PREVALENCE OF WEAK RhD PHENOTYPE in the blood donor population of Nairobi Regional Blood Transfusion Centre - Kenya

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ABSTRACT

BACKGROUND
The weak RhD phenotype is a form of RhD antigen that, in routine RhD typing, does not react by agglutination with potent monoclonal anti-D serum, but requires addition of antiglobulin serum to demonstrate the presence of the antigen. However, the weak D antigen can cause immunization or sensitization when a truly D-negative recipient is exposed to it. It is therefore crucial to correctly determine the RhD status of units in the blood donor pool of a transfusion service.

STATEMENT OF THE PROBLEM
The prevalence of the weak RhD phenotype is known to vary between races and countries, and the documented prevalence in one race or country is not applicable to others. The prevalence of the weak Rh-D phenotype has not been well documented in the Kenya population.

OBJECTIVES
The objective of the study was to determine the prevalence of the weak RhD antigen in blood donors at the RBTC in Nairobi. The study was also to explore the weak RhD antigen in relation to the gender and age of the donors in the population.

METHODS
Donor blood samples were typed by mixing monoclonal anti D with red cell saline suspensions in microtitre plates which were then spun at 2000 rpm for 1 minute. RhD negative samples were further tested by a tube agglutination method. Samples confirmed negative by the two methods were then tested by the indirect antiglobulin technique (IAT) in a Du test.

RESULTS
Of the 384 donor samples tested, 26 (6.8%) reacted negatively with anti D in the microtitre and tube tests. Eight (30.8% of negatives, and 2.1 % of total) of the 26 “negative” samples reacted positively by the IAT or Du test. There was no relationship between gender or age and weak RhD positivity.

CONCLUSION AND RECOMMENDATION
The prevalence of weak RhD was found to be 2.1 % in the donor population of the RBTC Nairobi Kenya. The Du test should be applied to all blood donor samples found to be RhD negative in routine blood typing.

INTRODUCTION

The weak RhD phenotype is a variant form of the RhD antigen that in routine RhD typing does not react by agglutination with potent monoclonal anti- RhD serum, but requires addition of antiglobulin serum to demonstrate the presence of D antigen. This weak form of RhD antigen was described in 1944 by Wiener and was formally referred to as Du antigen.[1,2] In 1946 Stratton termed this form of D as a weak expression of the RhD antigen.[3] The abnormality on the weak D red cells appears to be a quantitative variation. Weak RhD red cells have fewer D antigen sites per cell than normal RhD positive cells. The number of RhD antigen sites on the Rh (D)-positive red blood cell

is normally in the range of 9900 to 33000, but the weak D red blood cell has about 110 to 9000 antigen sites.[4,5] However, the antigens on the weak D red cells can cause sensitization or allo-immunization when a truly RhD negative person is exposed to them. This makes it very crucial to correctly identify this weakened form of D antigen in the blood donor pool to ensure recipient safety. The frequency of the weak RhD phenotype varies between races, and also depends on the method of determination.[6] The higher the frequency of the Du phenotype in a donor population, the higher is the risk of mismatches. The frequency of the weak D phenotype in whites is approximately 0.3% (3 in 1000).[7] It has also been established that the frequency of weak D among Blacks is higher than in Whites.[7] The purpose of this study was to determine the point prevalence of weak RhD phenotype in donated blood at the Regional Blood Transfusion Centre Nairobi (RBTC Nairobi)

MATERIALS AND METHODS

Blood samples from voluntary non- remunerated donors who had consented to participate in the study were collected in 6mls Ethylene Diamine Tetra-chloral acetic Acid (ETDA) tubes and delivered to the National Blood grouping laboratory. The samples were first typed by a microtitre method Those found to be RhD negative were then typed by tube method to confirm their D status. Samples confirmed as RhD negative by the two methods were then tested by the indirect antiglobulin method in a Du test. Positive and negative controls were included in all tests

RhD typing

- **Microtitre procedure**
  The microtitre plates were labeled appropriately with donor numbers. One drop of monoclonal anti-D was dispensed into all wells, and one drop of a 2% saline suspension of donor cells was added to respective wells. The plates were placed in the microtitre centrifuge and spun at 2000rpm for 1 minute. The plates were shaken for 1 minute, and the wells were examined visually with aid of magnifying mirror viewers. Samples not showing agglutination were regarded as RhD negative, and later retyped by tube agglutination method.

- **Tube agglutination procedure**
  Two drops of monoclonal IgG anti-D were placed in labeled tubes. Two drops of donor 5% red cell suspension in saline were added to respective tubes. All tubes were spun in a centrifuge at 1000 rpm for 1 minute. Absence of agglutination was taken as RhD negative

- **Du test (IAGT) procedure**
  All tubes showing no agglutination in the tube test were incubated at 37OC for 60 minutes and washed in saline 3 times. The supernatant from the last wash was gently discarded, and the cell button gently mixed. One drop of anti-human globulin (AHG) was added and gently mixed. Tubes were centrifuged at 1000rpm for 1 minute, and the contents were examined for haemolysis in the supernatant. The cell deposit was examined macro- and microscopically for agglutination. Samples showing agglutination, and or haemolysis were regarded as Du positive

RESULTS

Of the 384 blood donor samples grouped using monoclonal anti-D reagents 358 agglutinated directly with the anti-D in the initial micro-titre typing. When the 26 samples which did not react directly with anti- D in the first test were retyped using the tube method, there was no agglutination in any of the samples. When an indirect antihuman globulin test (the Du test) was performed on these samples, 8 of the 26 tubes showed agglutination. (Tables 1 and 2, and Figure 1)

Table 1: RhD antigen typing results (micro-titre plate method)

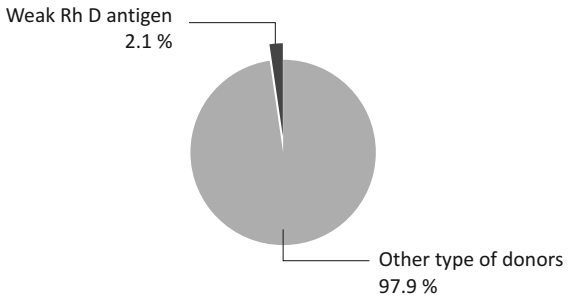
	Frequency	Percent (%)	Cumulative Percent
RhD antigen Positive	358	93.23	93.23
RhD antigen Negative	26	6.80	100.00
Total	384	100.00	-

Table 2: RhD antigen typing results (IAT/ DU Test)

	Frequency	Percent	Cumulative Percent
RhD antigen Negative	18	69.2	69.2
Weak RhD antigen Positive	8	30.8	100.0
Total	26	100.0	-

Figure 1: Weak RhD Antigen Prevalence

Prevalence of Weak Rh D antigen among blood donors

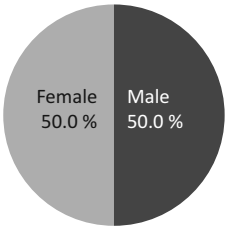


When the Du positive donors were segregated by gender, there was no significant difference in prevalence between males and females. (Table 3 and Figure 2)

Table 3: Weak RhD antigen Positive in relation to gender

Gender	Frequency	Percent	Cumulative Percent
Male	4	50	50
Female	4	50	100
Total	8	100	-

Figure 2: Weak RhD in relation to gender



When the weak D positive donors were segregated by age no correlation was found between age and Du status (Table 4 and Figure 3)

Figure 3: Weak RhD antigen Positive in relation to Age

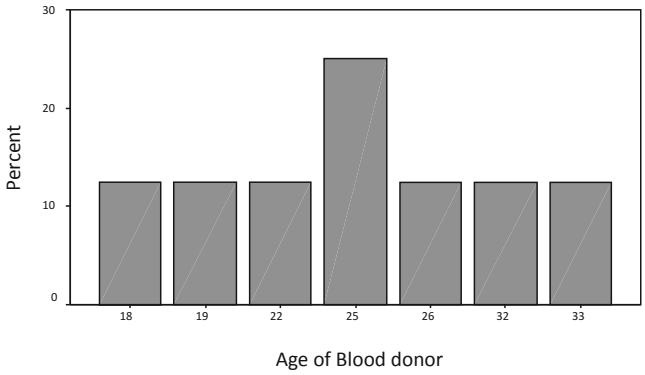


Table 4: Correlation between Age and Weak RhD antigen

		Weak RhD antigen	Age of Blood donor
Weak RhD antigen	Pearson Correlation	(a)	(a)
	Sig. (2-tailed)		
	N	8	8
Age of Blood donor	Pearson Correlation	(a)	1
	Sig. (2-tailed)		
	N	8	8

[a] Cannot be computed because the weak RhD antigen is a constant variable.

DISCUSSION

Extensive analysis has been done on Rh antigens, and presently, over 200 variants have been described.[8,9] Many of these may not be serologically distinguishable, and may require molecular analysis10. Many may also not be clinically important The D is the most immunogenic of the Rh antigens. It has been estimated that 20-30 % of RhD negative persons who receive significant volumes of RhD positive red cells make anti-D.[11,12] Transfusion of red cells bearing the weak D , which is a variant of the RhD antigen, may pose a risk of sensitization or allo-immunization in RhD negative recipients. Haemolytic disease of the newborn and of the fetus can also occur in pregnant RhD negative women carrying weak RhD positive babies.[13] Prevalence of weak RhD phenotype is known to vary between races,[6] and the documented prevalence in one race or country may not be applicable to others.

In general it has been observed that Blacks have higher prevalence of weak RhD than Whites.[7] While the prevalence of weak RhD phenotype has not been well documented in the Kenyan population, our study has revealed a prevalence of 2.1% for weak RhD phenotype in the Nairobi donor population. This figure is much lower than the 6.4% found in Ghana.[14] It is however higher than the 0.2-1% quoted for Caucasians,[7] as well as the 0.01%for Indians[15] and the 0.14% for Albanians.[16]

CONCLUSION AND RECOMMENDATION

The prevalence of weak RhD phenotype was found to be 2.1% in the donor population at the Regional Blood Transfusion Centre in Nairobi Kenya. It is recommended that all blood samples found to be Rh negative on routine saline grouping should be retyped in a Du test to avoid RhD mismatches. It is also recommended that similar studies to ours be carried out in other centres in Kenya to establish a national prevalence for Kenya, and in other parts of Africa to confirm the higher prevalence of weak RhD phenotype in Blacks.

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