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Evaluation of molecular typing and serological methods in solving discrepant results of weak and partial D (Rh) in South Egypt

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Abstract:

INTRODUCTION: Rh discrepancies produced by partial and weak D phenotypes are a problem during routine testing. Some blood units with weak and partial D expression may be missed by serology. Overcoming the limitations of serology can be achieved by molecular typing. Our objective was to evaluate currently used serologic methods with the molecular analysis in solving discrepant results of weak and partial D (Rh) in South Egypt.

PATIENTS AND METHODS: Fifty blood donor and patient samples with undetermined D phenotype were subjected to serology to define their phenotype using identification (ID)-Card "ID-partial RhD typing set" using six monoclonal anti-D panels, followed by molecular typing using polymerase chain reaction sequence-specific primer kit.

RESULTS: Molecular typing confirmed most of the serology results; two samples previously resolved as partial D Type 3 and DFR by serological methods were clarified by molecular techniques – one sample as weak Type 4 and the other sample as weak Type 3. Among the weak D alleles found in our study, Type 4 was the most common, with a frequency of 20%, followed by Type 3 (14%), Type 1 (8%), Type 2 (6%), and finally, Type 5 with a frequency of 3%. The most common types of partial D were partial D Type D5 (14%) and Type D3 (10%).

CONCLUSION: Our study identified D variants (weak D and partial D categories) of the antigen D and determined the frequency and composition of partial D and weak D alleles in our population. Molecular typing also confirmed most of the results obtained from serological methods.

Keywords:

Partial RhD, Rh genotypes, Rh phenotypes, Weak RhD

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Introduction

The most complex blood group system is the Rh blood group system, which composed of 54 antigens numbered RH1 to RH61 with seven numbers obsolete,[1] identifying by investigating the specificity of antibodies produced after blood transfusion or pregnancy.[2]

For clinical purposes, RhD variants are classified into one of three groups: partial D, weak D, and DEL, which are defined as D antigen or proteins. Partial D lacks D antigen epitopes and individuals with these types have the potential to develop alloanti-D, whereas weak D generally presents all D epitopes and does not pose the risk of developing alloanti-D.[3] A definition of serologic weak D phenotype is reactivity of red blood cells (RBCs) with an anti-D reagent giving no or weak reactivity

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in early testing but agglutinating moderately or strong with antihuman globulin.[4]

Serological RhD typing has always been challenging as the D antigen comprised a mosaic of different epitopes, the absence of one or more of these epitopes often results in weak detection using commercial anti-D,[5] making categorization based on their serologic reactivity alone impossible.[6]

Over the last decade, genetic data of the Rh locus has been described, and considerable variations in the RhD gene are known, clarifying discrepant laboratory RhD typing and confusing serologic interpretations.[7]

Using molecular testing methods can be better classified and identified individuals of weak D and partial D phenotypes, molecular typing can help prevent unnecessary Rh immunoglobulin prophylaxis for pregnant women with prevalent weak D Types 1, 2, 3, and 4; Rh-negative blood can be preserved for true Rh-negative persons; and blood donors with discrepant D typing due to weak D or partial D will not be classified as D negative.[3]

The aim of the present study was to find an answer to an important question which is how to approach partial and weak D identification (ID) in diagnostic use and if it is possible to distinguish between partial and weak D using commercially available anti-D reagent for routine use. Our aim also was to recognize the common subtype of weak D in our locality and approve the phenotypes identified by serological test using the molecular technique.

Subjects and Methods

Subjects

This study included a total of 50 samples. Thirty-nine of them were collected from blood donors and 11 were collected from patients recruited from the South Egypt Cancer Institute and Assiut University Hospital from October 2016 to May 2018.

All selecting blood samples had poor reactivity with column agglutination technique ID-Card "DiaClon ABO/D DVI+, + reverse grouping" (Bio-Rad, Cressier FR, Switzerland), and the tube method used in blood bank at the routine phenotyping procedure.

Methods

Sampling: an ethylenediaminetetraacetic acid tube (contains 2 ml) of fresh blood was withdrawn.

- Serologic method: These samples were tested serologically using Diaclon ABO/D (ID- card) (Bio-Rad, cressier FR, Switzerland) then using ID-Card ˝ID-Partial RhD Typing Set˝ with 6 microtubes containing polyspecific antihuman globulin [rabbit anti-IgG and monoclonal anti-C3d (cell line C139-9)], within the gel matrix. (Bio-Rad, cressier FR, Switzerland) where Serological identification of samples done by using six monoclonal anti-D panel.
- Molecular method: DNA was extracted from whole blood samples using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific #K0781).

Amplification of genomic DNA was performed for samples through single-tube method for all six polymerase chain reaction sequence-specific primer (5 weak primers and 1 partial primer) using Thermal Cycler (Creacon, Holand).

Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages when appropriate. The collected data were revised, then well organized, put in table form, and statistically analyzed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) version 23.0 for Windows.

Results

Total fifty subjects were included in the study their ages ranged from 18 to 57 years old with mean age ± SD of (38 ± 19), 34 (68.0%) of them were males and 16 (32.0%) females.

Serologic typing results

Serologic typing showed that 33 samples (66%) were identified as unresolved and 17 samples (34%) were identified as partial D.

Partial D samples were identified as the following: seven samples (14%) as Type V, fi ve samples (10%) as Type III, 3 samples (6%) as partial D Type IVa, and two samples (4%) as partial D type DFR, this is shown in Table 1. Figure 1 shows a case of partial D Type IVa.

Molecular typing results

Molecular typing showed that 27 samples (54%) were weak D variants, while 22 samples (44%) were

Table 1: Classification of the studied cases according to the serological typing

Serological typing	*n* (%)
Partial D (DFR)	2 (4.0)
Partial D Type III	5 (10.0)
Partial D Type IVa	3 (6.0)
Partial D Type V	7 (14.0)
Unresolved	33 (66.0)
Total	50 (100.0)

Data are presented as frequency and percent

unclassified (giving no bands) and 1 sample (2%) was partial D phenotype HMI. Among 27 samples of the weak D variants, 10 samples (20%) were weak D Type 4.0, 7 samples (14%) were weak D Type 3, 4 samples (8%) were weak D Type 1, 3 samples (6%) were weak D Type 2, and 3 samples (6%) were weak D Type 5 [Table 2 and Figure 2]. Figure 3 shows a case of weak D Type 5.

Twenty-two unclassified samples resulted by molecular typing, 7 samples of them were not resolved by either serological or molecular methods, while the remaining 15 samples had already been identified by serological methods. Two samples previously resolved as partial D Type III and DFR by serological methods were clarified by molecular techniques; one sample as weak Type 4 and the other sample as weak Type 3.

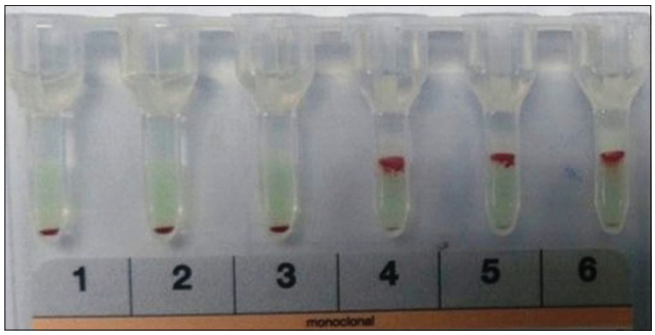

Figure 1: ID-Partial RhD card shows partial D Type IVa with positive reaction with reagent no. 4, 5 and 6 and negative reaction with reagent no. 1, 2 and 3

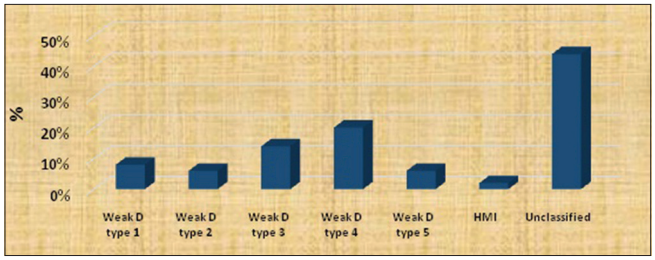

Figure 2: Classification of studied cases according to the molecular typing

Figure 3: The sample referred to as arrowhead shows: weak D Type 5 (size = 112 bp)

The molecular results of 33 unresolved samples which obtained by serological results have shown that 9 samples (27.27%) were weak D Type 4.0, 6 samples (18.18%) were weak D Type 1, 4 samples (12.12%) were weak D Type 1, 3 samples (9.09%) were weak D Type 2, also 3 samples (9.09%) were weak D Type 5, 1 sample (3.03%) was partial D (HMI), and 7 samples (21.21%) were unclassified, this is shown in Table 3.

Discussion

The D antigen is the most immunogenic and clinically important blood group antigen. Many alleles cause both qualitative and even quantitative variations in the expression of the D antigen on RBCs.[8]

There are two main classes of D variants: (1) weak D and (2) partial; these variations are mostly caused by mutations within the RhD gene, weak D class variants express the complete D antigen, but at estimated low quantities, partial D variants only express D antigens with partially complete structures.[5] The incidence of RhD variants differs between races and also depends on the method of determination.[9]

Serologic phenotyping is the standard test to ascribe transfusion strategies. RBCs with D variants may react in a different way depending on the typing method, the affinity of anti-D, and the serologic cutoff.[10]

Table 2: Classification of the studied cases according to the molecular typing

Molecular typing	*n* (%)
Weak D Type 1	4 (8.0)
Weak D Type 2	3 (6.0)
Weak D Type 3	7 (14.0)
Weak D Type 4.0	10 (20.0)
Weak D Type 5	3 (6.0)
Unclassified	22* (44.0)
Total	50 (100.0)

Data are presented as frequency and percent. *22 unclassified included (15 partial types by serological methods and 7 unresolved types by serological methods)

Table 3: Molecular results of 33 unresolved samples by serological typing

Molecular results	*n* (%)
Weak D Type 4.0	9 (27.27)
Weak D Type 3	6 (18.18)
Weak D Type 1	4 (12.12)
Weak D Type 2	3 (9.09)
Weak D Type 5	3 (9.09)
HMI	1 (3.03)
Unclassified	7 (21.21)
Total	33 (100.0)

Data are presented as frequency and percent

The different ethnic populations can also substantially have an effect on the molecular basis of D antigen. A cocktail of races has always been a characteristic of the Egyptian population. Egyptian ethnicity includes an admixture of the native African population, ancient Egyptians, Greeks, Romans, Jews, Persians, those of Arab ancestry, foreign invaders, immigrants, and other Mediterranean populations. Ethnic minorities include Bedouins located in the Eastern and Western desert and the Sinai Peninsula, as well as some Nubians gathered along the Nile in Upper Egypt.[11]

However, it is impossible to distinguish between these phenotypes by serological methods. Only molecular analysis will identify patients with D variants who are at risk for anti-D formation. Serologic phenotyping is the standard test to assign. Molecular study for blood groups was introduced more than 10 years ago as a significant aspect of immunogenetics. Their clinical application since then has been evolving.[12]

Our data showed that in South Egypt, most of the partial D variants by serological typing were partial D Type V (14%) and Type DIII (10%), followed by Type DIVa (6%) and DFR (4%) resembling black individuals.[13]

Egyptian study was done by Hussein and Teruya, 2013, reported that a higher frequency of D variants was characterized as partial D by serological typing. Moreover, they classifi ed partial D Type DIII category (60%) as the most frequent type, followed by Type DV (40%).

This was in agreement with the previous results who reported that the most common partial D in people of African descent is DIII, while the most common partial D in whites is DVI and DVII. The DV has been reported in multiethnic population.[13]

The serologic typing can be unsatisfying, e.g., in patients who have newly been transfused and those harboring large quantity of donor RBC. In all these cases, Rh genotyping is a choice.[14] Serologic detection of polymorphic blood group antigens and phenotypes provides valuable sources of appropriate blood samples for molecular studies.[15]

The serological analysis failed to detect allele D due to multiple causes complicating the determination of the D status. This includes the different monoclonal antibodies in food and drug administration-licensed reagents that can react differently with variant D antigens. In addition, the great number of different RhD genes, which can affect both the level of expression and potentially, the structure of the molecule and D-epitopes.[16] This may explain the unresolved samples which were obtained from the serological methods in this study.

Among the weak D alleles found in our study, Type 4.0 was the most common, with a frequency of 20%, followed by Type 3 (14%) and Type 1 (8%) followed by Type 2 (6%) and Type 5 (3%).

In agreement with our results, Ouchari *et al*., 2011, concluded that weak D Type 4.0 appears to be the most prevalent weak D in Tunisian population occurring with a frequency of 73.91% and the frequency of Type 5 and Type 11 were 21.7 and 2.17%, respectively.

Conversely, another Egyptian study done by Abdelrazik *et al*., 2012, that found weak D Type 4.2 (DAR) was the most prevalent among the Egyptian population constituting 44% of cases, followed by weak D Type 4.0 (22%), weak D Type 2 (10%), weak D Type 1 (4%), and weak D Type 17 (2%) which are the most prevalent elsewhere.

Our study detected 7 (14%) unclassified samples (which not resolved by serological methods or molecular typing), consistent with more than 80 partial D alleles have been described, most found in persons of European or African ancestry and the same as data from Asians, including Chinese populations.[17]

Serological phenotyping is the standard method to assign transfusion strategies, but it is impossible to properly define all samples that show weak reactions in D typing with serotyping alone. Molecular techniques provide a more specific classification of weak D and partial D.[18]

In this study, molecular typing solved most of the results obtained from serologic methods in the form of 26 samples (78.7%) from the total 33 (66%) unresolved samples resulted from serotyping. Also, two samples diagnosed by serological methods as partial D Type III and DFR when tested by molecular typing; they were revealed weak D Type 4 and Type 3, respectively.

It is important to identify the subtype of weak D Type 4. Actually, the management of weak D Type 4 varies depending on the subtype. Pregnant women and recipients of blood transfusions expressing the weak D Type 4.2 variant (DAR phenotype) should be regarded as D negative and require anti-D prophylaxis,[19] so ID of the Rh system is important to avoid the potential risk of erythroblastosis fetalis.[20]

Alloanti-D immunizations have not been observed in weak D Types 1, 2, and 3; therefore, carriers of these alleles may safely be transfused with D-positive blood.[21] A recent workgroup refrained from a recommendation of how to manage weak D Type 4.0 in the US, although a D-positive strategy was recommended in Europe[22] and has been recently adopted for Tunisia.[23]

Conclusion

In our study, serological methods identified D variants (weak and partial D categories) of the antigen D. Molecular typing confirmed most of the results obtained from serological methods and determined the frequency and composition of partial and weak D alleles in our locality.

These data will help us to implement the best alloimmunization anti-D prevention strategy in our locality to improve patient care, particularly for patients receiving long-term transfusion treatment.

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Conflicts of interest
There are no conflicts of interest.

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