Prenatal diagnosis for Bart's hydrops fetalis using real-time gap-PCR and high resolution DNA melting analysis from amniotic fluid

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Abstract
Couples at risk for having a child with homozygous alpha thalassemia-1 are prevalent in lower northern Thailand. To report the feasibility and accuracy of real-time gap-PCR with SYTO9 and high resolution DNA melting (HRM) analysis in prenatal diagnosis (PND) of alpha thalassemia-1 from amniotic fluid, twelve at risk pregnancies with their spouses were consecutively recruited to carry on PND with amniotic fluid sampling. Real-time gap-PCR with SYTO9 and HRM analysis was performed in 36 DNA samples from 12 amniotic fluids and 24 at risk couples’ bloods (12 families) to determine the alpha thalassemia-1 (Southeast Asian type) and normal allele. The tests were conducted in parallel with conventional gap-PCR followed by gel electrophoresis for alpha thalassemia-1 and VNTR assay for maternal DNA contamination. The dissociation curve analysis of alpha thalassemia-1 and normal alleles showed a peak of Tm at 90.37±0.13°C and 93.78±0.16°C respectively. Heterozygotes gave double peaks of Tm while homozygotes and normal samples resulted in single peak corresponded to their genotypes. All samples showed consistent results with conventional gap-PCR. 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 alpha thalassemia-1 PND.

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