Droplet-Based Digital PCR for Non-Invasive Prenatal Genetic Diagnosis for Alpha- and Beta-Thalassemia: a feasibility study

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September 28, 2020

Abstract

Objective The aim of this study is to develop ddPCR based-assay for detecting alpha (0)-thalassemia (SEA) and beta-thalassemia (HbE and 41/42 (-CTTT) from cell-free fetal DNA (cffDNA) extracted form maternal plasma. Design Feasibility study using sample collected from prenatal clinic. Setting Thailand. Population 46 couples who were identified to be carriers of alpha or beta thalassemia. Method Cell-free DNA from 46 singleton pregnant women were isolated and quantified using ddPCR with specially designed probes for each target allele. Allelic copy number (CNV) calculation and likelihood ratio test were used to classify the most likely fetal genotypes. Classification performances were evaluated against ground truth fetal genotypes obtained from conventional amniocentesis. Main outcome measures Concordance with fetal genotyping results from invasive technique. Sensitivity and specificity of ddPCR-based assays. Results CNV analysis of SEA deletion accurately classify fetal genotypes in 20 out of 22 cases with an AUC of 0.98 (95% sensitivity and 91% specificity) for the prediction of Hb Bart's hydrops fetalis. Application of sequential probability ratio tests to detect HbE and 41/42 correctly classified 12 out of 24 cases (10 out of 16 HbE and 2 out of 8 41/42) and provided inconclusive for 7 cases. Conclusion We showed that ddPCR-based analysis of maternal plasma is an accurate and effective NIPD for SEA deletion. Although the performance of ddPCR-based assay on HbE and 41/42 mutations is still not high enough for clinical application, our work should serve as a good foundation for future works in this field.

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