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The relationship between immunogenic red blood cell antigens and Human Immunodeficiency Virus infection

Glenda M. Davison*, Heather L. Hendrickse, Tandi E. Matsha

Department of Biomedical sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa

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ABSTRACT

Introduction: Evidence suggests that red cell antigens may act as receptors for viruses and bacteria and therefore could be associated with HIV infection. Previous studies have been controversial and therefore the aim of this exploratory study was to analyse the expression of immunogenic red cell antigens in HIV-seropositive individuals and to compare the results to negative donors from South Africa.
Methods: The expression of ABO, Rh, Kell and Duffy antigens from 119 HIV-seropositive patients was compared to 317 HIV-seronegative blood donors. Nucleic acid amplification testing and PCR were used to determine the HIV status and the ID-Gel Card Technology was used to determine the blood group antigen profile.
Results: There was no significant difference in the expression of A, B, AB, Duffy or Kel antigens between the two groups but significantly lower numbers of HIV+ individuals were O Rh Negative ($p = ,0.0001$). Analysis of those with a Duffy null phenotype revealed a significantly higher incidence of blood type A RH1-Positive, Dce/R^0r and B RH1-Positive, DcEe/R^2r within the HIV-seropositive group ($p = < 0.05$). None of the HIV-seropositive individuals were O RH1-Negative, dce/*rr*.
Conclusion: In conclusion these initial findings have demonstrated a decreased incidence of blood type O Rh1-negative in HIV + individuals which suggests that red blood cell antigens may play an important role in susceptibility to HIV infection. The relationship between red cell antigens and HIV infection however remains complex and therefore larger studies are required to confirm these results.

1. Introduction

It has been well documented that the expression or absence of various red blood cell antigens are not only major causes of blood transfusion reactions, but are also associated with infection and disease [1]. It has been hypothesized that during the process of acquiring pathogens, blood group antigens may act as receptors for a variety of viruses, parasites and bacteria [2,3]. A well recognised example of this phenomenon is the Duffy antigen which is a chemokine receptor, and acts as a receptor for *Plasmodium vivax*. Individuals who have been phenotyped as Duffy null (Fy(a-b-)) and do not express the antigen, exhibit natural protection against both *Plasmodium vivax* and *Plasmodium knowlesi* [2]. Others have also observed that individuals with blood type O are protected against severe Malaria due to decreased cell rosetting and adherence [4].

Blood group antigen expression has also been associated with a number of other diseases, and relationships between the ABO blood group system and malignancies such as gastric carcinoma [5], carcinoma of the cervix [6] and breast cancer [7] have been described. In these cases it is thought that increased expression of epidermal growth factor on the membrane of red cells in certain blood groups promotes tumour growth [8]. Evidence has further suggested that individuals with blood groups A, B and AB are susceptible to both arterial and venous thrombosis [9,10]. This phenomenon can be explained by the high levels of the pro-coagulant von Willebrand factor (vWF) and Factor VIII found in the plasma of individuals with these blood groups [10].

Viruses have also been associated with the expression or absence of red blood cell antigens [3] and recent evidence suggests that these molecules may influence HIV infection by acting as attachment receptors for the virus [11–13]. Others have proposed that the level of natural occurring antibodies might vary according to the red cell phenotype and therefore may also influence infection [11]. With this in mind, it has been hypothesized that individuals

* Corresponding author at: Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville 7530, Cape Town, South Africa.
E-mail addresses: davisong@cput.ac.za, glendamarydavison@gmail.com (G.M. Davison).

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who are group B and have anti-A antibodies may have increased levels of natural resistance whereas group O, probably due to a dosage effect, are less resistant [14]. An explanation for this is that during the budding process the HIV envelope becomes glycosylated by a blood group A glyosyltransferase [15,16] and consequently the presence of anti-A antibodies may destroy the virus before it is able to infect other blood cells. On the other hand, evidence suggests that naturally occurring antibodies are part of the innate immune system and that those who are group O and have anti-A, B and AB could be protected against infection [3].

Despite this, the relationship between red cell antigen expression and HIV infection remains unclear probably due to the inheritance of genetic polymorphisms which encode and regulate the expression of blood group antigens. However, there is enough evidence to suggest that red cell phenotype plays a role in the susceptibility and protection to infection by a variety of pathogens [3].

The incidence of HIV infection in South Africa is estimated to be 11.5% with 5.3% of the Western Cape population being infected [17]. The prevalence of HIV differs significantly by age, sex and socio economic status and analysis by the Human Science Research Council has demonstrated that the majority of HIV individuals are Black Africans [18]. Blood group antigen expression also varies across population groups and due to a lack of proper demographic data, studies examining the relationship between red cell antigen expression and HIV infection remains challenging.

With this limitation in mind our objective was to perform an exploratory study which aimed to investigate the red cell antigen expression of the most immunogenic antigens in a cohort of HIV-seropositive individuals and to compare this with a control group of HIV-seronegative blood donors.

2. Materials and methods

2.1. Study population and ethical considerations

This cross-sectional study was approved by the Faculty of Health and Wellness Sciences Ethics Committee of the Cape Peninsula University of Technology (CPUT HW-REC 2015/H14) and was conducted according to the Helsinki Code of Ethics. Informed signed consent was obtained from all voluntary and non-remunerated blood donors at the blood donor clinic and permission for data retrieval, without identifiers, was obtained from the Western Province Blood Transfusion Service. The HIV-seropositive blood samples were obtained with prior permission from the National Health Laboratory Service, Haematology laboratory, Groote Schuur Hospital and were received without identifiers and after all routine testing had been performed by the laboratory. A total of 436 (119 HIV-seropositive, 317 HIV-seronegative) individuals were included in this study.

2.2. Determination of blood group antigen profile

The ID-Gel Card technology from *BIO-RAD* (Dia-Med AG, Morat, Switzerland) was used to analyse the blood group antigen profiles (ABO1, ABO2, ABO3, RH2, RH4, RH1, RH3, RH5, KEL1, FY1 and FY2). Ten microliters (10 μl) of a 5% or 0.8% (Duffy antigen) RBC suspension was added to all microtubes of the ID-Gel Cards. After adding the appropriate anti serum and a 15 minute incubation the microtubes were centrifuged at 900 rpm for 10 minutes in the 009560 ID-Centrifuge 6 S (Dia-Med AG, Morat, Switzerland) and the results read and recorded. A positive result was reported when agglutinated cells formed a red line on the surface of the gel or agglutinates were dispersed in the gel. A negative result was recorded when a compact button of cells was detected on the bottom of the microtube.

2.3. Quality control and quality assurance

The specificity and sensitivity of the monoclonal antibodies present in the ID-Gel Cards was validated by comparing the phenotype results of 20 blood donor samples with those obtained from the Western Province Blood Transfusion Service. The equipment was calibrated by the *BIO-RAD* company according to a standard protocol before commencing with testing and an internal reproducibility control, a negative control and known positive and negative screen cells from *BIO-RAD* were included with each run. All reagents were stored between 2 °C and 8 °C in a temperature controlled fridge for optimal results.

2.4. Human Immunodeficiency (HIV) status

All samples were initially analysed using an enzyme-linked immunosorbent assay (ELISA) for HIV-1, HIV-2 and HIV-1 p24 antigen. Thereafter the HIV status was confirmed using Nucleic acid amplification testing for the presence of HIV-1 and HIV-2 RNA and viral load was determined using the polymerase chain reaction and reported as the number of copies of HIV DNA per millilitre of blood. Analysis took place at the Western Province Blood Transfusion Service and the National Health Laboratory Service which are both internationally accredited laboratories.

2.5. Statistical analysis

Microsoft Excel was used to capture the data of both groups and the results were statistically evaluated using the Statistical Package for the Social Sciences software version 24. The prevalence of blood group antigens was calculated for total HIV-seronegative and –seropositives. Odds Ratio and the Relative Risk for HIV infection and the respective 95% Confidence Interval for blood group antigens were estimated. The Odds Ratio and Relative Risk were obtained by calculating the probability (p) of infection based on the blood group antigens, dividing the number of seropositives by the total number of seronegatives. The p-value for certain categories was calculated using the non-parameter function. Conclusions were made based on the results obtained with a p-value of <0.05 representing statistical significance. If one of the groups had a zero (0) incidence, a p-value could not be calculated (CNBC).

3. Results

3.1. ABO and RH antigen expression

There was no significant difference in the incidence of blood group A, B and AB between the HIV-seropositive and –seronegative individuals however significantly fewer HIV-seropositive patients were blood group O (43% and 84% respectively; $p = < 0.0001$).

Based on the combined expression of the ABO and Rh blood typing data, there was a significant difference between the two groups in the expression of blood type A Rh1-Positive (prevalence = 31% vs. 9%; $p = < 0.05$) and no HIV-seropositive individuals were typed as blood type O Rh1-Negative (prevalence = 0% vs. 28%; p = CNBC) (Table 1).

Similarly, all the HIV-seropositive individuals were Rh1- and Rh4- compared to 30.3% (Rh1-) and 6.6% (Rh4-) within the HIV-seronegative group. In addition, there was a significant difference in the expression of the Rh3 antigen between the two groups (prevalence = 23.5% (HIV-positive) vs. 5.7% (control); $p = < 0.05$) (Table 1).

Based on the above phenotypes, no significant difference was detected in the expression of the Rh genotype DcEe/R^2r

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