Comparison of RhD status and fetal gender from maternal plasma using one and three exon method

Dep. Obs&Gynaecol., First Faculty of Medicine and General Teaching Hospital, Charles University, Prague, Czechia

Objective
The aim of the study was to perform noninvasive prenatal detection of fetal RhD gene (RHD) from plasma of RhD-negative pregnant women to detect RhD materno-fetal incompatibility together with fetal sex.

Methods
We tested 354 plasma samples of RhD negative mothers (range 10-38 wks). In the first pilot phase (2008-2012) 281 samples were tested for RHD exon 10 only. In the second phase (2012-2014) 73 samples were tested for three RHD exons (5, 7 and 10) together with DNA from buccal swabs of RhD negative mothers. 40 samples of maternal blood (range 7-32 wks) were also tested for fetal gender since 2012. Fetal DNA was isolated from 1ml of maternal plasma by QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany) and fetal status was determined by RT-PCR method.

Results
In the first phase (exon 10 only), 175 RhD+ and 97 RhD- results were detected, 9 plasma samples (3, 3%) were non-informative. 144 samples were postanatally verified (52, 9%) and 5 (3, 47%) false RhD negative (FN) and 4 (2, 77%) false RhD positive (FP) cases were then registered. In the second period (2012 - 2014) 73 samples were tested for three RHD exons (5, 7 and 10) together with DNA from buccal swabs of RhD negative mothers. 44 samples were RhD+, 28 RhD- and 1 sample (1, 37%) non-informative. No FN and FP results were recorded postanatally. 21 cases of 40 gender determination were medically indicated due to gonosomal recessive diseases risk - mostly Haemophilia A (8x) and Duchenne muscular dystrophy (5x). Male gender was determined in 21 tested fetuses. All cases were verified postnatally, no FP neither FN results were found. Six cases of gender determination before 10wks of pregnancy (2x7, 4x9) were proven correct (4x male), but the small number does not allow to draw any conclusions of testing accuracy.

Conclusion
Non-invasive RhD determination with three exons (5, 7, 10) together with DNA from buccal swabs of RhD negative mothers and fetal gender determination from 10th week of pregnancy provides diagnostic accuracy. Supported by the research grant RVO VFN64165.