Development of an ELISA method for the detection of HPV 16 in oral cancer patients

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Content:

Introduction: HPV infection has been associated with a subset of head and neck cancers and current evidence suggest that it may be an important risk factor for oral cancer. Using polymerase chain reaction (PCR) and sequencing, we recently demonstrated the presence of HPV in more than 50% of oral squamous cell carcinoma (OSCC) patients and that high-risk HPV is significantly associated with OSCC. Serological detection of HPV has been reported the most convenient method for detecting HPV. However, currently there is a lack of serological assays for the detection of the HPV. The HPV E6 viral oncoprotein is known to play crucial role in tumorigenesis, therefore detecting the presence of the E6 protein could be a useful biomarker for HPV detection.**Methods**: A pGEX plasmid containing HPV 16 E6 gene was ligated with KT3 oligonucleotide. Constructed plasmid was then transformed into Escherichia coli for production of the recombinant protein which was used as antigen in ELISA assay. ELISA was optimized using anti KT3 antibody to detect the recombinant antigen. HPV ELISA was performed on sera from 18 healthy and 15 OSCC patients obtained from the Malaysian Oral Cancer Database & Tissue Bank System (MOCDTBS) which is coordinated by Oral Cancer Research & Coordinating Centre (OCRCC). Sera that have net OD above calculated cutoff value were determined as HPV seropositive. Fisher's Exact test was used for statistical evaluation.**Results**: An ELISA method to detect the presence of HPV16 E6 protein was successfully developed. Using this method, 33.3% (5/15) of OSCC and 16.7% (3/18) of healthy patients were found to be HPV 16 seropositive. No significant association was found between HPV 16 seropositivity and OSCC occurrence (P value = 0.428).**Discussion**: Although there is a trend to support our previous findings using PCR where a larger proportion of OSCC patients were HPV positive in comparison to healthy individuals, our results using ELISA method did not show any statistical significance. This remains to be tested in a larger sample set to confirm pur preliminary result that could more representative of our patient population.

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