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## Comparative analysis of serologic and molecular methods for High Risk-HPV 16 detection in oral and oropharyngeal cancers in Sri Lanka

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Of the high risk human papilloma viral (HR-HPV) infections associated with oral cancer (OC) and oropharyngeal cancer (OPC), HR-HPV type 16 is the major cause of two thirds of all invasive OC/OPCs. Thus, detection of HPV16 infection in OC and OPC is critical. Blood and oral rinse samples of newly diagnosed, untreated OC (n = 146; C00-C06, C09) and OPC (n=104; C10) patients and age/gender matched non-cancer healthy controls (n = 250) were collected. Additionally, formalin-fixed paraffin embedded (FFPE) tumour tissue of OC and OPC patients and cancer suspected, negative individuals were used as controls. Anti-HPV 16 specific serum IgG antibody responses against HPV 16 virus like particles were assayed by in-house developed indirect ELISA. DNA was extracted from exfoliated oral cells and formalin fixed paraffin embedded (FFPE) tumour tissue samples and amplified by standard PCRs that targeted the conserved L1 open reading frame and E7 oncogene of HPV. NCBI BLAST service was used to analyse HPV L1 DNA sequences. Odds ratios (ORs) at 95% confidence interval were calculated using SPSS version 20.0. Of the OC/OPC patients tested, 24 (9.6%) were positive for HPV16 L1 PCR (OC; n = 11; 7.53% and OPC; n = 13; 12.5%) and 19 (7.6%) for HPV16 E7 (OC; n = 8; 5.48% and OPC; n = 11; 10.58%) while the control group screened negative. Yet, of the E7 HPV positive OC/OPC samples, all but one (n = 18) screened positive for L1 PCR. The 5 samples exclusively positive for L1 PCR may be due to the HPV16 E6 oncogene. Seropositivity for HPV16 by L1 ELISA was detected in 22 (8.8%) of the OC/OPC group (OC; n=9; 6.16% and OPC; n=13; 12.5%) and 2 (0.8%) of the control group. Of HR-HPV 16 positive OC/OPC, 91.6% scored positive in both ELISA and PCR while 8.3% were only positive in PCR. Hence, an acceptable agreement between serological and molecular assays for HR-HPV 16 detection was observed. The test group was more likely to have had exposure to HR-HPV 16 infection with a 3-fold and 5-fold increased risk of developing OC/OPC as detected by ELISA and PCR, respectively (ELISA; OR = 3.3; 95% CI = 1.231-17.352, L1PCR; OR = 5.1; 95% CI = 1.432-18.413 and E7PCR; OR = 4.8; 95% CI = 1.102-16.142). This study provides the first synonymous serologic and molecular evidence of HR HPV16 association with oral and oropharyngeal cancers in Sri Lanka.

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