Case Report

Rhesus-D zygosity and hemolytic disease of fetus and newborn

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Abstract:

Alloimmunization against the Rhesus-D (RhD) antigen still remains as a major cause of hemolytic disease of fetus and newborn (HDFN). Determination of paternal RhDzygosity is performed by molecular testing and is valuable for the management of alloimmunized pregnant women. A 30-year-old pregnant woman with AB negative blood group presented with two consecutive abortions and no history of blood transfusion. By application of the antibody screening, identification panel, and selected cells, she was found to be highly alloimmunized. RhDzygosity was performed on her partner and was shown to be homozygous for RhD. The sequence-specific priming-polymerase chain reaction used in this report is essential to establish whether the mother requires an appropriate immunoprophylaxis or the fetus is at risk of HDFN.

Key words: Rhesus-Dzygosity, hemolytic disease of fetus and newborn, anti-D

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Introduction

Hemolytic disease of fetus and newborn (HDFN) results from sensitization of the mother's immune system to paternal antigens on the red cells of fetus.[1] Despite the use of anti-D immunoglobulin prophylaxis, Rhesus-D (RhD) alloimmunization still remains as a major cause of HDFN.[2] In prenatal practice, the determination of the RhDzygosity in a D+ partner of a D negative woman with anti-D is performed by molecular testing and is of particular clinical interest in estimating the chances of the couple having a Dnegative off spring.[3-5] We report this article because of the accurate and unique clinical application of the sequence specific priming-polymerase chain reaction (SSP-PCR) as a rapid molecular test for the first prediction of zygosity and also the rarity and newness of the assay in Iran.

Case Report

A 30-year-old, AB negative pregnant woman without any blood transfusion record, with a history of two abortions (24th and 35th week) in two consecutive years and injection of Rh immune globulin (RhIG) after each abortion was referred to the Immunohematology Reference Laboratory of the Iranian Blood Transfusion Organization (IBTO) for further investigation. ABO-Rh was done by automated blood group analyzer (BIORAD Hemos SP π Twinsampler, Bio_Rad Laboratories, 3, boulevard Raymond Poincare-B.P.3, 92430 Marnes_la_Coquette_France,www.Bio-Rad.com). The antibody screening test and the antibody identification (ID) panel was performed by a patented and home-made "IBTO antibody Screen and ID panel" kit. By application of antibody screen panel, ID panel, and use of selected red blood cells with specific phenotype, she was found to be highly alloimmunized with RhD antigen of the fetuses from the previous pregnancies, having a titer of anti-D (IgG) equal to 1024. Her partner was also referred to Immunohematology Reference Laboratory of IBTO for further testing. The father had an Rh-positive blood group with a DcE phenotype of red blood cells. This would mean the father could have the possibility of Rh genotype homozygous Rh(D) DcE/DcE (R2R2) or heterozygous DcE/dcE (R2r").[6,7]

Paternal D-zygosity based on the detection of the hybrid Rhesus-box was analyzed by using inno-train SSP-PCR Zygofast kit (Inno-TRΔ in Diagnostic GmbH Niederhochstadter Str.62 D-61476 Kronberg/Tanus Tel. +49[0]6173-607930 Germany,www.inno-train.de).[8] The specimen requirements were 10 ml paternal whole blood collected in ethylene diaminetetraacetic acid. The term "SSP"describes a variant of PCR, in which only the sequence of the primer at the 3' end is responsible for specification of the allele to be identified.The RhD gene is flanked by two highly homologous DNA segments of approximately 9kilobase(kb), the so-called upstream (5' region) and downstream (3' region) Rhesus-boxes.[8] In haplotypes with a deletion of the RhD gene, the fusion of the two Rhesus-boxes generates the single hybrid Rhesus-box that is detected positively through amplification and zygosity testing. Therefore, the concept of a double-box detection is realized by performing 4 reactions: 2 upstream-box and 2 hybrid-box reactions [Figure 1].

If no specific product is present after PCR, the amplificate of the positive control must be clearly detectable. The amplificate of the internal control sequence has a size of 434 base pair (bp) in all reaction

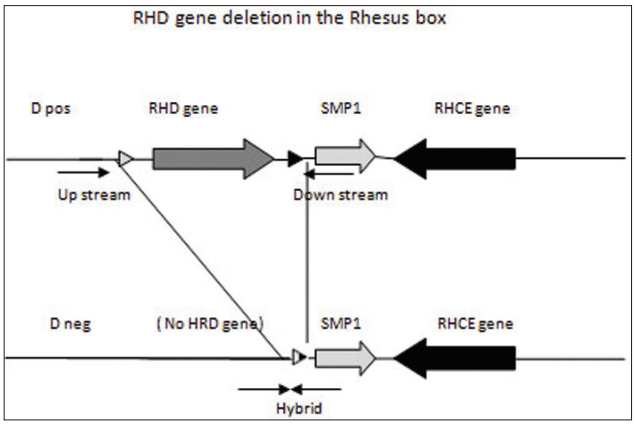

Figure 1: Genomic organization of RhD and RhCEgenes[8]

Reaction no.	1	2	3	4
PCR product	941 bp	1526 bp	937bp	1527bp
Specificity	Hybrid-Box	Hybrid-Box	Upstream-Box	Upstream-Box
dd	+	+	-	-
Dd	+	+	+	+
DD	-	-	+	+
Patent's result	-	-	+	+

The positive sign(+) marks the appearance and the negative sign(-)marks the absence of a DNA fragment.

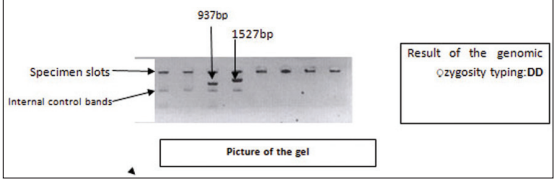

Figure 2: Genomic zygosititytyping

mixes. Evaluation of the result was performed by agarose gel electrophoresis. Then, the bands were visualized by intercalating ethidiumbromide and photographed for documentation. At the end, the zygosity is obtained by entering the pattern of the specific bands in the result protocol. The father's specimen was analyzed for D-zygosity by using inno-train D-zygofast kit. The PCR result showed that among 4 reaction mixes, only PCR products no. 3and 4 (937bp and 1527bp) are present. Thus,the genomic typing of the father was homozygous for RhD [Figure 2].

Discussion

Alloimmunization against the RhD antigen is still the most common cause of HDFN in many developing countries.Its frequency has been reduced by the introduction of anti-D prophylaxis in 1967. Previously, it accounted for death in 1:2200 affected infants, but the prophylaxis has reduced this to 1:21,000 in a Western population.[9] No data are available on the incidence of the disease in Iran.

The maternal patient's history in our case revealed injection of RhIG after each post-delivery cesarean section procedures, but the mother was confirmed to be highly sensitized to anti-D by serologic method.In a country like Iran, assessment of paternal RhDzygosity for the D antigen could prove to be most useful in pregnancies such as the one described.The SSP-PCR assay used in this report is useful in determining the exact genotype of an RhD-positive individual.The assay is >99% sensitive for RhD.It can help to predict the risk that a fetus will inherit RhD. Knowing whether the father is homo-or heterozygous for the D antigen is helpful in counseling parents as to the clinical action to be taken.A fetus of a heterozygous father has a 50% chance of inheriting the paternal alloallele to which the mother may be immunologically sensitized,while the risk is 100% from a homozygous father.[9] Since the current pregnancy also resulted in abortion during the 36th week of gestation and the father was shown to be homozygous for RhD, the physician did not recommend any further pregnancy in the future but suggested getting a surrogate mother could be highly considered. In the case of the heterozygosity, the decision to perform amniocentesis should balance the potential for disease and the risks associated with these invasive procedures.[9] It is to be noted that invasive procedures are associated with an increasing risk of transplacental hemorrhage, which could boost the maternal anti-D, enhancing the risk of severe HDFN.[3,5]

In this respect, further invasive procedures can be avoided by performing free fetal DNA typing in maternal plasma with a high degree of reliability.

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