jiangsu Chinese Han. Chin Med J 2012; 125(6): 1076-1081. doi: 10.3760/cma.j.issn.0366-6999.2012.06.021.
4. de Oliveira MC, Ido AA. Main erythrocyte antigens involved in the alloimmunization process. Open Sci J.2020;5(2).
5. Dubey R, Trigunait P, Pawar A, Yadav A. Haemolytic disease of the fetus and newborn: past, present and future considerations. Acta Scient Med Sci 2019; 3(10): 153-161 doi: 10.31080/ASMS.2019.03.0425
6. Karim F, Moiz B, Kamran N. Risk of maternal alloimmunization in Southern Pakistan–A study in a cohort of 1000 pregnant women. Transfus Apher Sci 2015; 52(1): 99-102. doi: 10.1016/j.transci.2014.12.002.
7. Bueno MP, Mitestainer MB, Da Silva JA, Benites BD, Roversi FM. Red-cell alloimmunization profile in multi transfused patients: Findings and insights of a blood transfusion service. Transfus Clin Biol 2021; 28(3): 258-263.doi: 10.1016/j.tracli.2021.04.006.
8. Howard H, Martlew V, McFadyen I, Clarke C, Duguid J, Bromilow I, et al. Consequences for fetus and neonate of maternal red cell allo-immunisation. Arch Dis Child Fetal Neonatal Ed 1998; 78(1): 62-66. doi: 10.1136/fn.78.1.f62.
9. Dajak S, Čulić S, Stefanović V, Lukačević J. Relationship between previous maternal transfusions and haemolytic disease of the foetus and newborn mediated by non-RhD antibodies. Blood Transfus 2013 ; 11(4): 528. doi: 10.2450/2013.0193-12.
10. Wong KS, Connan K, Rowlands S, Kornman LH, Savoia HF. Antenatal immunoglobulin for fetal red blood cell alloimmunization. Cochrane Database Syst Rev 2013(5). doi: 10.1002/14651858.CD008267.pub2.
11. Tian L, Hou L, Wang L, Xu H, Xiao J. HLA-DRB1* 09: 01 allele is associated with anti-E immunization in a Chinese population. Transfusion 2018; 58(6): 1536-1539. doi: 10.1111/trf.14568.
12. Hussain I, Naqvi AU, Amir S, Abbas G, Mehdi Q. Blood groups distribution pattern among limited adult human population of District Gilgit, Gilgit-Baltistan, Pakistan. Int J Pure Appl Zool 2018; 6(1): 18-24.
13. Owaidah AY, Naffaa NM, Alumran A, Alzahrani F. Phenotype frequencies of major blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) among blood donors in the eastern region of Saudi Arabia. J Blood Med 2020; 11:59–65. doi:10.2147/JBM.S236834.
14. Bakanay SM, Ozturk A, Ileri T, Ince E, Yavasoglu S, Akar N, et al. Blood group genotyping in multi-transfused patients. Transfus Apher Sci 2013 ; 48(2): 257-261. doi: 10.1016/j.transci.2013.01.009.
15. Flôres MA, Visentainer JE, Guelsin GA, de Souza Fracasso A, de Melo FC, Hashimoto MN, et al. Rh, Kell, Duffy, Kidd and Diego blood group system polymorphism in Brazilian Japanese descendants. Transfus Apher Sci 2014; 50(1): 123-128. doi: 10.1016/j.transci.2013.09.014.
16. Costa DC, Schinaider AA, Santos TM.. Frequencies of polymorphisms of the Rh, Kell, Kidd, Duffy and Diego systems of Santa Catarina, Southern Brazil. Rev Bras Hematol Hemoter 2016; 38: 199-205. doi: 10.1016/j.bjhh.2016.04.005.
17. Christian SG, Eze EM, Badom BM, Pepple IA, Simeon CA. Frequency Occurrence and Percentage Distribution of Rh C, Rh c, Rh E and Rh e Blood Group Amongst Pregnant Women Attending Antenatal Clinic in Port Harcourt, Nigeria. Eur J Med Health Sci 2021; 3(3): 50-54. doi: 10.24018/ejmed.2021.3.3.808.
18. Eze RI, Obeagu EI, Edet FN. Frequency of Rh Antigen C And c among pregnant women in Sub-Urban area in Eastern Nigeria. Madonna Uni J Med Health Sci 2021; 1(1): 19-30.