Frequency of variant D in Delhi, India

Sir,

The Rh blood group system has confounded the blood transfusionists for many years. Since the discovery of the D antigen by Landsteiner and Wiener in 1940, we have come a long way. At present 50 different Rh antigens are known. Despite the availability of large amount of information about weak D/partial D, it is still many a times not possible to categorize a D antigen negative blood group — so the term variant D is used to designate such cases. Limited literature is available for variant D in India. This study was conducted to assess the frequency of D variants in our population.

This study was conducted at the Regional Blood Transfusion Centre, Lady Hardinge Medical College and associated hospitals over the period January 2009 to July 2010. There were a total of 64,234 subjects (52,648 patients and 11,586 donors) of Indo-Aryan ethnic origin belonging to South East Asian region. Informed consent of the participating subjects was taken. Rh blood group typing of all samples was carried out by immediate spin tube technique with two antisera. The two antisera used were monoclonal IgM anti-D (Tulip Diagnostics Private limited, Goa, India) and polyclonal IgM+IgG anti-D blend (Tulip Diagnostics Private limited, Goa, India Lot No. 1050031 P). Samples that were negative by immediate spin tube method were further tested by Indirect Antiglobulin Test (IAT) and Gel card system (GCS) (ID Diaclon Anti-D, Diamed ID Microtyping System) for weak D. Equal volumes each of anti-D serum (IgM + IgG) and 5% red cell suspension were placed in a clean glass tube, mixed, and incubated at 37°C for 45 minutes and then centrifuged at 1000×g. The tube was gently resuspended and cell button observed for agglutination, which was confirmed microscopically. If the test red cells were agglutinated, the immediate spin tube test result was recorded as D antigen positive. If the test cells were not agglutinated (D antigen negative), cells were washed twice with large volumes of normal saline. After the final wash, the saline was decanted and two drops of antihuman globulin serum was added and the tube centrifuged at 1000×g for 1 minute. The cell button was resuspended and examined for agglutination macroscopically as well as confirmed microscopically. The samples showing agglutination after addition of AHG serum were considered weak D positive. Parallel controls were set up to rule out any DAT-positive samples.

Table 1: Summary of results of the study

Rh D status	Calculation	Percentage
D antigen positive	60,753/64,234	94.6
D antigen negative	3,481/64,234	5.4
Weak D positive	6/3,481 (out of D negative)	0.2
	6/64,234 (out of total population)	0.009
Partial D positive	1/3,481 (out of D negative)	0.03
	1/64,234 (out of total population)	0.002

For testing of weak D by gel card method, 1% red cell suspension of test sample was prepared in low ionic strength solution. Fifty microliter of 1% RBC suspension was dispensed in microtube of IgG card followed by the addition of 50 μL of monoclonal anti-D IgG (ID Diaclon Anti-D, Diamed ID Microtyping System). This was followed by incubation at 37°C for 15 min and centrifugation.

Partial D testing of D-negative samples was performed using the commercially available kit for Partial D (ID Partial Rh D typing test, Diamed ID Microtyping System, GmbH, Switzerland), which detects the following partial D variants: D-II, D-III, D-IVA, D-IVB, DV, DVI, DVII, DFR, DBT, and DHAR.

The result of our study is summarized in tables 1 and 2.

The incidence of variant D varies worldwide. There is a wide range of variant D positivity reported in the literature. Mak *et al.*, reported an incidence as low as 0.016% in Chinese donors, whereas Okrah *et al.* reported an incidence as high as 6.45% in African donors.[1,2] In our study, weak D comprised 0.2% of all D antigen-negative samples and 0.009% of total study population, whereas partial D comprised 0.03% of all D antigen-negative samples and 0.002% of total study population. Various authors have given the prevalence of weak D/partial D in their population. However, due to the lack of standard definition of weak D/partial D and the differences in the type of antisera used (monoclonal/polyclonal, single/blended), comparative analysis becomes very difficult.

At the level of blood banks, there is confusion as to how to proceed and what to call a sample that is negative on routine D antigen typing and positive on IAT/GCS. Furthermore, it has been adequately documented that D epitopes distribution differs with different geographic locales and ethnicities of the population.[3] It is being felt that the reagents produced in western countries may not be suitable for Indian population as D antigen is genetically controlled and major variations may exist in the D antigen profile of Caucasians and Indians.[4] Kulkarni *et al.* highlighted this fact by testing 42 confirmed partial D variants with seven commercially available anti-D reagents in India.[4] When monoclonal antiseras were used, only 59% partial D variant cells were picked up, however, polyclonal anti-D showed weak reaction with as many as 83% of partial D cells. Only two combinations picked up all partial D, whereas 90.5% available combinations of anti-D reagents did not detect partial D variants.[4]

The clinical significance of detecting weak D and partial D variants of Rh (D) lies in the fact that the D antigen is the most immunogenic of all protein antigens. The current opinion of the majority is that weak D/partial D subjects should be treated as D positive as donors to prevent alloimmunization if accidentally transfused to

Table 2: Summary of results of weak D/partial D typing of the 7 positive cases

Case number	Immediate spin tube test	Indirect antiglobulin test (weak D)	Direct Coomb's test (control)	Gel card microtyping system (weak D)	Partial D
Case 1-3	Negative	2+	Negative	2+	Negative
Case 4, 6	Negative	1+	Negative	3+	Negative
Case 5	Negative	2+	Negative	Negative	Positive
Case 7	Negative	3+	Negative	3+	Negative

D-negative recipients. Alloimmunization of D negatives can occur with weak D, while in child-bearing age can be disastrous and can lead to hemolytic disease of newborn. Newborns of D negative mother should be tested for D/weak D and Rh immunoglobulin is recommended for mothers of D/weak D positive infants in order to prevent immunization. On the other hand, partial D recipients should be considered as D negative else they will form antibodies against the missing epitopes of D antigen when transfused with D-positive blood. Use of IAT procedure for weak D typing can be dangerous as patients can be recorded as D positive when controls have been omitted/wrongly interpreted. If a mistyped D-negative female patient was then transfused with D-positive blood, the consequences due to the serious risk of alloimmunization would be more serious than if the test had not been performed.

Thus, even with limited resources in a developing country like ours, there should be a standard protocol for investigating every case of Rh-negative sample for weak D testing by either IAT or more sensitive GCS. Comprehensive national transfusion guidelines need to be laid down to standardize the protocol for D antigen testing for donors as well as patients to avoid misdiagnosis and to prevent transfusion-related complications.

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