

Prevalence of weak D phenotype among D negative C/E+ blood donors in Morocco

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ABSTRACT

Aims: Determination of D variants is important for selection of blood products and to prevent anti-D-related hemolytic disease of the fetus and newborn. The prevalence of weak D phenotypes varies significantly among different ethnic populations. In Morocco, D variants have not been fully characterized. The purpose of this study was to determine prevalence of weak D among D negative and C/E+ blood donors and distribution of Rhesus blood group antigens using serologic methods. **Methods:** A total of 15,865 blood samples from Moroccan blood donors were tested for D, C, c, E, e antigens by routine serologic methods. Among blood donors serologically D negative C+ and/or E+, 63 samples were tested for weak D by indirect antiglobuline test, enzymatic treated cells test and adsorption elution technique. **Results:** Among 63 samples tested, 10 were positive by serologic methods

(15.87%). Six samples identified as weak D by enzymatic test and four samples identified as Del by adsorption elution. Rhesus blood group antigens are distributed as DCcee: 38.85% and dccee :8.77%. **Conclusion:** The prevalence of D variants must be considered in transfusional and obstetrical strategy for RhD typing to ensure blood transfusion safety by optimizing the management of D-negative RBC units and Rh immune globulins.

Keywords: Anti-D immunization, D variant, Moroccan population, RhD, RBC serology, Transfusion, Weak D phenotype

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INTRODUCTION

The Rh blood group system is one of the most polymorphic systems in human. It is involved in the incompatible RBC transfusions conflicts and in the hemolytic disease of the newborn due to the maternal-fetal blood group incompatibility [1].

By year 2015, 58 Rh antigens have been identified. The most common and immunogenic are D, C, E, c,

and e [2]. The D antigen is encoded by the RHD gene while RhC, RhE, Rhc and RHe antigens are encoded by the RHCE gene [3, 4]. The RHD gene polymorphism leads to phenotypic polymorphism D variants including weak D, Del and partial D. The weak D phenotype corresponds to a decrease in the expression of D antigen [5]. The weakest RhD (Del) is detected by adsorption-elution test [6]. Partial D phenotype corresponds to qualitative changes in D antigen consistent with the absence of some epitopes [7].

More than 100 variant of D types have been reported in literature. The prevalence of D variants varies among populations. Between 0.2 to 1% of Caucasians carry D variant, while almost 30% of D negative Asians are phenotyped Del [8, 9]. There are few studies of RHD alleles among African populations. Wagner et al. reported a frequency of 31% of aberrant RHD allele among Africans of Mali while Kabiri et al. reported 0.4% in Morocco [10,11]. However, D variants are more frequent among subjects D negative RhC and/or RhE positive [12].

In transfusion medicine, the determination of these D variants is important to ensure blood safety. Their presence causes difficulties in hematology and blood transfusion laboratories. Related discrepancies observed depend on the anti-D reagents and/or serological techniques used. Some weak D and partial D are not detected by serology techniques. Transfused D negative patients with RBCs carrying these variants may develop alloantibodies against the D antigen [13, 14]. Immunohematological sensitive tests such as enzyme test, indirect antiglobuline test and adsorption-elution technique are necessary to detect some weak D.

Considering the above technical difficulties and the importance of the Rhesus system in transfusion medicine and hemolytic disease of the newborn, it is necessary to determine population frequencies of D variants. We aimed to determine the prevalence of weak D variants in Moroccan blood donors phenotyped D negative RhC and/or RhE positive.

MATERIALS AND METHODS

Rhesus phenotypes

From November 2011 to June 2012, blood samples were collected at the Regional Blood Transfusion Center in Casablanca (CRTSC) from 15,865 blood donors. Rhesus typing (D, C, E, c, e) is performed using automated analyzer (Olympus PK 7300, Japan) on red cells treated and suspended in 0.9% bromelain-PBS solution (1 g/μl) and incubated at 37°C for 60 minutes. The tests were performed with monoclonal antibodies: anti-D (clones n°: P3x61 + P3x21223B10 + P3x290 + P3x35, Diagast, France), anti-C (clones n°: 25513G8 * P3 + MS24, Diagast, France), anti-c (clone n°: 951, Diagast, France), anti-E (clone n°906, Diagast, France), and anti-e (clone n°: P3 GD512+MS63, Diagast, France).

Weak D Testing

Among blood donors phenotyped D negative, RhC and/or RhE positive, 63 samples randomly selected were tested by indirect antiglobuline test and papain test using the manufacturer's recommendations. The antibodies used were: anti-D (IgM/IgG blend, BGD00010, Fortress Diagnostics, UK) and an anti-IgG antiglobulin + polyspecific anti-C3d (clones n°18833 + 18896 + 12011D10, Diagast, France). Results were read by two observers independently. Samples typed as D negative by indirect antiglobuline test and enzymatic test, were tested for the Del phenotype by adsorption elution. RBCs washed 3 times with an isotonic solution of NaCl at 0.9% were incubated with anti-D (v/v) for 45 min at 37°C. After centrifugation and washing the pellet 6 times, RBCs were re-suspended in an isotonic solution (2v/v) and incubated at 56°C for 15 min. After a second centrifugation (400 g/5 min), the supernatant was incubated separately with 5% RBCs positive (D+) and negative (D-) suspensions controls. RBCs controls (D+ and D-) were provided by National Quality Control Laboratory of Morocco. After incubation at 37°C for 45 min, results were interpreted with the naked eye and microscopically by two observers.

RESULTS

Rhesus phenotype frequencies

A percentage of 9.5% (1519/15865) of samples were phenotyped D negative. Among them, 8.3% (127) were RhC and/or RhE positive. The DCcee was the most common phenotype. Table 1 gives the percentages of different Rhesus phenotypes.

Weak D phenotype frequency among RhD negative RhC and/or RhE positive individuals

63 D negative blood donors randomly selected are tested: 28 were RhC positive, 26 were RhE positive, and 9 were RhCE positive. Immunohematological sensitive tests revealed 10 weak D positive among 63 (15.87%) as given in Table 2.

DISCUSSION

This study revealed 15.87% of weak D among subjects C and/or E positive and previously documented as D negative. This percentage depends on immunohematological techniques, and reagents used (anti-RH1, antiglobulin, papain, bromelain) [15, 16]. The omission of weak D variants by immunohematological techniques has been reported by several authors in several countries [17–19]. D variants are found among D negative C and/or E positive blood donors [12]. It is recommended in Australia to use indirect antiglobuline test among C and/or E positive

donors to reduce alloimmunization, cost and time testing [19]. In Morocco, indirect antiglobuline test are not used to detect weak D. For transfusion safety, the donors phenotyped D negative RhC and/or RhE positive are classified as D positive donors, and D negative recipients in the blood transfusion center in Morocco, despite the lack of data on D variants. However, in Egyptian study, Eiman Hussein et al. suggested that D variants are more likely found in blood donors phenotyped Rhce [20].

In our study, the serological methodology has not distinguished between weak D and partial-weak D. The later represents 5–10% of weak D detected by serology [15, 21, 22]. Transfusion of partial-weak D recipients with D positive RBCs may induce alloimmunization against the missing epitopes. A typical example of these variants is DVI which may cause hemolytic disease of the newborn [23]. To prevent alloimmunization, the FDA recommends to use monoclonal anti-D which does not recognize DVI variant among recipients and pregnant women [24]. The alternative solution would be the RH genotyping [15].

Table 2: Serologic profile of weak D phenotypes

Samples	1	2	3	4	5	6	7	8	9	10
Phenotypes	Ccee	Ccee	ccEe	CcEe	ccEe	ccEe	CcEe	CcEe	Ccee	Ccee
IAT	-	-	-	-	-	-	-	-	-	-
Enzyme test	+	+	+	+	-	-	-	-	+	+
Adsorption-elution	NT	NT	NT	NT	+	+	+	+	-	-

+: Positive agglutination, -: No agglutination, NT: Not tested

RhD phenotyping showed 9.5% D negative blood donors in Casablanca. This percentage is consistent with previous studies [25]. The frequency of RhD negative varies between populations: 15% in Caucasians, 3–7% in Africans, 7–8% among Black Americans, and <1% in Asians [26–28]. Comparing different rhesus phenotypic frequencies, one can observe similarity between populations of Morocco, (DCcee: 38.85% dcee: 8.77%), Maghreb (DCcee: 37.55% dcee: 6.12%) and Arab Gulf (DCcee: 30.9% dcee: 7.3%) [29–31]. These results confirm previous studies concerning ABO system. Intermediate values between black Africans and Caucasians are explained by the history of migrations of different peoples in the Maghreb [32].

CONCLUSION

Immunohematological sensitive test revealed that 15.87% of blood donors RhD negative, RhC and/or RhE positive, previously documented as RhD negative were weak D. Our results suggest that it would be important for Blood Centers to continuously develop new strategies for Rh phenotyping. This will avoid unnecessary injections of immunoglobulins RhIG for weak D pregnant women and

Table 1: Rh phenotypes frequencies in Moroccan blood donors

Phenotypes	Number of blood donors	%
DCcee	6163	38.85%
Dccee	3119	19.66 %
DCCee	2523	15.9%
DccEe	1187	7.48%
DCcEe	1162	7.32%
DccEE	166	1.05%
DCCEe	26	0.16%
dccee	1392	8.77%
dCcee	96	0.6%
dccEe	28	0.18%
dCcEe	2	0.012%
dccEE	1	0.006%

transfusion of D negative patients with weak D RBCs, and therefore ensure better management of D negative blood stock. Since immunohematological techniques remain insufficient, RHD genotyping is recommended to identify RHD variants.

Author Contributions

Mariam EL Wafi – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Houria EL Housse – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Nadia Nourichafi – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Kamal Bouisk – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Mohamed Benajiba – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published

Norddine Habti – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published.

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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