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Does Rhesus C antigen provide a protective function against HIV infection?

L'antigène Rhésus C assure-t-il une fonction protectrice contre l'infection par le VIH ?

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ABSTRACT

Background and Objectives: Red blood cell antigens have been reported to be involved in the epidemiology of various diseases. Other studies have suggested that the Rhesus C antigen has a protective effect against HIV infection. However, there is limited information to support these findings. Therefore, this study sought to investigate whether there was an association between Rhesus C antigen and HIV infection.

Materials and Method: A clinical and laboratory based analytical cross-sectional study was carried out on 165 HIV positive samples from the Centre of Excellency at the Parirenyatwa Group of Hospitals and 165 HIV negative samples from National Blood Service Zimbabwe. Rhesus blood grouping for the 5 principal Rhesus antigens (D, C, E, c and e) was done using reagents supplied by Lorne Laboratories.

Results: A total of 330 HIV positive and negative samples were used for the study. One hundred and sixty-five (50%) of them were from HIV negative regular blood donors at the National Blood Service Zimbabwe and the other 165 (50%) were from HIV positive patients enrolled at the Centre of Excellency Clinic of the Parirenyatwa Group of Hospitals. Ninety-four (57%) and 71(43%) of the patients were males and females respectively. Forty-four (27%) and 46 (28%) of the HIV positive patients were in the 31-40- and 41-50-year age ranges respectively. Only 2 (1%) of the patients were over the age of 70 years. The study showed no association between Rhesus C antigen and HIV infection ($p>0.05$). E and C antigens were the least frequent Rhesus phenotypes in both HIV negative and HIV positive samples. The total number of probable genotypes containing the C gene was also very low at 57(17%). There were 33 (20%) of HIV negative donor samples with Rhesus C antigen, compared to 21 (13%) of HIV positive patient samples.

Conclusions: The study was unable to establish the link between the Rhesus C antigens with protection against HIV infection. Since Rhesus C antigen is of low frequency in the Black population, very few patients with this antigen were identified with HIV infection.

RÉSUMÉ

Contexte et objectifs : Il a été rapporté que les antigènes des globules rouges sont impliqués dans l'épidémiologie de diverses maladies. D'autres études ont suggéré que l'antigène Rhésus C a un effet protecteur contre l'infection par le VIH. Cependant, il existe peu d'informations pour étayer ces conclusions. Par conséquent, cette étude a cherché à déterminer s'il y avait une association entre l'antigène Rhésus C et l'infection par le VIH.

Matériels et méthode : Une étude transversale analytique clinique et en laboratoire a été réalisée sur 165 échantillons positifs pour le VIH du *Centre of Excellency at the Parirenyatwa Group of Hospitals* et 165 échantillons négatifs pour le VIH du *National Blood Service Zimbabwe*. Le groupage sanguin Rhésus pour les 5 principaux antigènes Rhésus (D, C, E, c et e) a été effectué à l'aide de réactifs fournis par *Lorne Laboratories*.

Résultats : Un total de 330 échantillons positifs et négatifs pour le VIH ont été utilisés pour l'étude. Cent soixante-cinq (50 %) d'entre eux provenaient de donneurs de sang réguliers séronégatifs du *National Blood Service du Zimbabwe* et les 165 autres (50 %) provenaient de

patients séropositifs inscrits à la *Centre of Excellency at the Parirenyatwa Group of Hospitals*. Quatre-vingt quatorze (57%) et 71 (43%) des patients étaient respectivement des hommes et des femmes. Quarante-quatre (27 %) et 46 (28 %) des patients séropositifs avaient respectivement 31-40 ans et 41-50 ans. Seuls 2 (1%) des patients avaient plus de 70 ans. L'étude n'a montré aucune association entre l'antigène Rhésus C et l'infection par le VIH (p>0,05). Les antigènes E et C étaient les phénotypes rhésus les moins fréquents dans les échantillons VIH négatifs et VIH positifs. Le nombre total de génotypes probables contenant le gène C était également très faible à 57 (17 %). Il y avait 33 (20 %) échantillons de donneurs VIH négatifs avec l'antigène Rhésus C, contre 21 (13 %) échantillons de patients VIH positifs.

Conclusions : L'étude n'a pas pu établir de lien entre les antigènes Rhésus C et la protection contre l'infection par le VIH. L'antigène Rhésus C étant peu fréquent dans la population noire, très peu de patients porteurs de cet antigène ont été identifiés comme infectés par le VIH.

INTRODUCTION

The Rhesus blood group is one of the most important blood group systems in transfusion medicine practice because of its involvement in some transfusion related reactions. Apart from A and B antigens of the ABO blood group, Rhesus antigens are the next most immunogenic. There are currently more than 40 Rhesus antigens, with *D, C, c, E* and *e* being the principal antigens which are dealt with in routine Blood Transfusion Practice.[1]

Some antigens such as Kidd and Duffy are transmembrane glycoproteins which are known to aid in the transportation of substances across the cell membrane.[1] Although a lot has been said about blood group antigens, their exact function is still not clearly understood. Several studies are now associating these antigens with certain disease states.[2, 3] Rhesus antigens are an integral part of the red blood cell membrane. Individuals with no Rhesus antigens (Rhnull) have been found to develop a haemolytic anaemia known as Hereditary Stomatocytosis.[4] The structure of Rhesus antigens has been reported to be similar to that of ammonia transporter /methylamine permease (Amt/Mep). The Amt/Mep proteins are also found in yeast, bacteria and simple plants. The Amt/Mep proteins are responsible for ammonia transportation. This is believed to be one of the functions of the Rhesus antigens in human beings. Therefore, there is more to blood group antigens than challenges in blood transfusion alone.[5]

Since Rhesus antigens have been linked to possible transmembrane transportation of microorganisms, studies have also associated some of these antigens with human immunodeficiency virus (HIV) infection. One of such studies linked the red blood cell antigens with possible protection against HIV infection[6]. Some blood group antigens are known to act as receptors or ligands for bacteria, parasites and viruses and many research studies have linked red blood cell antigens with HIV epidemiology.[7]

Some studies have indicated the significance of red blood cells in the pathogenesis of HIV. Red blood cells are thought to enhance the viral infectivity by binding free viruses and viral immune complexes, thereby cross-infecting HIV-susceptible cells. The Duffy antigen receptor for chemokines (DARC) has been reported as a binding site for HIV-1 on red blood cells. This binding increases infectivity of susceptible immune cells. Another study linked Rhesus C, Lu^b and P_1 antigens with protection against HIV infection.[8, 9, 10, 11]

As Zimbabwe is in one of global HIV endemic regions, and based on the above findings, this study was found appropriate to establish the role of Rhesus C antigen in HIV infection in the country. The objective of this study was to determine the frequencies of Rhesus C antigen in HIV positive and negative samples and determining its role in HIV infection.

MATERIALS AND METHODS

A clinical and laboratory based cross-sectional study was carried out on samples obtained from the Parirenyatwa Centre of Excellency (CoE) and the National Blood Services Zimbabwe (NBSZ), from February 2019 to April 2019. Ethical approval to conduct the study was granted by the Joint Research Ethical Committee of the Parirenyatwa Group of Hospitals and the then College of Health Sciences (*JREC/372/18*).

Permissions to access patient data, collect blood samples and perform laboratory tests were granted by the Clinical Director of Parirenyatwa Group of Hospitals (PGH), the Director of the Centre of Excellency (CoE) and the Chief Medical Laboratory Scientist, respectively. Access to stored donor blood samples at National Blood Service Zimbabwe (NBSZ) was granted by the Planning, Information and Research Manager at NBSZ.

The study was carried out in the Safety, Health, Environment and Quality (SHEQ) laboratory at the NBSZ. Blood samples from HIV negative blood donors at NBSZ and HIV positive patients from CoE were used.

To further maintain strict confidentiality during the study, collected data was used for research purposes only. All the results were saved on memory storage devices and password protected computer that was only accessible to the researchers.

The *inclusion criteria* were samples from HIV negative regular blood donors and those from HIV positive CoE-enrolled patients. The e*xclusion criteria* were samples from HIV negative and first-time blood donors.

A calculated sample size of 303 was determined using the *Dobson's Formula.* To maintain confidentiality, HIV positive samples were assigned new study identification numbers, and HIV negative donor samples retained their original donor identification numbers in addition to new study numbers.

Rhesus phenotypes were determined on patient and donor red blood cells using commercial antibodies to the five principal Rhesus antigens (D, C, c, E and e). The phenotyping technique was based on agglutination for positive reactions as outlined in the Lorne Laboratories Limited manufacturer's instructions. Positive and negative controls for C antigen were also done at the same time to validate the test results.

Data was captured and analyzed using the Microsoft Excel and IBM SPSS *version 19* software, respectively. Descriptive statistics were used to analyze both categorical and continuous variables.

Comparison of proportions of blood group antigens between HIV positive and negative samples were conducted using *Pearson's chi-square test ($\chi 2$)*, and the results were considered significant at $p < 0.05$.

RESULTS

A total of 330 HIV positive and negative samples were used for the study. One hundred and sixty-five (50%) of them were from HIV negative regular blood donors at the National Blood Service Zimbabwe and the other 165 (50%) were from HIV positive patients enrolled at the Centre of Excellency Clinic at the Parirenyatwa Group of Hospitals.

Ninety-four (57%) and 71(43%) of the patients were males and females respectively. Most of the HIV positive patients, (44 (27%)) and 46 (28%), were in the 31-40- and 41-50-year age ranges respectively. Only 2 (1%) of them were over 70 years old (**Table 1**).

Table 1: Demographic Characteristics of HIV positive patient Samples (*n= 165*)

Characteristic	n	%
Participant Gender : (N=165)		
Male	94	57
Female	71	43
TOTAL	**165**	**100**
Patients Age Group: (N=165)		
0 – 10 years	8	5
11 – 20 years	22	13
21 – 30 years	17	10
31 – 40 years	44	27
41 – 50 years	46	28
51 – 60 years	20	12
61 – 70 years	6	4
71 – 80 years	2	1
TOTAL	**165**	**100**

The study showed no association between Rhesus C antigen and HIV infection ($p>0.05$) (**Table 2**). The C and E antigens were the least frequent Rhesus phenotypes in both HIV negative and positive samples (**Tables 2 & 3**). There were 33 (20%) of HIV negative donor samples with Rhesus C antigen compared to 21 (13%) of HIV positive patient samples. The total number of probable genotypes containing C gene were also very low at 57(17%) (**Figure 1**).

Table 2: Distribution of Rhesus antigens in HIV Negative and Positive blood samples.

Principal Rhesus antigens	HIV-negative (n=165) n (%)	HIV-positive (n=165) n (%)	p value
D	157 (95.2)	158 (95.8)	0.792
C	34 (20.6)	27 (16.4)	0.321
c	161 (97.6)	163 (98.8)	0.410
E	26 (15.8)	31 (18.8)	0.467
e	162 (98.2)	164 (99.4)	0.314

Table 3: Rhesus phenotypes in HIV Negative and Positive blood samples

Phenotypes	HIV-Negative (n=165) n (%)	HIV-Positive (n=165) n (%)
D, c, e	115 (70)	116 (70)
D, c, E, e	14 (8)	25 (15)
D, C, c, e	19 (12)	18 (**11**)
D,C, c, E, e	9 (5)	3 (2)
D, C, e	5 (3)	0 (0)
D, c, E	3 (2)	3(2)

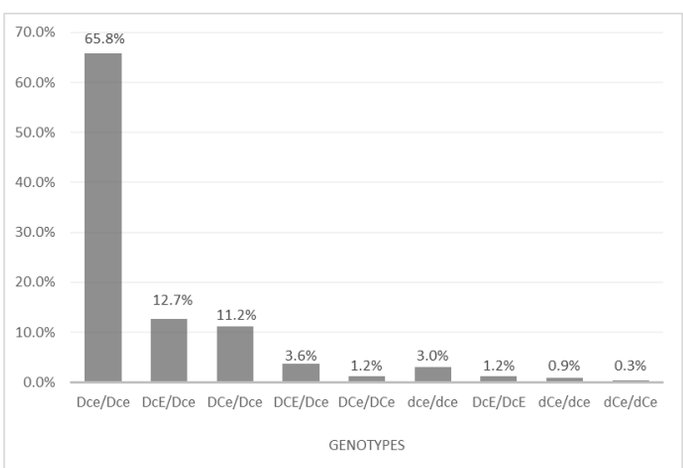

Figure 1: Frequencies of most probable Genotypes in study samples (n=330).

DISCUSSION

Although the calculated sample size was 303, the total number of participant samples exceeded this. Therefore, the results were not affected by the sample size. An equal number of HIV negative donor samples and HIV positive patient samples were used to justify the outcome of the study results.

There were more male than female patients with HIV infection. Some studies have shown that women have lower risk of HIV/AIDS than men.[12] However, previous studies have shown that Zimbabwean females were at higher risk of HIV infection than males, and women in Sub-Saharan Africa carried a disproportionally high burden of HIV/AIDS.[13, 14] The majority of HIV positive patients were in the 31-50 years age range. This could be a direct result of increased sexual activities associated with the age group, as indicated in a previous study in the country.[15] Only two HIV positive patients lived beyond 70 years. This could be due to strict antiretroviral (ARV) treatment adherence. There has been a remarkable improvement in life expectancy of people living with HIV across the globe[1.6]

The study showed no association between Rhesus C antigen and HIV infection, although some studies have indicated an association of blood group antigens with HIV epidemiology.[6] In the general population, the frequencies of Rhesus C and E antigens are very low. Therefore, the apparent association of Rhesus C antigen with HIV negativity had more to do with the antigen's low prevalence in the general population, especially in Blacks.[1] Rhesus C and E antigens are antithetical to Rhesus c and e respectively. That is, Rhesus C and c antigens are always present together or one of them may be present, and there is never a situation where both are absent. The same applies to Rhesus E and e antigens. Since Rhesus c and e are high frequency antigens, Rhesus C and E are respectively very low frequency antigens in the Black population.[1, 17, 18] In a similar study on the role of Rhesus D antigen, Rhesus D positive people appeared to be more susceptible to HIV infection than their Rhesus D negative counterparts. It was argued that more than 95% of Black people have the D antigen. Therefore, it was expected to have more HIV positive people in Rhesus D positive than Rhesus D negative individuals.[19]

The exact role of Rhesus antigens remains largely unknown, except for the removal of ammonia from the red blood cells by the Rhesus associated antigen (RhAG) and the maintenance of cell membrane. Another possible function of Rhesus antigens is transportation of carbon dioxide (CO^2) and oxygen (O^2).[4, 20] It has also been argued that Rhesus antigens, as non-glycosylated transmembrane proteins, may prevent any interaction of the antigens with HIV virus.[18, 21]

The probable genotypes containing C gene were very low. This finding is consistent with frequencies of C gene in the Black population as published in many literature sources. However, in a recent study, the frequency of Rhesus C antigen in an Indian population was found to very high while that of Rhesus c antigen was low.[21, 22, 23] Therefore, it appears the distribution of Rhesus blood group antigens vary according to ethnic groups.

Limitations: The study excluded other antigens, $\mathbf{P_1}$ and $\mathbf{Lu^b}$ that were also thought to be associated with possible protection against HIV infection.

CONCLUSION

The study was unable to establish the link between Rhesus C antigen with protection against HIV infection. Since Rhesus C antigen is of low frequency in the Black population, very few HIV positive patients with this antigen were identified. The study also showed decreased frequencies of Rhesus C gene in created probable genotypes in both Rhesus positive patient and HIV negative donor samples in the study.

RECOMMENDATIONS

The role of red blood cell antigens is still a topical issue which requires continuous research work. Therefore, more research should be carried out in this area in different ethnical population groups.

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