Multiplex ligation-dependent probe amplification (MLPA): next step in the molecular diagnosis of thalassemias

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OBJECTIVE: Investigating for different deletion/duplication mutation in those suspected to be carrier of alpha and or beta globin gene mutation is needed to identify the underlying genetic cause in more than 70% of alpha-thalassemia and nearly 10% of beta-thalassemia cases. MLPA is a recently developed, simple technique, suitable for rapid gene dosage study allowing more than 40 different specific target sequences to be detected and quantified.

METHODS: Couples referred to Kawsar Genomics Center for PND and remained unknown after investigation for common deletion and point mutation in alpha and beta globin genes using Gap-PCR and direct sequencing, further investigated by MLPA methods using SALSA MLPA Kits P140B2 and P102B1, purchased from MRC Holland (Amsterdam, the Netherlands). After denaturation, hybridization and ligation, PCR-amplification was performed with the specific SALSA primers. Capillary electrophoresis of PCR products performed using ABI-3130 genetic-analyzer. Data Analysis was performed using GeneMrker V1.6.

RESULTS:MLPA study of beta-globin gene cluster revealed a variety of diverse deletion patterns in 19 suspected cases. Deletion pattern was compatible with the Sicilian deletion in 12 (63.15%) which was confirmed with Gap-PCR. Four different deletions were detected in the remaining 7 cases which should be further characterized. Duplication of 4 to 6 probes was detected in 2 cases. Alpha-globin gene study showed 8 different pattern of deletion in 18 cases. Anti3.7 triplication also was found in 8 different cases.

CONCLUSIONS:MLPA can help us to increase accuracy of prenatal diagnosis for alpha and beta-thalassemia especially when we face with cases suspected to have unknown deletion/duplication.

Keywords: thalassemia, MLPA, PND, Iran

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