ISSN: 2692-5516

Research Article

Advances in Hematology and Oncology Research

Serological Detection of Some Rhesus D Polymorphisms among Rhesus Negative Sudanese Blood Donors

Dalia Adel Ezzet[1], Ahmed Elhadi Elsadig[*1], May Mohammed Ali[2] and Alfatih Aboalbasher Yousif[3].

[1]*Haematology Department, Faculty of Medical Laboratory Sciences, Alzaeim Alazhari University, Khartoum, Sudan.*

[2]*Haematology and Immunohematology Department, College of Medical Laboratory Sciences, Sudan University of Science and Technology, Khartoum, Sudan.*

[3]*Pathology Department, Ibra Hospital, Ministry of Health – Oman.*

[*]Corresponding author
Ahmed Elhadi Elsadig, Haematology Department, Faculty of Medical Laboratory Sciences, Alzaeim Alazhari University, Khartoum, Sudan.

Submitted: 04 Jun 2022; **Accepted:** 09 Jun 2022; **Published:** 20 Jun 2022

Citation: *Dalia Adel Ezzet, Ahmed Elhadi Elsadig, May Mohammed Ali and Alfatih Aboalbasher Yousif. (2022). Serological Detection of Some Rhesus D Polymorphisms among Rhesus Negative Sudanese Blood Donors. AdvHemaOnco Res, 5(1), 102-105.*

Abstract

Background: *Rhesus (Rh) blood group, mainly the D phenotype is subject to polymorphisms associated with several Rh D variants. The identification of such variants may prevent blood reactions due to Rh incompatibilities.*

Material and Methods: *This descriptive cross-sectional study was conducted in Khartoum North, Khartoum-Sudan during the period from December 2021 to March 2022. A total of 100 blood donors who had Rh D negative grouping results were recruited for this study. All samples were tested for weak D and Rh-Del phenotypes by indirect antihuman globulin and adsorption-elution methods respectively. In addition, Rh CE typing was performed for all samples, then the association between Rh CE phenotype and Rh D variants was tested by Chi square test. P values of < 0.05 were considered statistically significant.*

Results: *Among the tested donors, 7% were Rh-Del, 5% were weak D while the remaining 88% were truly Rh-negative phenotype. All Rh-Del subjects were Rh CE positive; 80% of weak D were Rh CE positive, while only 11% of truly Rh-negative donors were Rh CE positive. Statistically significant association was observed between Rh CE positive phenotype with Rh Del and weak D phenotypes, while truly negative Rh D phenotype is associated with negative Rh CE phenotypes (All P values < 0.05).*

Discussion: *Variant D phenotypes were observed in this study population. Therefore, attention for this category among Rh D negative donors is needed to avoid allo immunization in truly negative patients.*

Keywords: Adsorption, Elution, Rhesus Del, Rhesus negative, Weak D

Introduction

Rh blood group system is an important blood group system after the ABO system. In which the people are classified according to the presence or absence of the Rh antigens mainly the D antigen or Rh factor. This blood group consists of 49 defined antigens, among which the five antigens: D, C, c, E, and e are the most important. Rhesus positive indicates the presence of the D antigen, while Rh negative term indicates its absence. The D antigen is encoded by the RHD gene, while C, c, E, and e antigens are encoded by the RHCE gene [1]. Beside the standard phenotypes, RH locus is susceptible to certain genetic polymorphisms, these polymorphisms associate with the expression of serologically different Rh D phenotypes referred to as D variants including; partial D, weak D, Rh-Del and Rh null phenotypes [2].

Rh-Del phenotype is characterized by expression of very low density of D antigen on red cells membrane. It has been reported that, transfusion of Rh Del blood may cause both primary and secondary allow immunization in Rh-negative recipients [3-6]. Due to the very low density of the antigen, serological detection of Rh Del phenotype is only possible by adsorption/elution technique [5,7].

D antigen which does not react with anti-D at immediate spin, or after incubation at 37 °C, but reacts at anti-human globulin (AHG)

phase is described as weak D phenotype. This phenotype expression result from substitution in the transmembrane or intracellular domains of the Rh-D gene, with consequent quantitative under-expression of the protein [2]. Theoretically, weak D blood may cause immunization and/or reactions in truly negative recipients, but actually many weak D subjects can be transfused with D positive cells with no significant harm [8].

The objective of the current study was to detect weak D, Rh Del phenotypes among Rh-D negative Sudanese blood donors in some blood banks at Khartoum North. Moreover; to find out the association between these variants' phenotypes and Rh CE phenotype. This would provide valuable information for better management of transfusions among Rh-negative blood recipients.

Materials and Methods

This is a cross-sectional; laboratory-based, descriptive study conducted in 100 Rh-D negative blood donors at Ahmed Qasim hospital, Aldroushab Specialist hospital and Ali Abdulfattah hospital blood banks in Khartoum North city during the period from December 2021 to March 2022.

Indirect Antihuman Globulin Test for Weak D (Du method)

One drop of 3–5% washed red cells in normal ionic strength saline was mixed with two drops of monoclonal anti-D (IgM + IgG) in a glass test tube and incubated at 37°C for one hour. After incubation, agglutination was read. Then, the red cells were washed three times with normal ionic strength saline. Two drops of antihuman globulin (AHG) serum were added, the tube was spin at 3000 rpm for 15 seconds. Then agglutination was read, positive agglutination recorded as weak D. Samples showed negative antihuman globulin test were then subjected to adsorption-elution test for Rh-Del detection.

Adsorption-Elution Technique for Rh-Del

Elution reagent was prepared with the following ingredients {acid-glycine (0.1M glycine-HCL buffer - pH 1.5) / EDTA (10% EDTA with ratio of 4: 1)}.

Adsorption Technique

Equal volumes (200µL) of washed red cells and monoclonal anti-D (IgM + IgG) were mixed and incubated at 37°C for one hour. After incubation, six times washes were performed thoroughly using normal saline. The supernatant of the last wash was reserved for further check.

Elution Technique

sensitized, washed RBCs (from the previous step) was then incubated with equal volume of the elution reagent at room temperature (22–24°C) for one minute. Then, 28 µl of 1 MTRIS-NaCL was added to the mixture and the tube was placed in the centrifuge at 1000 rpm for one minute, then solution eluate was collected from the top of the tube. The pH of the product was adjusted by 1MTRIS-NaCL to (7.0--7.4).

The eluate was used for indirect anti-human globulin testing using RhD-positive cells. Eluate that agglutinate RhD-positive cells indicates Rh-Del phenotype, while no agglutination was considered truly RhD-negative phenotype. The supernatant of the last wash of the adsorption step was used as control, and it gave negative AHG results with RhD-positive and negative RBCs.

Rh CE serotyping

Anti-CDE reagent was used for Rh CE typing, all samples were typed by immediate spin tube method technique.

Statistical Analysis

Data were analyzed using SPSS program (Statistical Package for Social Science) version 23. Frequencies and percentages were calculated for the tested phenotypes. Association testing was done by Chi square test. P values < 0.05 were considered statistically significant.

Results

Among the tested donor samples, 7% of Rh-negative donors were Rh Del, 5% were weak D phenotype, while the remaining 88% were truly Rh D negative. Rh CE typing was done for the 100 Rh negative samples to find out the association Rh CE and Rh D variants. Statistically significant associations were observed between Rh CE and Rh Del and weak D phenotypes; while true Rh D negative phenotype was significantly associated with negative Rh CE phenotype (All P values < 0.05) (Table .1).

Table 1: Association of Rh CE antigens with Rh D phenotypes (Rh D negative, Rh DEL, Weak D)

Rh D phenotypes	Rh CE		P. value
	Rh CE (+)	Rh CE (-)	
Rh D negative (n=88)	10 (11%)	78 (89%)	0.000
Rh DEL (n=7)	7 (100%)	0 (0%)	
Weak D (n=5)	4 (80%)	1 (20%)	

Discussion

Rh incompatibilities are among the significant causes of hemolytic transfusion reactions. Rh negative recipients are at risk for these reactions if the transfused blood possess Rh antigen, even in small amount, which is the case in the D variants phenotypes. Therefore; careful identification of Rh D phenotype on the blood units to be transfused for negative recipients, is of great importance. In this study, the existence of Rh-Del and weak D phenotypes were serological tested in 100 Rh-negative Sudanese donors from some blood bank centers at Khartoum North. The Rh-Del type was observed in 7% of the studied population which is comparable to frequencies obtained in some previous studies. These include studies done in North India and Malisya which demonstrated that; in Rh D-negative donors, Rh-Del phenotype frequencies were 1.5% and 2.3% respectively [9,10]. At the other hand, our finding is quite higher than what had been observed in other nationalities like the 0.1% in German donors and > 1% in Austrians and Brazilians [11,12].

Higher frequencies were observed in other studies including a study from Yangon, Myanmar which demonstrated that, 15.8% were Rh-Del phenotype and 9% were weak D phenotype [13]. Comparable Rh-Del frequencies were observed in different Chinese populations ranging between 2.4% up to 23.3% [14].

Another finding of our study is that; weak D phenotype represent 5% of Rh-negative blood donors, this finding is agree with previous studies in Nigeria and African countries which reported prevalence rates ranging from 0.95 to 4.9% [15-17]. While another study done in Kano; northwest Nigeria observed that, 10.6% of participants were typed as weak D, which is higher comparing to other African countries (2.0–2.6%) [18,19]. However; D variants reported among African-Americans in the USA represents 9.8%, as well as in Canadian population 36% and Brazil 8.4% [20-22]. These variations in prevalence across different populations could be explained by the ethnic diversities. Moreover; Rh-Del is characterized by very low density of antigen D, so it might be misidentified and the actual frequency could be higher than what has been observed.

Third finding of this study is the association of Rh CE positive phenotype with Rh-Del and weak D phenotypes. This finding is in lin3 with previous observations in Thai and Chinese nationalities [23,24]. A limitation of this study is that; the Rh-CE serotyping was done by a combined anti-CE sera, while the c and e antigens were not determined due to reagents in availability in the Sudan.

Conclusion

Among the Rh-D negative donors, variant D phenotypes were observed. Therefore, attention for this category among Rh D negative donors is required to prevent allo immunization in truly negative patients.

Data availability

All data underlying the results available as part of the article and no additional source data are required.

Authors Contribution

Ahmed and Dalia designed the study, Dalia performed data curation, formal analysis and writing of the original draft. Supervision, writing review and editing were performed by Ahmed. Mai performed methodology, writing review and editing. Elfatih analysed the data, writing review and editing. All authors read and approved the final version of the paper.

References

1. Dean, L. (2005). The Rh blood group in: Blood Groups and Red Cell Antigens. National Center for Biotechnology Information.
2. Flegel, W. A. (2011). Molecular genetics and clinical applications for RH. Transfusion and Apheresis Science, 44(1), 81-91.
3. Shao, C. P., Wang, B. Y., Ye, S. H., Zhang, W. L., Xu, H., Zhuang, N. B., ... & Xu, H. G. (2012). DEL RBC transfusion should be avoided in particular blood recipient in East Asia due to allosensitization and ineffectiveness. Journal of Zhejiang University Science B, 13(11), 913-918.
4. Yang, H. S., Lee, M. Y., Park, T. S., Cho, S. Y., Lee, H. J., Lim, G., ... & Park, K. U. (2015). Primary Anti-D Alloimmunization Induced by. Annals of laboratory medicine, 35(5), 554-556.
5. Yasuda, H., Ohto, H., Sakuma, S., & Ishikawa, Y. (2005). Secondary anti-D immunization by Del red blood cells. Transfusion, 45(10), 1581-1584.
6. Kim, K. H., Kim, K. E., Woo, K. S., Han, J. Y., Kim, J. M., & Park, K. U. (2009). Primary anti-D immunization by DEL red blood cells. The Korean journal of laboratory medicine, 29(4), 361-365.
7. Nuchnoi, P., Thongbus, J., Srisarin, A., Kerdpin, U., & Prachayasittikul, V. (2014). Clinical and laboratory update on the DEL variant. Laboratory Medicine, 45(4), 285-290.
8. Brecher, M. E., Linden, J. V., & Roseff, S. D. (2005). Blood collection, storage and component preparation. American Association of Blood Banks Technical Manual. 15th ed., Bethesda, Maryland, 799-818.
9. Kulkarni, S., Parchure, D. S., Gopalkrishnan, V., & Madkaikar, M. (2018). Screening for DEL phenotype in RhD negative Indians. Journal of Clinical Laboratory Analysis, 32(3), e22288.
10. Ramli, S., Kamarudin, E., & Bahari, M. (2017). Serological and Molecular Detection of RhD DEL Phenotype in National Blood Centre, Malaysia. Pertanika Journal of Science & Technology, 25.
11. Flegel, W. A., Von Zabern, I., & Wagner, F. F. (2009). Six years' experience performing RHD genotyping to confirm D− red blood cell units in Germany for preventing anti-D immunizations. Transfusion, 49(3), 465-471.
12. Wagner, F. F. (2013). RHD PCR of D-negative blood donors. Transfusion Medicine and Hemotherapy, 40(3), 172-181.
13. Wah, S. T., Chi, S. N., Kyaing, K. K., Khin, A. A., & Aung,

T. (2020). Serological Detection of Rh-Del Phenotype among Rh-Negative Blood Donors at National Blood Center, Yangon, Myanmar. Advances in Hematology, 2020.
14. Yin, Q., & Flegel, W. A. (2021). DEL in China: The D antigen among serologic RhD-negative individuals. Journal of Translational Medicine, 19(1), 1-14.
15. Maryam, D. U., Mukhtar, I. G., Yusuf, A. A., & Salisu, A. I. (2021). High prevalence of serological weak D phenotype and preponderance of weak D type 4.0. 1. genetic variant in a Nigerian population: implications for transfusion practice in a resource-limited setting. Hematology, Transfusion and Cell Therapy.
16. Nwauche, C. A., & Ejele, O. A. (2002). Is DU Phenotype Testing A Necessity In Blood Bank Practice In Nigeria? Nigerian Journal of Clinical Practice, 5(2), 120-122.
17. Ahmed, S. G., Kangu, M. B., & Abjah, U. A. M. (2009). The role of du testing in scaling down the burden of Rhesus-D negative transfusion in Northern Nigeria. Int J Third World Med, 8(2).
18. Touinssi, M., Chapel-Fernandes, S., Granier, T., Bokilo, A., Bailly, P., & Chiaroni, J. (2009). Molecular analysis of inactive and active RHD alleles in native Congolese cohorts. Transfusion, 49(7), 1353-1360.
19. Ouchari, M., Polin, H., Romdhane, H., Abdelkefi, S., Houissa, B., Chakroun, T., ... & Jemni Yacoub, S. (2013). RHD* weak partial 4.0 is associated with an altered RHCE* ce (48C, 105T, 733G, 744C, 1025T) allele in the Tunisian population. Transfusion Medicine, 23(4), 245-249.
20. Wang, D., Lane, C., & Quillen, K. (2010). Prevalence of RhD variants, confirmed by molecular genotyping, in a multiethnic prenatal population. American journal of clinical pathology, 134(3), 438-442.
21. Denomme, G. A., Wagner, F. F., Fernandes, B. J., Li, W., & Flegel, W. A. (2005). Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion, 45(10), 1554-1560.
22. Zacarias, J. M. V., de Figueiredo Pereira, E. M., Visentainer, J. E. L., Guelsin, G. A. S., de Melo, F. C., & Sell, A. M. (2016). Frequency of RHD variants in Brazilian blood donors from Parana State, Southern Brazil. Transfusion and Apheresis Science, 55(1), 120-124.
23. Srijinda, S., Suwanasophon, C., Visawapoka, U., & Pongsavee, M. (2012). RhC phenotyping, adsorption/elution test, and SSP-PCR: the combined test for D-elute phenotype screening in Thai RhD-negative blood donors. International Scholarly Research Notices, 2012.
24. Chen, Q., Li, M., Li, M., Lu, X. S., SUN, J., & LIU, Z. (2012). Molecular basis of weak D and DEL in Han population in Anhui Province, China. Chinese Medical Journal, 125(18), 3251-3255.