Usage of Real–time PCR for quantitative analysis of cell–free fetal DNA in maternal blood circulation

Abstract

The presence of fetal DNA in maternal plasma may represent a source of genetic material which can be obtained non-invasively. We analyzed methodological influences and characterized the concentrations of cell-free fetal DNA (cff DNA) circulating in maternal plasma at different gestational ages in physiological pregnancies. These values were compared with ones from pathological pregnancies.

We investigated 238 independent samples from single male-bearing physiological pregnancies of different gestation age and small number of samples from pathological pregnancies. In other 47 pregnancies, the samples were collected three times during pregnancy (at all trimesters) to evaluate the kinetics of cff DNA. DNA extraction methods were compared: the manual (Roche) with manual (Qiagen) and automated (Roche) with manual (Roche). Cff DNA was amplified using Real-time PCR method and Y-specific sequences SRY and DYS14. The amount of total free-cell DNA circulating in maternal plasma was determined by amplifying of the GAPDH sequence.

The elevation in the concentration of cff DNA during pregnancy with the highest value in the third trimester was observed independently on the DNA extraction method and on the Y-specific amplified sequence. There was also documented the increase of the percentage of fetal DNA in total cell-free DNA in maternal plasma during trimesters of pregnancy (8.3, 10.7 and 23.2 % in average).

We discuss methodological problems and describe statistical parameters of cff DNA concentrations in maternal plasma during pregnancy as the basic information for comparison with pregnancies having a pathological outcome.

Key words:

cell–free fetal DNA, maternal plasma, non – invasive prenatal diagnosis, pregnancy, Y chromosome, Real–time PCR

Kľúčové slová:

voľná fetálna DNA, materská plazma, neinvazívna prenatálna diagnostika, tehotenstvo, Y chromozóm, Real–time PCR