Usefulness of prenatal detection of RhD typing by molecular analysis in Indians

Sir,

The Rh blood group system is one of the most polymorphic systems known in humans. The Rh locus is on the short arm of chromosome 1p34 - p36 and consists of two closely linked, highly homologous structural genes RHD and RHCE encoding D and CcEe antigens respectively. The prenatal determination of fetal blood group status early in pregnancy imparts considerable benefit to both the mother and the fetus. Prenatal diagnosis of fetal RhD status by polymerase chain reaction (PCR) exploits the structural differences between the RHD and RHCE gene. Here, we have evaluated the usefulness of fetal RhD typing by PCR using chorionic villus (CV) tissue DNA.

Transabdominal CV sampling was done in 15 cases between nine to twelve and a half weeks gestation for prenatal diagnosis of thalassemia after informed consent. The DNA of these family members along with their CV DNA was used for RhD typing by PCR. The following primers viz. A1 (5’TGTGTGTAAACCCACTTC3’), A2 (5’ACATDCCATTTCCG3’), A3 (5’AAACCCACTCACTGCAAA3’), A4 (5’ATGCTGAGATTCTTCCG3’) were used for PCR according to the method of Bennett et al 1993. RhD negative cases show a 136bp fragment, whereas RhD positive cases show two fragments (136 bp and 186 bp).

Analysis of the RhD genotype in chorionic villus tissue DNA samples from 15 fetuses and their families was performed. There was complete agreement on the results of RhD typing of chorionic villus sample with the cord blood tested after delivery by serological methods [Figure 1].

Despite the widespread use of prophylactic anti-D, Rh-alloimmunization continues to occur in pregnancy. Severe alloimmunization could warrant exchange transfusion to prevent bilirubin encephalopathy in newborns. The incidence of Rh-alloimmunization has declined significantly from 5% to 1.7% after the initiating the practice of administration of anti-D immunoglobulin to all-RhD negative women registered in the antenatal clinic. The incidence can be further reduced by use of prophylactic anti-D antenatally. Molecular typing of RhD is not yet routinely done in India. We have demonstrated that the fetal RhD genotype can be reliably and rapidly determined from CV tissue DNA by PCR. This could facilitate decision-making, when the mother is RhD alloimmunized and the father is heterozygous for the D allele. A negative result enables early reassurance for the couple that the baby will be unaffected and that serial intrauterine transfusions will not be required. The knowledge that the fetus is RhD positive allows for undertaking a more cautious approach. Molecular RhD typing can be performed with CV sample or amniotic fluid sample. The results can be obtained within 24h of sample collection in laboratories having DNA-based testing facilities. RhD PCR can also be useful to indicate requirement of antenatal anti-D prophylaxis in RhD negative women.

Kulkarni SS, Gorakshakar AC, Colah RB, Gupte SC, Mohanty D

Red Cell Serology and Hematogenetics, Institute of Immunohaematology, 13th Floor, New Multistoreyed Building, KEM Hospital Campus, Parel, Mumbai - 400 012, India

Correspondence:
Dr. Roshan B. Colah, E-mail: colahrb@icmr.org.in

References