Combining serology and molecular typing of weak D role in improving D typing strategy in Egypt

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BACKGROUND: Rh discrepancies are a problemduring routine testing because of partial and weak D phenotypes. Some blood units with weak and partial D expression may escape detection by serology. Limita-tions of serology can be overcome by molecular typing. The objective of study was to compare currently used serologic methods with molecular analysis to determine the potential application of molecular methods to improve D typing strategies and to estimate the fre-quency of weak D types among the Arab population.

STUDY DESIGN AND METHODS: Fifty blood donorand patient samples with discrepant results of D pheno-typing were subjected to routine serology to define the D phenotype including monoclonal anti-D immunoglobu-lin M and indirect antiglobulin test. Commercially avail-able panels of monoclonal anti-D were used for identification of partial D and weak D phenotypes. Genomic DNA was evaluated using allele-specific amplification polymerase chain reaction with sequence-specific primers to define weak D type.

RESULTS: Molecular typing confirmed most of theserology results; three samples that were not clear-cut serologically were identified by molecular typing, two samples as weak D Type 4.2 (DAR), and one sample as weak D Type 4.0. Another two samples identified by serologic panel as weak D were unresolved by molecu-lar typing. A sample with partial D Type II by serology revealed a Weak D Type 4.0 by molecular typing. Results interestingly showed the high frequency of weak D Type 4.2 (DAR) in Egypt.

CONCLUSION: *RHD*molecular typing can solve dis-crepancies during routine testing due to partial and weak D phenotypes for better transfusion outcome.

Blood grouping by serology is superior for routine typing and has made transfusion safe, but the experts in the field are well aware that blood incompatibility remains a significant problem in transfusion medicine and that these problems reflect certain inherent limitations of hemagglutination-based testing.[1-3] These include the weak reactivity of certain clinically significant antibodies, weak expression of some red blood cell (RBC) antigens, the lack of universal test methods for antibody detection and identification, and the subjective nature of the tests performed.[2,3] Addi-tionally, there are a number of issues related to reagents themselves, and these also are expressed as technology limitations and they include, of course, the variability in the reagents, the lack of reagent-grade antibodies, and the different reactivity of monoclonal antibodies (MoAbs) compared to polyclonal antibodies.[3]

The Rh blood group system is the most polymorphic of the human blood groups; more than 50 different Rh antigens have been identified by investigating the speci-ficity of antibodies produced after blood transfusion or pregnancy.[4-6] The ability to clone complementary DNA (cDNA) and sequence genes encoding the Rh proteins has led to an understanding of the molecular bases associated with some of the Rh antigens.[5]

RhD variants are classified for clinical purposes into one of three groups: partial D, weak D, and DEL, which are defined as D antigen or proteins, partial D lack D antigen epitopes, and individuals with these types have the

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potential to develop alloanti-D, whereas weak D generally present all D epitopes and does not pose the risk to develop alloanti-D.[4,7] Some weak D type do not imply that carriers will not be immunized by exposure to normal D through transfusion or pregnancy as weak D Types 4.0, 4.2 (DAR), 11, and 15 and described to be prone to anti-D alloimmu-nization and thus should be considered as partial D. The differentiation and identification of D type is important for selection of blood products and to prevent anti-D–related hemolytic disease of the fetus and newborn.[8]

Rh discrepancies are a problem during routine testing because of partial D or weak D phenotypes.[8,9] Panels of MoAb are being developed to identify D phenotype when there is anomalous D typing results; however, molecular characterization offers a more specific classification of weak and partial D type.[8,9]

Several assays for blood group genotyping of patients and donors have recently been developed to predict the blood group antigen profile of an individual, with the goal of reducing risk or helping in the assessment of the risk of hemolytic disease of the newborn and hemolytic transfu-sion reactions.[10,11] They include polymerase chain reac-tion (PCR)–restriction fragment length polymorphism, allele-specific PCR, sequence-specific PCR as single or multiplex assays, and real-time quantitative PCR.[12,13] The aim of this study was to compare currently used serologic methods with molecular analysis of weak D type to deter-mine the potential application of molecular methods to improve D typing strategies and to investigate the fre-quency of weak D type in Egypt.

MATERIALS AND METHODS

Blood samples

This study included 50 blood samples collected over a period of 18 months (from September 2010 to February 2012) selected from routine D typing tests performed in Fayoum University Hospital Blood Bank with discrepant results of D phenotyping or weak reactivity. Thirty-three samples were collected from blood donors and 17 samples were collected from patients. Initial criterion for selecting blood samples was poor reactivity with different Food and Drug Administration (FDA)-approved anti-D reagents and gel technology used in blood bank at the routine pheno-typing procedure with positive indirect antiglobulin test (IAT).

Serologic analysis

Routine Rh typing was done using the immediate-spin tube technique with FDA-approved anti-D reagent (DiaClon ESD1, DiaMed, Cressier, Switzerland). Nonreac-tive samples were tested with different anti-D blend using two gel matrix techniques (DiaMed and Grifols, Düdin-gen, Switzerland) and tube IAT.

Weak reactive samples and samples showing reactiv-ity in IAT were further tested with a panel of nine MoAbs (D-Screen, Diagast, Loos, France) for identification of partial D in gel matrix technique (DiaMed-ID micro typing system, DiaMed).

Molecular analysis for weak D types

DNA was extracted from whole blood samples using DNA blood mini kit (QIAamp, Qiagen, Hilden, Germany). Amplification of genomic DNA was performed for samples with weak D phenotype with PCR–sequence-specific primers typing kit (BAGene DNA-SSP kit, BAG Health Care, Lich, Germany).

Statistical analysis

Data were statistically described in terms of frequencies (number of cases) and percentages when appropriate. All statistical calculations were done using computer pro-grams (Microsoft Excel 2003, Microsoft Corp., Redmond, WA; and Statistical Package for the Social Sciences, Version 15 for Microsoft Windows, SPSS, Inc., Chicago, IL).

RESULTS

Serologic typing results

Serologic typing showed that 78% (39 of 50) of the samples were weak D phenotype while 16% (eight of 50) were partial D phenotype and the other 6% (three of 50) of the samples were unresolved according to the reactivity pattern with the monoclonal anti-D panel. Serologic results and the frequency of different partial D phenotypes in donor and patient samples are summarized in Table 1.

Molecular typing results

Molecular typing results were 82% (41 of 50) of the samples as weak D type while 18% (nine of 50) gave no amplification product and this could be partial D or weak type not targeted by the kit.

TABLE 1. Serologic typing results in donor and patient samples

Samples	Weak D	Partial D Type VI	Partial D Type IVb	Partial D Type II	Partial D Type DFR	Unresolved	Total
Blood donors	25	4	0	1	1	2	33
Patients	14	1	1	0	0	1	17
Total of samples	39	5	1	1	1	3	50
Percent	78	10	2	2	2	6	

TABLE 2. Molecular typing results in donor and patient samples

Samples	Weak D Type 4.2 (DAR)	Weak D Type 4.0	Weak D Type 2	Weak D Type 1	Weak D Type 17	No amplification product	Total
Blood donors	15	5	5	0	1	7	33
Patients	7	6	0	2	0	2	17
Total of samples	22	11	5	2	1	9	50
Percent	44	22	10	4	2	8	

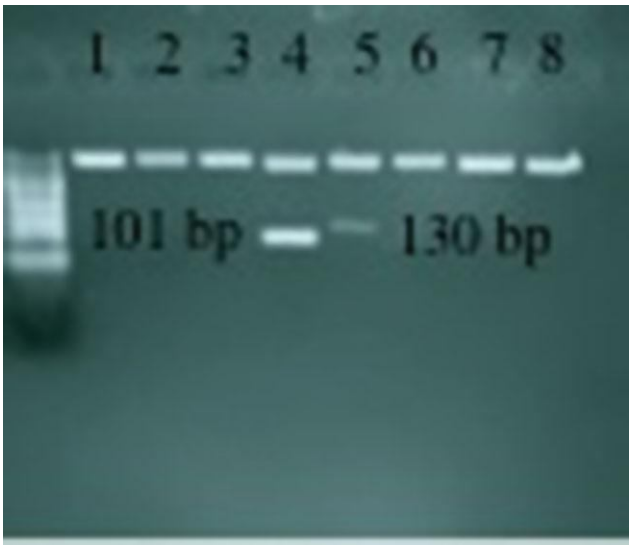

Fig. 1. A case of weak D Type 4.2 (DAR) with two bands one at Lane 4 (size = 101 bp) and the other band at Lane 5 (size = 130 bp).

Molecular results and frequency of different types of weak D in donor and patient samples are summarized in Table 2. Figure 1 shows a case of weak D Type 4.2 (DAR).

Correlation between serologic and molecular typing results

Molecular typing confirmed most of the results obtained from serologic identification. For the three unresolved samples, molecular typing identified two samples as weak D Type 4.2 (DAR) and one sample as weak D Type 4.0. Another two samples identified by serologic panel as weak D were unresolved with the molecular typing. Another sample with Partial D Type II by serology revealed a Weak D Type 4.0 by molecular typing. Table 3 shows number of samples and serology reactivity strengths.

DISCUSSION

The RhD polypeptide is a highly immunogenic protein present on the RBC surface of approximately 85% of Cau-casians, more than 90% of Africans, and nearly 100% of Asians. Some of the more than 200 *RHD* alleles lead to a reduced or variable expression of D antigenic epitopes on the RBC surface.[12] Patients with these aberrant alleles may be mistyped by serology because many of the alleles do not react equally with all anti-D typing reagents.[14-16] FDA-approved reagents and technologies are used in the clini-cal laboratory to ensure the accurate assignment of the D antigen for potential transfusion recipients and pregnant women but discrepancies are observed when laboratories change methods or reagents.[17-19] It is important to resolve these discrepancies to determine appropriate anti-D pro-phylaxis for pregnant women and the Rh status for trans-fusion recipients at risk of making anti-D.[15] In most cases, molecular analyses can be used to identify *RHD* alleles that can be deemed D+.[15,16]

In our study, molecular typing confirmed most of the results obtained from serologic identification. For three samples that were not clear-cut serologically, molecular typing identified two samples as weak D Type 4.2 (DAR) and one sample as weak D Type 4.0. Another two samples identified by serologic panel as weak D phenotypes remained unresolved by molecular typing, because they lacked specific amplification products. Another sample with partial D Type II by serology revealed a weak D Type 4.0 by molecular typing. The difference between the serol-ogy and molecular results gives an indication that the molecular typing is more specific than serologic identifi-cation and the use of molecular typing kit for partial D can be of great importance and more confirmatory.

In this study, weak D Type 4.2 (DAR) was the most prevalent among the Egyptian population constituting 44% of cases, whereas weak D Type 1, 2, and 3, DVII, and DV are the most prevalent elsewhere.[20] The difference in frequency between studies could be attributed to genetic differences between different ethnic groups.[20,21] Addi-tional studies are needed to estimate the frequency of dif-ferent D types among the Arab population. The use of molecular typing techniques for partial D is highly recom-mended for more confirmation and evaluation of results. Currently, we cannot recommend molecular *RHD* typing of all donors and recipients. Based on our results with weak D Type 4.2 (DAR) being the most prevalent and described to be prone to anti-D alloimmunization,[20] D typing strategy would be improved in Egypt with the least possible cost by combining serology and molecular typing for donations for girls and women of childbearing age. Pregnant women and transfusion recipients expressing

TABLE 3. Number of samples and serology reactivity strengths*

Molecular type	Number of samples	Immediate spin tube	Solid phase	IAT	Serologic interpretation (monoclonal typing kit)
Weak D Type 4.2 (DAR) (n = 22)	6	0	2+	3+	Weak D
	5	0	1+	2+	Weak D
	4	1+	1+	3+	Weak D
	3	0	0	3+	Weak D
	2	1+	0	2+	Weak D
	1	0	0	1+	Unresolved
	1	1+	1+	3+	Unresolved
Weak D Type 4.0 (n = 11)	5	0	1+	3+	Weak D
	4	1+	2+	4+	Weak D
	1	1+	1+	2+	Partial D
	1	0	1+	3+	Unresolved
Weak D Type 2 (n = 5)	3	0	0	2+	Weak D
	2	1+	1+	3+	Weak D
Weak D Type 1 (n = 2)	2	0	1+	2+	Weak D
Weak D Type 17 (n = 1)	1	0	0	2+	Weak D
Unclassified (n = 9)	3	2+	2+	3+	Partial D
	3	1+	2+	3+	Partial D
	1	2+	2+	3+	Partial D
	1	1+	1+	2+	Weak D
	1	0	0	2+	Weak D

* Score of agglutination ranges from 0 to 4+ for samples is indicated.

the weak D Type 4.2 (DAR) should be regarded as D– for prenatal and transfusion management.[20]

It should be realized that at present applied genotyp-ing approach is still not replacing serology, but is only an addition to Rh serology.[22] Most, if not all, blood banks will be reluctant to abandon serology. However, when hun-dreds of thousands of donors have been genotyped in the near future, it might become clear that serology can be safely omitted. Moreover, the recognition of *RHD* genes in donors that are falsely typed as D– by serology already shows that *RHD* genotyping of donors is superior to RhD serology.[2,23]

Nowadays, the costs for genotyping assays are exceeding the costs of agglutination assays but many other costs can be saved. For instance, reduction in alloimmunization will largely reduce the numbers of advanced serologic investigations required. An economic analysis is needed to estimate the net effects.[2,24] The geno-typing approach fits perfectly well in the general trend in medicine where medical care will become more and more personalized and genotyping will become part of the diag-nostic work-up of patients to identify their disease suscep-tibility, treatment response, or risk for adverse reactions to certain drugs.[2]

In conclusion, correctly defining all samples that show weak reactions in D phenotyping as weak D or partial D is not possible with serology alone. Molecular genotyping gives us more confidence in what we do regarding confirmation of unusual findings and leads to lower incidence of immunization through better match-ing of donor and patient, lower acute and delayed trans-fusion reactions, and lower requirement for extended reference serology and helps in recognition of weak and partial D recipients that can be immunized by D+ blood and also recognition of weak D type, which cannot make anti-D and can receive D+ blood. Further work is needed to unravel the frequency of different weak D types in the Egyptian population and other Arab nations.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any organiza-tion regarding the material discussed in the manuscript.

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