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 72 DNA samples from 24 amniotic fluids and 48 at risk couples’ bloods (24 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 86.75 ºC and 92.02 ºC 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, beta thalassemia, prenatal diagnosis