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Deletion analysis of the *RB1* gene in retinoblastoma patients in Sri Lanka

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Retinoblastoma (RB), a tumour, affecting children aged less than 5 years has a prevalence of 1 in 20,000 with twenty cases/ year predicted in Sri Lanka. Unilateral RB (60%) presents on average at 24 months and bilateral RB (40%) presents around 15 months. A family history is reported in 10%. Many cases, diagnosed late, require enucleation. Genetic testing has not been available locally, but may enable better targeting of screening for patients and their siblings and reduce the need for enucleation in affected cases. Mutations of *RB1* gene lead to inactivation of pRB (retinoblastoma protein) and loss of its function. Different mutations of *RB1* gene include nonsense (37%), frameshift (20%), splice site (21%), missense (5%), deletions/ duplications of one or several exons or even the entire gene (15%) and mutations in the promoter region (1%). Hypermethylation of the promoter region is also found in the tumours of some retinoblastoma cases. About 5-15% of retinoblastoma patients have microscopic or submicroscopic deletions, which includes the entire or substantial parts (one or several exons) of the *RB1* gene. The objective of this study was to identify the presence of germline copy number variations of the *RB1* gene or any of its exons in retinoblastoma patients. Primers were designed for the 27 exons and promoter region of the target gene (*RB1*) and a control gene (Cystic fibrosis transmembrane conductance regulator - *CFTR*) to compare the copy number of both genes using gene ratio analysis copy enumeration PCR (GRACE-PCR). The peak height of the melting curve was analysed for calculation of the ratio. The ratio of the peak height of the melting curve of the target (*RB1*) to the control gene (*CFTR)* was calculated for patients and normal individuals separately. A ratio of patient to normal of 1 indicates the patient is deletion negative. A ratio of 0.5 indicates a deletion and a ratio of 1.5 indicates a duplication. Seven exons (exons 3, 4, 5, 6, 8, 9 and 10) of 32 patients and 3 more exons (exons 11, 12 and 16) of 16 patients who have no germline mutation identified from targeted amplicon sequencing, were tested. One case had a deletion for all 10 exons, while one case each had deletions of exon 11 and exon 12. In conclusion, three RB cases out of 32 patients (9%) have a deletion of one or several exons which is similar to world wide data and further testing is ongoing. Genetic testing helps to determine the recurrence risk and to target intensive screening to at risk family members. This can contribute to balance the resource limited healthcare services of developing countries.

Key words: Germline, Mutation, Large deletion, *RB1*, Retinoblastoma

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