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Development of a modified Polymerase Chain Reaction - Oligonucleotide ligation assay (PCR-OLA) to detect the commonest mutations IVS1-1 (G to A) and IVS1-5 (G to C) in Sri Lankan beta-thalassemic patients

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Beta-Thalassemia is an autosomal recessive disorder characterized by reduced production or absence of functional beta globin chains. It is mostly caused by point mutations or by small deletions within the β - globin gene. This disease is common in Sri Lanka and its economic and social cost is high due to the patients' life long need for monthly blood transfusions and Iron chelation therapy. There is currently an urgent need for the development of a rapid, reliable and effective population-based presymptomatic screening method for β-thalassemia. Although there are several mutations that cause β -thalassemia, studies have shown that in Sri Lanka, two mutations [IVS1-1 (G to A) and IVS1-5 (G to C)] are predominant and constitute 83% of all beta-thalassemic mutations. A modified form of the Polymerase Chain Reaction-Oligonucleotide ligation assay (PCR-OLA) assay was developed to screen the two predominant beta-thalassemic mutations in Sri Lanka.

Selected population of β -thalassemic patients attending the Anuradhapura General Hospital and Kurunegala Teaching Hospital for blood transfusions was chosen for the study. The hotspot regions of the β-globin gene containing ~98% of known mutations in Sri Lankan β -thalassemic patients were amplified using DNA extracted from the blood samples. Two primers were designed to anneal adjacent to each other on the PCR amplified target DNA. The nucleotide at the 5' end of the downstream primer (mutation specific primer) is positioned at the mutated site. Ligation of the upstream primer with the downstream primer will occur only if the target DNA carries the mutation. After ligation, products were detected by PCR using specific pan handle primer sets that anneal to panhandles in the downstream and the upstream primer. Amplification products of 112bp and 109bp indicated the presence of IVS1-5 (G to C) and IVS1-1 (G to A) mutations, respectively. Samples that were positive (n=19) were also analyzed by single stranded conformation polymorphism (SSCP) which showed different banding profiles when compared to normals. Due to the ability of this method to accurately detect single base mutations; the modified PCR-OLA technique (PCR-OLA-PCR) can be used as a better screening method for presymptomatic diagnosis of β -Thalassemia in Sri Lanka.

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