Human Papillomavirus Detection in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

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Abstract

Human papillomavirus is associated with a subset of head and neck cancer (HNCC) which 90 % of it is Oral Squamous Cell Carcinoma (OSCC). The purpose of the research is to detect human papillomavirus (HPV) in OPMDs and OSCC using immunohistochemistry (IHC).

A total of 118 formalin-fixed paraffin embedded tissues were selected comprising of normal oral mucosa (NOM) (n=9), oral epithelial hyperplastic tissue (OEH) (n=31), oral potentially malignant disorders (OPMDs) cases (n=32) and OSCC cases (n=46). The cases were retrieved from 3 different places which are Oral Pathology Laboratory, Faculty of Dentistry, National University of Malaysia; Institute of Medical Research, Kuala Lumpur and Tuanku Mizan Hospital, Kuala Lumpur. The IHC staining was manually performed using HPV antibody (Clone K1H8, Dako) following manufacturer's instruction and assessed semi-quantitatively (positivity and staining intensity) between all groups. Positive and negative controls were used to validate the IHC run.

All data were then analysed using SPSS version 23.0 and p values <0.05 were considered significant. HPV positive was found higher in OSCC (34.78%) compared with OEH (3.22%) and OPMDs (3.12%). In this present study, HPV were increased following degree of malignancy.

The finding suggests that HPV plays important role in OSCC.

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Introduction

The incidence of oral cancer remains low in UK and USA, approximately only about 2% of the population. In contrast, the incidence of oral cancer is higher in some developing countries such as India and Sri Lanka, with approximately 40% of the total number of cancer cases there.[1] Oral squamous cell carcinoma (OSCC) is a malignant tumour of the squamous epithelium which is the most common primary malignancy of the oral cavity.[2]

One of the viral aetiologic factors of oral cancer is human papillomavirus (HPV). Human papillomavirus has characteristic small, non-enveloped, DNA viruses and member of the papillomaviridae family found integrated into the host genome, non-integrated or a combination of it in infected tissue.[3] HPV DNA was detected more frequently in younger individuals (≤ 53 years old) with range 94.3% were positive for HPV 16.[4] According to Umudum et al. (2005)[5], HPV plays an important role not only in tumor process initiation but also in metastatic disease development. The purpose of this research is to detect the expression of human papillomavirus in OPMDs and OSCC using immunohistochemistry method.

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Materials and methods

A total of 118 formalin-fixed paraffin embedded tissues (FFPET) was selected retrospectively comprising of test group OSCC cases (n=46), OPMDs (n=32), OEH (n=31) together with control group NOM (n=9) from Oral Pathology Laboratory, National University of Malaysia, Institute Medical Research, Kuala

Lumpur and Tuanku Mizan Hospital, Malaysia. Insufficient tissues and samples with no definite diagnosis of OSCC and OPMDs will be excluded from the research.

Inclusion criteria are samples come from new cases and the patients never undergo with any anticancer treatment before. FFPET specimens were then processed in the oral pathology laboratory, National University of Malaysia for immunohistochemistry staining. An ethical approval was brought from PPUKM Medical Research and Ethics Committee, National University of Malaysia with approval number UKM 1.5.3.5/244/DD/2015/013(2).

Immunohistochemistry

4 µm FFPET tissue cut by microtome were placed on glass slides and were then deparaffinised in the oven at 60°C for 30-60 minutes in the oven and 20 minutes in waterbath at 95°C.Slides were incubated with peroxidase blocking agent for 5 minutes and were rinsed with PBS (Phosphate Buffer Saline).

Slides were incubated with the antibody overnight at 4°C for HPV. Slides were rinsed with PBS and were incubated with secondary antibody (HRP Envision, Dako) for 30 minutes. Slides were then incubated with DAB for 5 minutes. Slides were counterstained with hematoxylin eosin for 1 minute and then were rehydrated in alcohol and immersed in xylene. The mounting agent was put on slides (DPX, Merck, Germany) and a cover glass was put on top of the slide. Negative control was stained with omitting primary antibody. Cervical cancer with HPV positive was used for HPV positive control.

Semi-quantitative analysis

Semi-quantitative analysis was done referring to Sailan (2010)[6], Abrahaoet al.[7] and Tarakji et al. (2010)[8]. Positive IHC expression for HPV antigen will be identified by positive brown staining inside the nucleus (internuclear) or next to nucleus (para nuclear) in epithelial cells which are called koilocytes.

The percentage of positive cells were assessed by 2 independent inter-observer and scored as 0= no expression, 1= weak staining, 0-25% or total cells show positive staining, 2= moderate staining, 25-75% of the cells show positive staining, 3= strong staining, >75-100% cells show positive staining. To asses inter-observer reliability, Cohen's Kappa was settled and reflected a high level of inter-observer agreement (K=0.83).

The results are expressed in both number of positive cases and percentage of positive cells after cell counting using Image J software using the picture taken under the microscope with 400x magnification (Olympus) connected with the camera (Q Imaging, Q30795, Canada).

Statistical analysis

All data were coded and analysed by using SPSS for windows version 23.0 with 95% confidence interval (p<0.05). The Kolmogorov-Smirnov analysis was used to test the normality of the data. Kruskal-Wallis test was used to compare positive expression of HPV between all groups.

The post-hoc test was applied by using Mann-Whitney test in order to find the exact result between each group. Pearson Chi-square was used to compare HPV staining intensity between all groups.

Results

Figure 1 shows HPV positivity in NOM, OEH, OPMDs and OSCC using immunohistochemistry method in 400x magnification. HPV positive was found highest in OSCC (34.78%) compared with OEH (3.22%) and OPMDs (3.12%). In NOM, HPV positive were found in 2 cases over 9 archived cases (28.57%). Weak staining intensity was most common intensity found in all groups.

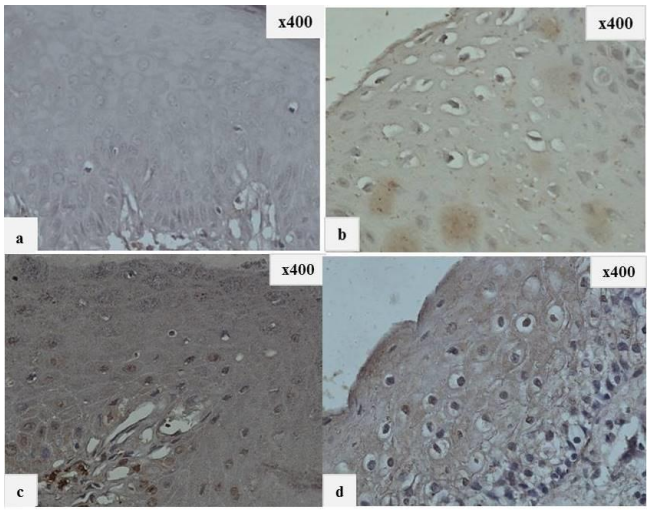

Figure 1. (a) HPV in NOM (b) HPV in OEH (c) HPV in OPMDs (d) HPV in OSCC.

There is a significant difference between HPV positivity with all groups. There are significant differences between NOM and OSCC

(p<0.05), OPMDs and OSCC (p<0.005) and between OEH and OSCC (p<0.005). No significant differences between NOM and OEH neither between NOM and OPMDs (p>0.05) as shown in Table 1.

Tissue groups	HPV positivity (%)	p value	HPV staining intensity				Total	p value
			Negative	Weak	Moderate	Strong		
NOM	28.57%	<0.001	9	0	0	0	9	<0.01
OEH	3.22%		30	1	0	0	31	
OPMDs	3.12%		30	1	0	0	31	
OSCC	34.78%		29	10	3	3	45	

Table 1. HPV expression in each tissue groups.

There are no significant differences between HPV positivity and tumour site, neither between HPV positivity and malignancy grading in OSCC (p>0.05) as shown in Table 2.

Variables	Classification	HPV		p value
		Negative	Positive	
Tumour site	Buccal mucosa	10	5	
	Lateral of tongue	14	5	
	Tip of the tongue	0	1	p= 0.522
	Alveolar ridge	4	2	
	Lip	1	1	
	Floor of the mouth	1	0	
	Palatal	0	1	
Broder's grading	Well-differentiated OSCC	17	6	p = 0.155
	Moderately differentiated OSCC	10	9	
	Poorly differentiated OSCC	3	0	

Table 2. Comparison between HPV expression and histopathological variables in OSCC.

Discussion

In the current study, HPV positive was detected in normal oral mucosa (28.57%) that lies in agreement with previous studies[3,9,10].

HPV is possible to be found in normal tissues without any symptom in apatient due to latent period of the virus. These findings disagree with those reported by Bouda&Gorgoulis (2000)[11] and Saghravanian et al. (2011)[12] that found HPV did not appear in normal oral mucosa. The percentage of HPV presence in normal oral mucosa seemed to be higher than OPMDs group due to the insufficient number of archived normal oral mucosa samples compared to other groups.

The study revealed that HPV was detected also in normal oral mucosa by immunohistochemistry method. This evidence contradicts with Blioumi et al. (2014) that stated only PCR method which was able to detect HPV in normal oral mucosa due to the very low concentration of HPV or the latent type of HPV in normal oral mucosa. Here, the presence of HPV was able to be detected by using immunohistochemistry method, although still unable to identify the specific type of HPV.

Our study revealed that 3.22% of OEH and 3.12% of OPMDs cases are positive for HPV, which is very low compare to OSCC (34.78%). Our finding is quite similar with Agrawal et al. (2013)[13] that found 10% of oral epithelial dysplasia were positive for HPV and 35% of OSCC were positive for HPV. These two findings show that HPV is an important causal for OSCC, but not in OPMDs and OEH. Mattila et al. (2012)[14] reported 15.7% of oral atrophic lichen planus were positive for HPV while Stokes et al. (2012)[15] reported 38.46% of HPV positive in oral dysplasia which both are higher than our finding. This might happened due to different methods of detecting HPV presence.

Human papillomavirus is associated with a subset of head and neck cancer (HNCC) which 90% of it is Oral Squamous Cell Carcinoma.[16]The low prevalence of HPV in OPMDs (3.12%) and OEH (3.22%) in our study consintents with previous studies [12,17,18] that found 0% of HPV presence in OPMDs lesions. These findings suggest that HPV may not play important role in OEH and OPMDs lesions. Histopathologic parameter of tumour site and malignancy grading are not associated with HPV presence and HPV staining intensity in OSCC.

Conclusions

The high prevalence of HPV (34.78%) in OSCC concludes that HPV is one of the important aetiological factors for OSCC, but not in OPMDs. HPV may play role in oral tumour progression. Immunohistochemistry method can be used to detect the presence of HPV not only in OEH, OPMD and OSCC tissues but also in normal oral mucosa.

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Declaration of Interest

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