Prenatal diagnosis for beta thalassemia using real-time PCR and high resolution DNA melting analysis from amniotic fluid

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Abstract

Couples at risk for having a child with compound heterozygous hemoglobin (Hb) E / beta thalassemia are prevalent in lower northern Thailand. Homozygous beta thalassemia and compound heterozygous beta$^{0/+}$ / beta$^{0/+}$ thalassemia can be found in one out of 10,000 pregnancies. To report the feasibility and accuracy of real-time PCR with SYTO9 and high resolution DNA melting (HRM) analysis in prenatal diagnosis (PND) for beta thalassemia from amniotic fluid, 50 at-risk pregnancies with their spouses were consecutively recruited to carry on PND with amniotic fluid sampling. Real-time PCR with SYTO9 and HRM analysis was performed in 150 DNA samples from 50 amniotic fluids and 100 blood samples from at-risk couples (50 families) to determine the beta thalassemia mutation. The assays were performed with Bio-Rad CFX 96 using precision melting software. There were 6 HRM protocols designed to cover all documented beta thalassemia mutations. The mutations were identified with known melting curve pattern confirmed by direct DNA sequencing from previous study. The tests were conducted in parallel with amplification refractory mutation system (ARMS) for beta thalassemia mutation and Variable Number Tandem Repeat assay for maternal DNA contamination in amniotic fluid. The beta^{0} thalassemia mutations detected were CD17 (A-T), IVS1nt1 (G-T), IVS1nt5 (G-C), CD41 (-C), CD41/42 (-CTTT), CD71/72 (+A) and 3.4 KB deletion. The beta^{+} thalassemia mutations detected were -31 (A-G), -28 (A-G) and Hb E (CD26; G-A). Each mutation had unique melting curve profile. HRM analysis revealed 12 pregnancies (24%) with compound heterozygous Hb E / beta^{0} thalassemia and 3 (6%) with compound heterozygous Hb E / beta^{+} thalassemia. All samples showed consistent results with ARMS. There were no maternal DNA contaminations detected in both methods. These concordant data, together with cost-effectiveness benefit of this new intervention, worth reforming routine beta thalassemia PND.

Keywords: real-time PCR, high resolution melting analysis, beta thalassemia, prenatal diagnosis