Weak D Types in the Egyptian Population

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Abstract

Patients with the most common weak D types 1, 2, and 3 can be safely considered D positive. We evaluated 1,113 Rh-negative Egyptian samples for weak D expression to propose a cost-effective strategy related to D variant testing. D variants were tested using polymerase chain reaction with sequence-specific priming. Fifty samples were D variants (4.5%): weak D type 4.2 (32%), weak D type 4.0/4.1 (16%), and weak D type 15 (2%). Fifteen (62.5%) of 24 samples were identified serologically as partial D. We also studied the probability of the development of anti-D in 52 Rh-negative children with thalassemia who were receiving units for which weak D was not tested. Anti-D alloimmunization was observed in 63.5% of patients with thalassemia. It is prudent to implement weak D typing in Egyptian donors. Weak D variants of Egyptians are significantly different compared with Caucasians. Ethnicity must be taken into consideration when developing clinical and prenatal strategies related to D variants.

The molecular basis of D antigen varies substantially in different ethnic populations. Egypt has always been a country characterized by a mix of races. Egyptian ethnicity comprises an admixture of the indigenous African population, ancient Egyptians, Jews, Greeks, Romans, Persians, those of Arab ancestry, foreign invaders, immigrants, Turks, Armenians, and other Mediterranean populations. Evidence suggests that these different ethnic influences are distributed homogeneously in the delta and Nile valley, where the overwhelming majority of Egyptians live (99.6%). Ethnic minorities include Bedouins in the Eastern and Western desert and the Sinai peninsula, as well as some Nubians clustered along the Nile in upper (Southern) Egypt.

Numerous studies have characterized the different RHD alleles in whites, Africans, and Asians, but none have been conducted among the Middle Eastern population. Approximately 7% to 8% of Egyptians are serologically D negative. D negative phenotype is prevalent in whites (15%-17%) and less common in black Africans (5%) and Asians (0.5%). In whites, the D-negative phenotype arises from deletion of the entire RHD gene, whereas Africans and Asians often have an inactive or silent RHD. Major advances in cloning of the Rh system gene have challenged the way that D status is assigned. RHD variants are classified into 3 groups: weak D, DEL, and partial D alleles. They occur in an estimated 0.2% to 1% of whites. In black Africans, the frequency of the D variant is much greater, with the most prevalent being partial D. In Asians, 10% to 30% of D-negative donors are of the DEL phenotype as shown by studies conducted in China, Japan, and Taiwan. Weak D primarily results from single point mutations in the transmembranous or intracellular regions of RHD, reflected in reduced quantities of the normal D antigen.
D antigen. More than 50 different molecular weak D types have been described. Weak D types 1, 2, and 3 represent more than 90% of all weak D in whites.

Patients who carry weak D types 1, 2, 3, and 4.1 are not sensitized when exposed to the D antigen and can be safely considered D positive, thus saving the use of some D-negative units and RBC immune globulin prophylaxis during pregnancy. Anti-D alloimmunization has only been documented for weak D types 4.0, 4.2 (DAR), 11, and 15.

DEL RBCs express very low quantities of D antigen that cannot be detected on routine serologic testing and can only be detected with adsorption elution techniques. They are less frequent in whites (0.27%) and are often found in Asian ethnic populations. It is well established that RBC units with DEL phenotype can cause alloimmunization in D-negative recipients. Many blood centers in Europe have applied programs to implement RHD molecular screening for DEL donors. In contrast with weak D, partial D variants are caused by mutations in the extracellular regions and replacement of RHD axons by their RHCE counterparts, leading to altered or new epitopes. Patients with partial D may develop anti-D when exposed to D antigen.

Usually transfusion of 200 mL or more of D-positive RBCs causes allo-anti-D production in 75% to 80% of Rh-negative recipients. More recent data demonstrated an inverse correlation between the number of transfused units and the probability of antibody formation, underscoring the importance of transfusion of weak D. This issue is controversial because not enough reports have been published in the literature to give conclusive evidence. The aim of this study was to determine the frequency of various weak D types among Egyptians and evaluate the serologic issue in the Egyptian population based on serologic techniques. We also studied to estimate the incidence of partial D in the Egyptian population based on serologic techniques. We also studied the probability of anti-D development in 52 Rh-negative children with β-thalassemia who received Rh-negative RBC units for which weak D was not tested.

Serologic D Typing

Routine D typing was performed using monoclonal blended DiaClone anti-D reagents (Diaimed, Cressier sur Morat, Switzerland) containing IgG and IgM antibodies (cell lines TH-28 and MS-26), which react in the indirect anti-globulin test (IAT) with most weak D and partial D RBCs, including DVI. It was performed using the IS tube method according to the process described in the American Association of Blood Banks (AABB) Technical Manual following the manufacturer’s recommendations. Samples that were not agglutinated using the tube method were further tested with the IAT using the manufacturer’s recommendations (anti-IgG/C3d, polyspecific antihuman globulin, Millipore, Billerica, MA), and results were interpreted microscopically. Appropriate controls were used. Samples that were weakly agglutinated with the tube method or that reacted only on the IAT were classified as D variants.

Molecular Study

DNA was isolated from peripheral blood with the PCR purification kit following the manufacturer’s instruction (QIAquick, Qiagen, Hilden, Germany).

Kit Design

The basic material for typing with the BAGene DNA-SSP kits is purified leukocytic DNA. The test uses the PCR-SSP procedure. This method is based on the fact that primer extension and hence successful PCR relies on an exact match at the 3’ end of both primers. If the primers entirely match the target sequence, amplification is produced, which is subsequently visualized on agarose gel electrophoresis.

Partial D Identification

Another group of 24 samples with D variants were studied serologically in gel cards with the advanced partial D typing kit (ID-partial RHD-typing kit, Bio-Rad, Hercules, CA), following the manufacturer’s recommendations. The kit included commercially available panels of monoclonal anti D (cell lines: LHM76/55 [IgG], LHM77/64 [IgG], LHM70/45 [IgG], LHM59/19 [IgG], LHM169/80 [IgG], and LDM1 [IgM]).
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Analysis of Patients With Thalassemia

Screening for anti-D alloantibody was performed on patients with thalassemia using a gel method (Diamed-ID microtyping system).

Results

Molecular Identification of Weak D Phenotypes Using PCR-SSP

Fifty (4.5%) of 1,113 Rh-negative samples were classified as D variants. Molecular typing of the 50 D variants revealed that 16 (32%) were weak D type 4.2, 8 (16%) were weak D type 4.0/4.1, and 1 (2%) sample was weak D type 15

| Table 1 | Different Weak D Types Identified by SSP-PCR in 50 Samples With D Variants

<table>
<thead>
<tr>
<th>RHD Variant</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak D type 4.2</td>
<td>16 (32)</td>
</tr>
<tr>
<td>Weak D type 4.0/4.1</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Weak D type 15</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Partial or other rare weak D</td>
<td>25 (50)</td>
</tr>
</tbody>
</table>

Serologic Identification of Partial D

Using a panel of 6 monoclonal antibodies, all samples with partial D could be characterized according to the reactivity chart

| Table 2 | Partial D Kit Reactivity Chart

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Cell Line</th>
<th>DII</th>
<th>DIII</th>
<th>DIVa</th>
<th>DIVb</th>
<th>DV</th>
<th>DVI</th>
<th>DVII</th>
<th>DFR</th>
<th>DBT</th>
<th>DHAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LHM76/655</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>LHM77/64</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>LHM70/45</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>LHM59/19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>LHM169/80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>LDM-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

A weaker reaction can be observed with this antibody in comparison with the other 5 serum samples.

Patients ranged in age from 3 to 17 years with a mean of 12.3 ± 5.0 years. All patients received regular ABO- and D-matched transfusions for a mean of 6.6 ± 2.7 years (range, 2-13 years). Patients exclusively received prestorage leukofiltered blood transfusions for 4 years before the analysis. The vast majority of patients had long-term exposure to nonleukofiltered or bedside leukofiltered blood (1-9 years). Transfusion of Rh-negative RBC units that were not tested for weak D caused alloimmunization in 33 (63.5%) of 52 patients. Patients were a mean of 15 ± 3.5 years old and received a mean of 247.7 ± 44.8 RBC units (range, 212-298 units) for 10.2 ± 2.2 years (range, 7-13 years). Patients with no anti-D received significantly fewer transfusions (P < .05) at a mean of 30 ± 71 transfusions (range, 10-144 units) for 7 ± 3 years (range, 2-8 years). A total of 18 (54.5%) of 33 alloimmunized patients were girls, including 2 siblings.

Analysis of Patients With Thalassemia

A total of 52 Rh-negative children with β-thalassemia were included in this study, of whom 29 (55.8%) were boys.

Discussion

One of the major challenges in clinical transfusion practice is to avoid anti-D alloimmunization with the least possible costs while avoiding wastage of D-negative units and Rh immune globulin. It is well documented that patients with partial D phenotypes are at risk for production of anti-D. In contrast, patients with the most common weak D types—1, 2, and 3—are not at any risk for sensitization when exposed to D-positive RBCs and could safely receive transfusions of D-positive blood without the need for Rh immune globulin prophylaxis.10 Anti-D alloimmunization has only been documented for weak D types 4.0, 4.2 (DAR), 11, and 15.18 However, distinction among these phenotypes is impossible serologically. Only molecular analysis will identify patients with D variants who are at risk for anti-D production.

Serologic phenotyping is the standard test to assign transfusion strategies. RBCs with D variants may react differently depending on the typing method, the affinity of anti-D, and the serologic cutoff.28 The monoclonal anti-D has been in routine use at Cairo University Blood Center since 2000. Repeated testing of all Rh-negative units by IS tube test is typically carried out both before screening as
well as before the release of blood units. It has been reported that high-potency monoclonal anti-D reagents can detect D variants that are difficult to detect with less sensitive techniques. However, 4.5% of Egyptian samples were missed on routine serotyping and were only detected with the IAT. The monoclonal IgM and IgG blend clones are routinely used in the United States, whereas most European centers do not use monoclonal anti-D and do not perform IAT for weak D. It has been noted that the current monoclonal Food and Drug Administration–licensed anti-D serologic reagents are designed not to react in direct testing with partial DVI RBCs, which is the most common partial D in whites.

According to AABB, weak D testing is not required for patients or pregnant women but is mandatory in blood donor and cord blood testing. Molecular analysis for blood groups was introduced more than 10 years ago as an important aspect of immunogenetics. Their clinical application since then has been evolving. RHD molecular strategy varies considerably between the United States and Europe. In the United States, it is performed to resolve discrepant serologic results, to aid complex antibody identification, and to differentiate allo- from autoantibodies. RHD genotyping in many European centers, predominantly in Germany, is used for D variant testing in patients and pregnant women, in patients who have had a transfusion, and in patients with D typing discrepancies; in donors for DEL and D+/D− chimeras; and to determine RHD zygosity.

Because in most white patients RHD alleles are caused by a few weak D–variant types, molecular assays of these phenotypes would characterize most samples. Flegel recommended that patients carrying the prevalent weak D types 1, 2, and 3 can be classified as D positive, saving 3% to 5% of all D-negative units. Likewise, when pregnant women are genotyped to identify D variants, 3% to 5% of all anti-D injections can be spared. This service has been applied routinely in Germany since 2001. Pham and colleagues recommended that this strategy be applied in other countries to avoid wasting D-negative units. In our study, D variant prevalence was 4.5% of all D-negative samples (50/1,113). Molecular assay of the 50 D variants revealed that weak D type 4.2 was the most frequent (32%), followed by weak D type 4.0/4.1 (16%), and weak D type 15 (2%). Only 8 (0.7%) of 1,113 cases were weak D type 4.0/4.1, and the PCR-SSP platform was unable to distinguish between weak D type 4.0 and 4.1. The remaining 25 samples were not identified and probably constituted partial D or other rare weak D types.

To estimate the incidence of partial D in Egyptians, another group of 24 samples with D variants were screened serologically for detection of partial D with the advanced partial D kits, which enable the characterization of many known partial D phenotypes and facilitate differentiation of weak D from partial D. In our study, 62.5% of D variants were characterized as partial D; the DIII category was the most frequent, followed by DV. The most common partial D in whites are DVI and DVII, and the most common in people of African descent is probably DIII. The DV has been reported in white, black, and Japanese people. Wagner and colleagues proposed that frequency data of D variants in specific populations, based on DNA analysis, can be used as a basis to implement optimal transfusion and obstetric programs.

Based on our data, with the routine use of the advanced ID-partial D typing kit, 50% to 62.5% of patients with D variant can be characterized serologically. Further implementation of weak D genotyping is necessary to appropriately characterize the remaining RHD variants. This approach can ensure blood transfusion safety and would allow a better use of D-negative units and Rh immune globulin without adding much to the costs.

Whether RBCs with D variants are capable of stimulating antibody production when transfused to D-negative recipients has been debated. For more than 60 years, it has been known that transfusion of RBC units with D variants could cause sensitization in D-negative recipients, but the first molecular workup was described in 2000. Sensitization with minor amounts of D antigen was reported previously. The only clinical trial to assess the risk of RBCs with weak D to stimulate anti-D in D-negative recipients reported no antibody production in 49 patients who received transfusions of 68 units that harbored D variants. In our group of patients, transfusion of Rh-negative RBC units that were not tested for weak D caused alloimmunization in 33 (63.5%) of the 52 patients with thalassemia who received multiple transfusions. The rate of alloimmunization in our group of patients was considerably high. Not all D-negative patients can make anti-D when receiving D-positive RBC transfusions. Whether an allo–anti-D is induced depends on the immunologic condition of the transfusion recipient. The immunomodulation role of WBCs might have contributed to the high incidence of anti-D observed in our patients. The vast majority of those patients were exposed to nonleukofiltered blood or bedside filtered blood, which is a suboptimal leukoreduction method. D alloimmunization incidence also increased with the number of units transfused. A high incidence of anti-D has been reported in African Americans and in individuals of mixed ethnic backgrounds because of the common occurrence of D variants. In a previous study conducted to determine the prevalence of RBC alloimmunization in 272 Egyptian patients with β-thalassemia, D alloimmunization was observed in 34.5% of all Rh-negative patients. In this study, 80% of all anti-D developed in patients over 18 years. In a study conducted at another university hospital in Cairo, 19.6% of...
patients with thalassemia were alloimmunized, of whom 13% developed anti-D. A high incidence of anti-D in thalassemia patients was also noted in another Iranian study. The high incidence of anti-D in our group of transfusion-dependent patients with thalassemia underscores the importance of IAT in the D typing of Egyptian donors. Detection of all D-negative donors who harbor clinically relevant D variants, however, is impossible serologically. Polin and colleagues proposed that all apparently D-negative donors should be screened with genotyping methods to avoid missing potentially immunogenic D variants. Routine screening of first-time donors for RHD has been implemented in some European centers.

The rationale for this strategy is to eliminate the risk of D sensitization and hence improve the safety of blood transfusions. The cost benefit of this policy has been debated for years. The application of an affordable, automated, higher, and faster throughput genotyping technology to transfusion practice might help to resolve this contention. It could also improve patient care, especially for those receiving long-term transfusion therapy.

In summary, weak D variants among Egyptians are significantly different compared with whites, but were found to be similar to those seen in black Africans. Population ethnicity must be taken into consideration when developing transfusion and prenatal strategies related to D variants. It is prudent to systematically implement weak D typing in Egyptian donors.

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