DETECTION OF WEAK D (D^u) PHENOTYPE AMONG Rh-D NEGATIVE MALES AND FEMALES IN KUMASI, GHANA

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ABSTRACT
Weak Rh D phenotypes are very frequent in Africans. They are capable of causing alloimmunization in Rh D-negative individuals. Some weak Ds may elude routine typing using direct agglutination techniques. This study aimed at determining the prevalence of weak D phenotype among Rh-negatives, using indirect antiglobulin technique. A total of 400 donors between the ages of 16 and 35 years who were grouped by the blood bank were randomly sampled over a period of 2 months. Three hundred and sixty nine (92.25%) were typed as Rh D-positive and 31 (7.75%) RhD-negative. Two (6.45%) of the Rh D-negative donors were weak D positive while 29 (93.55%) were weak D negative. Among the males 25 (9.43%) were Rh D-negative and 240 (90.57%) RhD-positive. Two (8%) of the 25 males were weak D positive. Among the females, 6 (4.44%) were Rh D-negative and 129 (95.56%) RhD- positive. This implies that, there are people in Kumasi with weak D phenotype which cannot be detected by the direct monoclonal anti-D agglutination. Consequently, indirect antiglobulin test may be indicated for such individuals typed Rh D-negative. This study has shown the need for a comprehensive policy on appropriate testing of donors and newborns, and management of Rh D-negative mothers in the Region. This should include weak D testing of all Rh D-negative blood donors before transfusion in Rh D-negative patient.

Keywords: *Rhesus D, weak Rh D, indirect antiglobulin test, Kumasi-Ghana*

INTRODUCTION

The Rh blood-group system is clinically important because antibodies against Rh antigens are involved in haemolytic disease of the newborn, haemolytic transfusion reactions, and autoimmune haemolytic anaemia (Issitt, 1989; Issitt and Telen, 1996). Individuals are classified as Rh-positive and Rh-negative according to the presence or absence of the D antigen on the surface of their red cells (Issitt, 1989), which is the most immunogenic, and therefore, of critical importance for the blood transfusion strategy, and proper management of Rh-negative gravid women.

The RhD blood group antigen has been shown to be subject to many phenotypic variations (Jones *et al.*, 1995; Jones *et al.*, 1996) and the frequency of weak D phenotype varies with the method used, the reagent used, and the racial mix tested (Division of Medical Laboratory Science, 2001). The incidence of weak D phenotype is high among blacks. The frequency of the weak D phenotype in whites is approximately 0.2% to 1% (Flegel *et al.*, 2000; Wagner *et al.*, 2000), up to 10% in Africans (Cheesbrough, 2000), 7.5% among the Yoruba of South-Western Nigeria, 0.75% in Kenya (Lyko *et al.*, 1992), 0.6% in the United Kingdom (Nwauche *et al*., 2003) and approximately 0.23% in USA (Mollison *et al.*, 1997). Nwauche et al., (2003) found 0.95% among adult females in Port Harcourt, Nigeria. These varied results from Nigeria depicted heterogeneity in the prevalence of weak D, even among somewhat homogenous entities.

Weak D phenotypic expression is known to arise from three mechanisms (Tippett, 1988). In one of these mechanisms, referred to as gene interaction, there is a suppressive effect of the C gene when in trans to the D gene (e.g., Dce/Ce). The second is when part of the D antigen is missing (partial D). Thirdly, the presence of an aberrant form of D (e.g., at the molecular level) would result in weak phenotypic expression. The term 'Weak D' actually refers to red cells with the aberrant RhD protein, expressing reduced membrane surface D antigens. The partial Ds, sometimes give weak phenotypic reactions in serologic procedures. Because serologic distinction is currently not possible, many laboratories would detect some partial D's as weak D. It is known that many partial D phenotypes present as normal D types (e.g. react strongly with routine anti-D) so they are often not classified until they make anti-D.

The number of D antigen sites on the Rh D-positive red blood cells is normally in the range of 9900 to 33000 (Issitt and Telen, 1996) and the weak D phenotype appeared to be a quantitative variation in the number of D antigen sites on the red blood cell (Mollison et al., 1997). Primarily, the weak D is the result of point mutations that cause amino acid changes predicted to be intracellular or in the transmembrane regions of RhD and not on the outer surface of the RBC (Wagner *et al.*, 1999). These mutations, they indicated, affect the efficiency of insertion, and therefore, the quantity of RhD protein in the membrane. The inefficient insertion, reflected in the reduced number of antigen sites on these red blood cells, and explains why the indirect antiglobulin test (IAT) may be required for detection (Wagner *et al*., 1999). It was confirmed latter that, most weak D types, including prevalent ones, carry altered D antigens (Wagner *et al.*, 2000).

In the laboratory, serologic typing techniques employing the use of anti-D sera are mainly used to detect the presence of the Rh D antigens on the red blood cells of individuals. The number of samples classified as weak D depends on the characteristics of the typing reagent (Westhoff, 2004). Weak D individuals (who are actually Rh -positive) were commonly mistyped as Rh-negative through the use of polyclonal antibodies with grave consequences (Calhun and Petz, 1995). However, with the advent of monoclonal anti-D reagents, most weak D individuals are now typed as Rh-positive. It is now becoming evident that there are some immunogenic weak D samples that would not be detected by direct agglutination. Antihuman globulin test may therefore need to be performed to detect weak D red cells in individuals who initially type as Rh-negative. The objective of this study therefore, is to determine the prevalence of weak D (D^u) phenotype among Rh-negative males and females in Kumasi.

MATERIALS AND METHODS

Materials used

Blood bags containing anticoagulant Citrate Phosphate Dextrose Adenine solution, CPDA-1 (USP), AGARY® blood bag (Agary Pharmaceutical Ltd, Shandong, China) was used; RhD and ABO typing was done using kits from DiaMed-ID Micro Typing Technique (Lorne Laboratory

Ltd-Great Britain); Weak D. (Du) was typed using indirect anti-globulin technique (Biotech Laboratory Limited, UK) and HbsAg, HCV, HIV were determined using new rapid point-of-care tests, the Determine^{TM} series (Abbott, Abbott Park, IL).

Donor Selection (Inclusion/Exclusion Criteria)
Donors were selected whose blood pressure were between 90-140/60-90 mmHg and body weight of 50kg and above. They were also screened for HbsAg, HCV, and HIV-antibodies and must have tested negative. Their Hb values were also12g/dl (for females) or 13g/dl (for males).

Those excluded from donating blood fell within the following categories:

- Taking drugs for high blood pressure or heart failure
- Having Hb below 12g/dl (for females) or 13g/dl (for males)
- Tested positive for HbsAg, HCV, and HIV -antibodies.
- Have had jaundice, liver disease, epilepsy, diabetes, duodenal or gastric ulcer, asthmatic and tuberculosis
- Drug addict, or taken self injected drugs
- Sickle Cell Disease e.g.: SS, SC, Sβ-thal
- Prostitute and/or homosexual
- Experienced severe weight loss within the last six months.

Subjects and sample preparation
This study included 400 adult men and women with an age range 16 to 35 years who had been grouped by the blood bank. They include 265 males and 135 females voluntary blood donors living in Kumasi. The blood donors were those routinely bled by the staff of blood bank unit of Komfo Anokye Teaching Hospital, Kumasi. The blood was collected into blood bags containing Citrate Phosphate Dextrose Adenine and mixed. After the donation exercise, the units of blood were grouped for ABO and Rh status and kept in the blood bank at between 4-6^{o}c. This study had the approval of the Management of the Transfusion Medicine Unit to use the donor blood already grouped. Three ml of the banked blood was collected into plain test tubes and re-tested for ABO and RhD typing using the DiaMed-ID Micro Typing Technique (Lorne Laboratory Ltd-Great Britain). Weak D (Du) was typed using indirect anti-globulin technique (Biotech Laboratory Limited, UK) on all Rh-negative subjects. The results were recorded: (+) for agglutination and (-) for no agglutination. A known donor of blood group 'O' Rh D positive was used as a positive control; another known donor of 'O' Rh D negative was used as a negative control.

RESULTS
Figure 1A shows the frequency of RhD-negative and RhD-positive among blood donors. Of the subjects 92.25% (369/400) were RhD-positive and 31 (7.75%) RhD-negative. Among the females, 4.44% (6/135) of them were RhD-negative with 95.56% (129/135) being RhD-positive (Figure 1B). Of the males, 9.43% (25/265) were RhD-negative and 90.57% were RhD-positive (Figure 1C).

Out of the 31 Rh-negative subjects, 25, representing 6.25% of the 400 were males whilst 6 (1.50%) were female (Table 1). Table 2 shows the prevalence of weak D phenotype among the Rh-negative males and females. Out of the 31 apparently negative subjects, 6.45% (2/31) were detected to be weak D positive with the other 93.55% being negative (Figure 1D). Both samples were from males, thus 8% (2/25) of the Rh-negative males were weak D-positive. No weak D was detected in the 6 Rh-negative females.

DISCUSSION
The finding of 7.75% prevalence of Rh-negative among donors in this study (Fig 1A) is in keeping with available records of low prevalence of Rh-negative in other African countries. Among Kenyans 3.9% has been reported and 1-6% among Nigerians (Nwauche *et al.*, 2003). The low incidence of Rh-negativity contrasts markedly with high figures obtained elsewhere, for

example, about 15% - 17% among Europeans (Race and Sanger, 1975) and 15% in the USA population (Mollison *et al*., 1997). This could be due the high *RHD* gene frequency among Africans (Flegel and Wagner, 2002), and for that matter, Ghana.

Weak D prevalence among Africans has been said to be as high as up to about 10% (Cheesbrough, 2000). Consistent with this, is the finding of a 6.45% weak D prevalence among the subjects involved in this study (Fig 1D). This is however, a little below the 7.5% prevalence reported among the Yoruba of South-Western Nigeria (Nwauche *et al*., 2003). On the other hand, the 6.45% is high, compared to those found in some other populations; for instance, approximately 0.4% among the Caucasians (Flegel *et al*., 2000). The relatively small number of Rh-negative samples obtained could account for the high weak D prevalence obtained in this investigation. However, it should be noted that RhD frequency depends on ethnicity. In Africans, the frequency of aberrant *RHD* alleles, which encode weak D is much higher (Flegel and Wagner, 2002). This could also account for the high weak D prevalence obtained in this study.

Though the two weak D donors were men, it may not be suggestive that weak D is more prevalent among males than females. It should be noted there were only 6 Rh-negatives out of 135 females. This should compare with our finding that 25 of the 265 males were Rh-negative.

Considering the fact that monoclonal anti-D antisera, were used in the direct agglutination test it is obvious that the two samples that showed weak D phenotypic reactions had low antigen D density. This attests to previous observation that donors who are carriers of some weak D may pass unnoticed in tests based on direct agglutination with monoclonal anti-D (Wagner *et al.*, 2001). The results of this study, however, questions the opinion that there is no need to test Rh negative samples for weak D when using monoclonal IgM anti D (Cheesbrough, 2000).

The implications of the findings in this study are far-reaching. From previous reports (Gassner *et al.*, 2005; Wagner *et al.*, 2005), it can be inferred that units of blood from the two weak D positive donors observed pose the potential risk of alloimmunizing Rh-negative recipients. If the two samples were from individuals of Rh-negative mothers having no preformed anti-D, by the procedures of some laboratories they would be typed as Rh-negative by direct agglutination test. Rh immune prophylaxis would not be given to the mother, possibly resulting in her being antigen D-alloimmunized. Therefore the universal acceptance of weak D typing of individual who type as Rh-negative with direct agglutination technique is justified by the results of this study. Neglect of such a procedure may result in unnecessary incidence of preventable haemolytic disease of the newborn with its severe clinical implications.

Sometimes when an Rh-negative woman becomes pregnant it becomes necessary to determine the partner's Rh status to assist in the proper management of the woman if there is the possibility of the baby being Rh-positive. By using only direct agglutination, if the partner's weak D red cells are not detected, the result will be similar to earlier instances mentioned above.

Because of the rarity of Rh-negative donors, carefully selected patients may be tested for weak D when typed as Rh-negative by direct agglutination. That may be considered when no Rh-negative blood is available. In that case it should be borne in mind that current knowledge indicates that majority of individuals with a weak D phenotype can safely receive Rh-positive blood and do not make anti-D (Westhoff, 2004). If the two subjects who typed as weak D positive required blood transfusion, they could therefore receive Rh-positive blood in the absence of Rh-negative blood unit. The question that comes to mind is, what, if the weak D reaction is due to a partial D? In our African setting it may not be a problem. Only about 5-10% of the weak Ds are partial Ds, most of

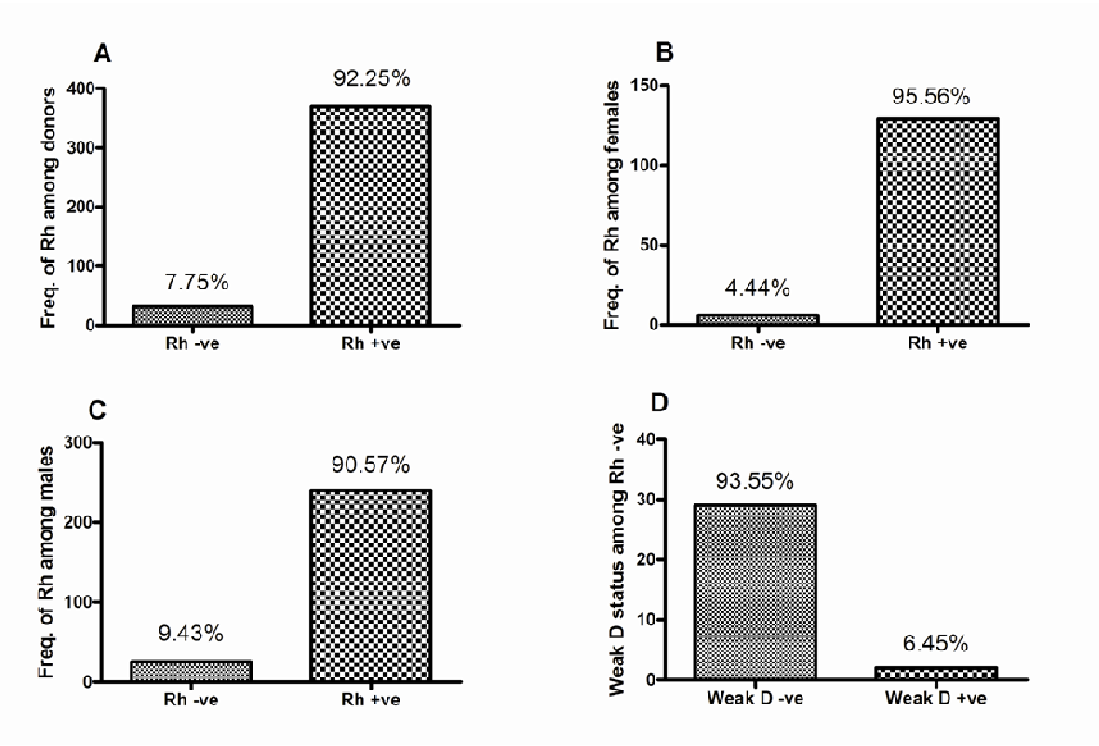

Fig 1. Characteristics of the Rhesus D among donors. A is the frequency of Rh –ve and Rh +ve among the whole donor populations; B is the frequency of Rh –ve and Rh +ve among the female donors; C is the frequency of Rh –ve and Rh +ve among the male donors and D is the frequency of the apparently negative subjects who reacted positive and negative to the weak D test.

Table 1: Prevalence of Rh-negative among males and females

	Rh-negative		Rh-positive		Total	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Male	25	6.25	240	60.00	265	66.25
Female	6	1.50	129	32.25	135	33.75
Total	31	7.75	369	92.25	400	100

Table 2: Prevalence of weak D phenotype among Rh-negative males and females

	Rh-negative		Weak D-positive		Weak D-negative	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Male	25	80.65	2	6.45	23	74.19
Female	6	19.35	-	-	6	19.35
Total	31	100	2	6.45	29	93.55

which are D^{VI} Rh variant (Shulman, 2002; Shulman, 2005). The D^{VI} is more frequent among Caucasians but not Africans. Because RhIg is not completely without risk (HCV and unknown variant Creutzfeldt-Jakob disease), is costly, and is derived from human source material that is in short supply, it is prudent policy to avoid its unnecessary use (Shulman, 2005).

CONCLUSION

This study has found that 6.45% of blood donors who were typed as Rhesus negative are actually weak D positive. Because of the potential risk of alloimmunization caused by such red cells, indirect antiglobulin testing must constitute part of the routine typing of all blood donors. It would therefore, be prudent for policy makers and transfusion units to carefully consider the issue of the incidence of the weak D and come up with policies for its testing. Also, the universal acceptance of weak D testing of newborns is valid and need to be implemented. Finally, donors and husbands of Rh negative women who type as Rh -negative may need to be typed for weak D, even when monoclonal anti-D is used.

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