205/A

Characterization of known and unknown mutations of human beta globin genes of Sri Lankan beta thalassemic patients using allele specific priming and Single Stranded Conformational Polymorphism techniques

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Beta thalassemia is a highly heterogeneous disorder resulting from more than 200 beta globin gene mutations, which are population based. It is characterized by reduced synthesis of the hemoglobin beta chains that leads to microcystic/hypochromic anemia, an abnormal peripheral blood smear with nucleated red blood cells and reduced amount of hemoglobin A (Hb A). Beta thalassemia is a severe health problem in Sri Lanka as treatment and management of such patients is a major drain on the annual health budget. Mutation analysis of the gene encoding beta globin is useful for prediction of the clinical phenotype. There is currently an urgent need for the development of a rapid, reliable and effective population based screening method. In this study, a selected population of beta-thalassemic patients attending the Anuradhapura and Ratnapura General Hospitals for blood transfusions were selected to characterize the mutations. A panel of allele specific primers based on known mutations (IVS1-1, IVS 1-5, CODONS 8/9, 41/42, 16 and 15) in beta-thalassemic patients from Sri Lanka were designed and tailored to investigate the presence of the above mutations. Single Stranded Conformational Polymorphism (SSCP) technique that detects both known and unknown mutations was developed subsequently. Hotspot regions of the beta globing genes (481bp), that contains 98% of known mutation in beta thalassemic patients (n=30) were amplified. To make them amenable to SSCP, amplified products of hotspot regions were split into two overlapping segments of 238bp and 268bp. SSCP analyses of these overlapping segments showed additional banding patterns compared to normal individuals. Off these, 6 DNA samples were sequenced and revealed the presence of mutations. The SSCP protocol developed in this study is a good method to detect the presence of mutations in the hot spot region of the beta globin gene and in combination with allele specific PCR, is an ideal method for presymptomatic diagnosis.

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