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Original Article

Factors associated with mortality of oral squamous cell carcinoma (OSCC) patients managed at Hospital USM

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Abstract This study was to determine the sociodemographic and clinicopathological factors that were associated with mortality of OSCC patients managed at Hospital USM. The prevalence of high-risk human papillomavirus (HPV) in these patients and its association with epithelial proliferation rate were also determined. A retrospective study was conducted whereby medical records of patients diagnosed with OSCC and tissue specimens from 2005 to 2015 were studied. Formalin-fixed paraffin-embedded (FFPE) tissue specimens were evaluated for histological grading of OSCC, p16 overexpression and Ki-67 immunostaining. Descriptive statistics, simple and multiple logistics regressions were used for data analysis. Prognostic factors for mortality includes male gender (AOR=10.89; 95% CI: 1.99, 59.65; $p = 0.006$), alcohol consumption (AOR = 16.45; 95% CI: 1.36, 59.65; $p = 0.028$), not receiving treatment (AOR = 5.88; 95% CI: 1.03, 33.61; $p = 0.046$) and late stage (T3, T4) at presentation (AOR = 4.85; 95% CI: 1.12, 21.02; $p = 0.035$). Significant association was found between high-risk HPV positivity and higher epithelial proliferation rate expression ($p < 0.003$) in the OSCC tissue specimens.

Keywords: Human papillomavirus; mortality; oral squamous cell carcinoma.

Introduction

Squamous cell carcinoma of the oral cavity is the sixth most frequent malignant tumour (Ajila *et al*., 2015) and is a fatal disease with up to 50% of mortality rate (Mehrotra and Yadav, 2006). The carcinomatous changes involving the oral mucosa are due to a multifactorial aetiology, which includes smoking, tobacco use, alcohol consumption, *paan*, betel quid, viral stimuli, and some genetic and epigenetic changes (Chaturvedi, 2012; Vargas-Ferreira *et al*., 2012; Ali *et al*., 2017). It has been established in recent studies that high-risk human papillomavirus (HPV) has a causative role of oral squamous cell carcinoma (OSCC) (Vargas-Ferreira *et al*., 2012; Kerishnan *et al*., 2016; Jiang and Dong, 2017).

For more than 10 years, Hospital USM has served as the main oncologic centre for the referral and management of OSCC patient mostly from the east-coast region of Malaysia. There is limited data available on the prevalence and survival status of OSCC patients managed in this hospital. The main objective of this study was to determine the sociodemographic and clinicopathological factors that were associated with mortality of OSCC patients from 2005 to 2015. The prevalence of high-risk human papillomavirus (HPV) in these patients and its association with epithelial proliferation rate were also determined.

Materials and methods

The design of the study was retrospective and had been approved by Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/16050184). Sample size calculation was done using PS software version 3.0.10 (Dupont and Plummer, 1997).

The inclusion criteria were patients who were diagnosed with OSCC in the oral

cavity at Hospital USM during the time period from 2005 to 2015, availability of their clinicopathological data in medical records and availability of primary tumour specimen for p16 and Ki-67 immunohistochemical staining. The exclusion criteria were recurrent cases of OSCC, tumours arising from tonsillar and posterior pharynx, other histopathological types of head and neck malignancy arising from salivary glands or odontogenic origin, missing data or not enough tissue for immunohistochemistry.

All formalin-fixed paraffin-embedded (FFPE) specimens were evaluated by haematoxylin and eosin (H&E) staining to confirm OSCC. A total of 57 OSCC cases from 2005 to 2015 were included and 41 FFPE specimens were tested for presence of high-risk HPV and epithelial proliferation rate analyses by p16 and Ki-67 immunohistochemistry (IHC).

Sociodemographic and clinicopathological factors

Sociodemographic data were retrieved through Medical Record Department of Hospital USM, which included age, gender, ethnicity, smoking habit, alcohol consumption, betel quid use and family history of cancer. Clinical data comprised of anatomical site, size of a tumour, treatment received, the duration of diagnosis and treatment and patient's survival status. Classification of TNM and staging group at the time of diagnosis (according to WHO) data were interpreted based on computed tomography (CT) scan reports. Histopathological data included OSCC histological grading, surgical margin involvement, presence of bone involvement, lympho-vascular and perineural invasion.

p16 immunohistochemistry

IHC staining was done using p16 CINtec Histology Kit (Code No. 9517; Ventana Medical System Inc., AZ, USA) according to the manufacturer's protocol. For positive control, tissue known for high-risk HPV positivity was used. For negative control, no application of the primary antibody on the tissue specimen was done. Two oral pathologists independently reviewed all the p16 IHC slides and found agreement as to consider p16 positivity only if there were clear nuclei and cytoplasmic staining in more than 70% of tumour cells (Jordan *et al.*, 2012; Bhosale *et al*., 2016) . IHC results were scored based on both staining intensity (weak = 1, moderate = 2 and strong = 3) and percentage of positive cells (0-100%). Inter-rater reliability for two pathologists rating was determined by Kappa score.

Ki-67 immunohistochemistry

Ki-67 staining was done using Ki-67 antigen (clone MIB-1, code: IR62661, Dako, USA) according to the manufacturer's protocol. For positive control, normal tonsillar tissue was used. For negative control, no application of the primary antibody on the tissue specimen was done. Manual cell counting of stained nuclei was done using 6 x 6 grid on slide images. The percentage of positive cells was determined by a total of positive nuclei cells divided by total cell number and multiplied by 100 (Bologna-Molina *et al*., 2011). More than 50% of positive cells were considered Ki-67 positive while less than 50% of positive cells were considered negative for Ki-67 (Hwa *et al*., 2015; Liu *et al*., 2015).

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS, IBM, and Chicago, USA version 24). Prognostic factors of mortality for OSCC patient was analysed by using simple and multiple logistic regression. Descriptive data were analyzed using frequency and proportion parameters for the prevalence of high-risk HPV. Inter-rater reliability for two pathologists rating was determined by Kappa score. Chi-square test was used to obtain the epithelial proliferation rate difference between HPV positive and non-HPV OSCC.

Results

There were 123 cases of OSCC notified and registered in Hospital USM, Kelantan during 2005 to 2015. Of these, only 57 cases completed the criteria, which were included in this study. Sixty-six cases were excluded from this study, as these were cases of recurrences of a primary tumour and incomplete records. The mean age at the time of diagnosis was 62.50 (14.07) years. Majority of them were of Malay ethinicity (84.2%). There were nearly equal gender distribution with male and female, 52.6% and

47.4%, respectively (Table 1). Age, ethnicity, family history of cancer, smoking and betel quid habits were not significant contributors to mortality in OSCC patients managed at Hospital USM (p>0.05) (Table 2).

Table 1 Sociodemographic features of patients with OSCC (n = 57)

Variables	n (%)
Age (years)	62.50 (14.07)*
Gender	
Male	30 (52.6)
Female	27 (47.4)
Ethnicity	
Malay	48 (84.2)
Indian	1 (1.8)
Chinese	6 (10.5)
Others	2 (3.5)
Smoking	
Chronic smoker	7 (12.3)
Ex-smoker	24 (42.1)
Non-smoker	26 (45.6)
Betel quid	
Chronic user	4 (7.0)
Ex-user	12 (21.1)
Non-user	41 (71.9)
Alcohol	
Chronic user	1 (1.8)
Ex-user	4 (17.0)
Ocassional user	1 (1.8)
Non-user	51 (89.5)

**Mean (SD)*

Multivariable analysis showed that male patients with OSCC had higher risk i.e. 10 times odds of dying compared to female. (AOR = 10.89; 95% CI: 1.99, 59.65; p = 0.006) (Table 3). Concerning the risk factors of OSCC, 54.4% of the patients were smokers (either active or previous history of exposure) and only 28.1% practiced or previously had a habit of betel quid chewing. Majority of the patient (89.5%) did not have history of alcohol consumption (Table 1). Nevertheless, history of alcohol consumption was found to be statistically significant. Patients with history of alcohol consumption had 16 times odds of dying compared with non-alcoholic patients. (AOR = 16.45; 95% CI: 1.36, 59.65; p = 0.028) (Table 3). However, this finding did not consider the possibility of existing cofounding factors i.e. smoking status of the involved patients.

The most common site of OSCC was tongue (45.6%) followed by buccal mucosa (26.3%). Majority of the cases (68.4%) were at stage 4 at the time of diagnosis and 70.2% of cases histologically presented as well differentiated OSCC (Table 4). Based on the analysis, the stage of tumour was statistically significant with those who were in late stage (T3, T4) had 5 times odds of dying as compared to those who were in early stage (T1, T2) (AOR = 4.85; 95% CI: 1.12, 21.02; p = 0.035) (Table 3). Histological grading of well differentiated OSCC was statistically significant as those patients who were not having well differentiated OSCC i.e. moderately or poorly differentiated histological grading, had 8 times odds of dying (AOR = 8.00; 95% CI: 1.35, 47.37; p = 0.022) (Table 3).

Out of 42 (73.7%) patients who had undergone treatment, 25 (59.5%) of them were still alive at the time of the study as compared to only 4 (26.7%) of patients who did not undergo treatment. Majority of patient who did not undergo treatment (73.3%) had already died. Management for these patients mostly involved surgical intervention (43.9%) with 19.3% were a combination of surgery and radiation therapy followed by surgery alone (12.3%). Only a handful (8.8%) of patients received triple treatment modalities, which included a combination of surgery, radiation and chemotherapy (Table 4). The variable of treatment received, or no treatment was significant. The result showed that those who did not receive treatment had almost 6 times odds of dying as compared to those who had received treatment (AOR = 5.88; 95% CI: 1.03, 33.61; p = 0.046) (Table 3).

Several important prognosticators in OSCC includes surgical margin clearance and involvement of bone, lympho-vascular and perineural invasion. Most of the alive patients in this study demonstrated clear surgical margins (75%) and no involvement of bone (68.4%), lympho-vascular (68.4%) or perineural invasion (66.7%) (Table 5).

Other less common but significant prognosticators in management of OSCC is the presence of high-risk HPV. In this study, a total of 9.8% (n = 4) cases were found to be HPV positive, whereas 90.2% (n = 37) were HPV negative. Inter-rater reliability (Kappa score) for two pathologists rating was

0.84. There was no correlation found between HPV positivity and sociodemographic factors of OSCC patients. However, there was a significant association between HPV positivity and higher epithelial proliferation rate expression (Ki-67) as compared to non-HPV OSCC (p< 0.003) (Table 6).

Table 2 Factors associated with mortality of OSCC patients using simple logistic regression (n = 57)

Variables	Alive, n(%)	Died, n(%)	Crude OR (95% CI)	*p*-value
Age (years)			0.10 (0.96,1.03)	0.851
Gender				
Female	17 (63.0)	10 (37.0)	1	0.086
Male	12 (40.0)	18 (60.0)	2.55 (0.76,7.43)	
Ethnicity				
Malay	24 (50.0)	24 (50.0)	1	0.760
Non-Malay	5 (55.6)	4 (44.4)	0.80 (0.19,3.34)	
Smoking				
Yes	13 (41.9)	18 (58.1)	1	0.143
No	16 (61.5)	10 (38.5)	0.45 (0.16,1.31)	
Family history				
No	19 (51.4)	18 (48.6)	1	0.922
Yes	10 (50.0)	10 (50.0)	1.06 (0.36,3.13)	
Betel quid				
User	10 (62.5)	6 (7.9)	1	0.276
Non-user	19 (20.9)	22 (53.7)	1.93 (0.59,6.30)	
Alcohol use				
User	5 (83.3)	1 (2.9)	1	0.127
Non-user	24 (25.9)	27 (25.1)	5.63 (0.61,51.50)	
T classification				
T1, T2	17 (65.4)	9 (12.8)	1	0.048
T3, T4	12 (38.7)	19 (15.2)	2.99 (1.01,8.84)	
Well differentiated SCC				
Yes	24 (60.0)	16 (40.0)	1	0.040
No	5 (29.4)	12 (70.6)	3.60 (1.06,12.19)	
Treatment				
Yes	25 (59.5)	17 (20.6)	1	0.035
No	4 (26.7)	11 (73.3)	4.04 (1.10,14.84)	

Table 3 Factors associated with mortality of OSCC patients using multiple logistic regression

Variables	Alive, n (%)	Died, n (%)	Crude OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Gender						
Female	17 (63.0)	10 (37.0)	1	0.086	1	
Male	12 (40.0)	18 (60.0)	2.55 (0.76, 7.43)		10.89 (1.99, 59.65)	0.006
Alcohol use						
Yes	5 (83.3)	1 (2.9)	1	0.127	1	
No	24 (25.9)	27 (25.1)	5.63 (0.61, 51.50)		16.45 (1.36, 59.65)	0.028
T classification						
T1, T2	17 (65.4)	9 (12.8)	1	0.048	1	
T3, T4	12 (38.7)	19 (15.2)	2.99 (1.01, 8.84)		4.85 (1.12, 21.02)	0.035
Well differentiated						
Yes	24 (60.0)	16 (40.0)	1	0.040	1	
No	5 (29.4)	12 (70.6)	3.60 (1.06, 12.19)		8.00 (1.35, 47.37)	0.022
Treatment						
Yes	25 (59.5)	17 (20.6)	1	0.035	1	
No	4 (26.7)	11 (73.3)	4.04 (1.10, 14.84)		5.88 (1.03, 33.61)	0.046

Forward LR was used, no multicollineary and no interaction. Hosmwer Lemeshow Test, *p*-value = 0.901. Classification table 78.9% correctly classified. ROC curve 85.2%.

Table 4 Clinicopathological features of OSCC patients (n = 57)

Variables	**n (%)**
Tumour site	
Tongue	26 (45.6)
Buccal mucosa	15 (26.3)
Floor of mouth	2 (3.5)
Palate	7 (12.3)
Lips	3 (5.3)
Mandible	4 (7.0)
Histological grading	
Well differentiated	40 (70.2)
Moderately differentiated	15 (26.3)
Poorly differentiated	2 (3.5)
T classification	
T1	5 (8.8)
T2	21 (36.8)
T3	4 (7.0)
T4a,4b,4c	27 (47.4)
N classification	
N0	12 (21.1)
N1	14 (24.6)
N2a,2b,2c	27 (47.4)
N3	4 (7)
M classification	
M0	51 (89.5)
M1	6 (10.5)
TNM staging	
Stage 1	3 (5.3)
Stage 2	4 (7.0)
Stage 3	11(19.3)
Stage 4a, 4b, 4c	39 (68.4)
Treatment received	
Surgery	7 (12.3)
Surgery + Chemotherapy	2 (3.5)
Surgery + Radiotherapy	11 (19.3)
Surgery + Chemotherapy + Radiotherapy	5 (8.8)
Radiotherapy	8 (14.0)
Radiotherapy + Chemotherapy	6 (10.5)
Chemotherapy	3 (5.3)
No treatment	15 (26.3)
Patient status	
Alive	29 (50.9)
Dead	28 (49.1)

Table 5 Surgical specimens features associated with dead and alive patients status (n = 25)

Treatment outcome	**Surgical margins involvement**		**Bone invasion**		**Lymphovascular invasion**		**Perineural invasion**	
	No	Yes	No	Yes	No	Yes	No	Yes
Alive	12 (75%)	5 (55%)	13 (68.4%)	4 (66.7%)	13 (68.4%)	4 (66.7%)	14 (66.7%)	3 (75%)
Dead	4 (25%)	4 (45%)	6 (31.6%)	2 (33.3%)	6 (31.6%)	2 (33.3%)	7 (33.3%)	1 (25%)

Table 6 Association of HR HPV status with Ki-67 status (n = 41)

HR HPV status	Ki-67 status		*p*-value*
	Negative *n* (%)	Positive *n* (%)	
Negative	30 (81.1%)	7 (18.9%)	**0.003***
Positive	0 (0.0%)	4 (100.0%)	

***** Fisher's Exact test. The assumption of Fisher's Exact test because the expected frequency of less than five is more than 20% of cells.

Discussion

The most common malignancy of the oral cavity and mobile tongue is squamous cell carcinoma arising from the mucosal epithelium. The epidemiology of OSCC varies between specific geographical regions as evidenced by marked variation in incidence. Cigarette smoking, alcohol consumption, chewing or dipping of smokeless tobacco; either by mixing it with areca nut and other substances (e.g. slaked lime, betel inflorescence, condiments, sweetening agents and spices) to create betel quid, have been established as the aetiologies for OSCC development (Takata and Slootweg*,* 2017). High-risk HPV, 16 and 18, on the other hand, is a recognized aetiological factor for oropharyngeal carcinoma (OPSCC) but is only seen in 3% of OSCCs (Seethala and Stenman, 2017). In Asian populations, OSCC commonly affects the buccal mucosa due to tobacco chewing and betel quid chewing (Takata and Slootweg*,* 2017). The prevalence of OSCC was common among Malaysian females (67%) and of Indians ethnicity (49.5%) mainly due to their habit of chewing betel quid (Kerishnan *et al*., 2016). For Malays and Chinese group, tobacco smoking and alcohol consumption were the significant risk factors (Zain *et al*., 1999; Tan *et al*., 2000).

During the 11-year period (2005-2015) of the present study, 5-8 new cases of referral per year were received at Hospital Universiti Sains Malaysia (Hospital USM) and male gender was contributory in the survival status of patient i.e. either dead or alive status (p = 0.006). In a previous study, it was reported that the death of males (n = 3200) was more than females (n = 1610) in oral cavity cancer (Siegel *et al*., 2016). This particular trend of higher cancer mortality rates and lower survival rates among males as compared to females, had been studied previously (Cook *et al*., 2011). The ten cancers with highest male to female ratio (MRR) were lip, larynx, hypopharynx, oesophagus, urinary bladder, tonsil, oropharynx, floor of mouth, tongue and nasopharynx. They suggested that these MRR results were more strongly related to aetiology rather than prognosis i.e. indulgence in certain habits that are more gender prone such as smoking and alcohol consumption in males and betel quid in females. Gender disparities in cancer survival also involves other factors i.e. natural history of disease, access to medical care, response to treatment or combination of these.

Those who had a habit of alcohol consumption showed an increased risk of death as compared to non-alcoholic OSCC patients. However, the smoking status of the alcoholic user was not determined; therefore, the increased deaths might not be attributable to alcohol consumption alone. A previous study has shown that cessation of alcoholic habit in OSCC patients significantly reduced mortality (Jerjes *et al*., 2012). Another study showed that alcohol consumption contributes to 3.2% to 3.7% of cancer-related deaths (Nelson *et al*., 2013). Counselling on cessation of alcohol consumption and smoking should be emphasized as a part of overall management of OSCC. Continuation of these habits negatively affects overall survival as it predisposes to increases toxic side effects from radiation therapy, reduce treatment efficacy, increases risk for disease recurrences and development of second primary tumours (Browman *et al*.,1993; Warnakulasuriya, 2009; McCarter *et al*., 2016).

Tumour stage also plays an important role in prognosis of OSCC patients managed at Hospital USM. It was noted that T3, T4 stages had a higher risk of deaths as compared to T1 and T2 stages. This result was consistent with a study that had similar outcome (p = 0.03) of T3 and T4 stages (Jardim *et al*., 2015). Previous study also indicated that poorer prognosis was associated with an increase in the size of the tumour (Grimm, 2012). This is probably due to higher risk of nodal metastasis and local recurrence with larger tumours (Punhani *et al*., 2017).

Studies have shown that histological grading i.e. degree of differentiation; significantly affects prognosis. Tumours which demonstrated moderate to poorly differentiated OSCC had a poorer prognosis in comparison to well differentiated tumours. The present result was consistent with a study which found that a poorly differentiated tumour had an approximately three-fold risk of deaths than other histological grades (Thomas *et al.*, 2014). They also reported the risk of death was 42% higher in moderately differentiated tumours. Similar findings were reported whereby the well differentiated SCC was shown to have a better prognosis, whereas moderate and poorly differentiated tumours were significantly related to deaths (p = 0.001) (Jerjes *et al*., 2010).

The possible reason for poorer prognosis in moderate to poorly differentiated tumours could be due to its association with late stages (T3, T4) of tumour presentation. In this study, late stage presentation (T3, T4) was related to moderately differentiated tumours (28%). None of the early stage tumours (T1, T2) were histologically graded as moderately or poorly differentiated. Other studies have documented that moderate to poorly differentiated tumours have a significant correlation with advanced stages (T3, T4) of the tumour (p = 0.02) (Jardim *et al*., 2015; Sawazaki-Calone *et al*., 2015).

Histological assessment of degree of differentiation in these tumours should be carried out routinely in combination with cytological parameters i.e. cellular pleomorphism, mitotic activity and nuclear aberrations. Woolgar and Triantafyllou (2009) had discussed extensively regarding histological grading systems of OSCC and highlighted several key parameters that serve to supplement the WHO grading system. Assessment of pattern of invasion i.e. degree of keratinocyte dyscohesion of the advancing front of the tumour and tumour-host interface i.e. stromal inflammatory cell reaction were recommended as part of the proposed multifactorial grading system.

Other additional pathological parameters considered to be better prognosticators includes maximum diameter of tumour, reconstructed tumour thickness i.e. maximum depth of invasion by the tumour, histological type of carcinoma either conventional, papillary, verrucous, basaloid, adenosquamous, acantholytic or spindle cell carcinoma, involvement of overlying skin, histological assessment of nodal metastases and presence of extracapsular spread (Helliwel and Woolgar, 2013).

It is worthy to note that higher frequency of patients to be alive when there was no involvement of surgical margin, bone, lympho-vascular and perineural invasion (Table 5). Complete excision of the tumour with sufficient margin is an essential clinical prognosticator. Nason *et al*. (2009) reported that survival chance improved by 8% with each 1 mm of clear margin. In a previous study, it was reported that positive involvement of surgical margin has a higher association with recurrence and poor outcomes (Capote-Moreno *et al*., 2010). A clear margin is believed to be associated with good prognosis, but this could not assure better survival outcome based on this single prognosticator alone. The presence of moderate, severe epithelial dysplasia or carcinoma in situ within 5 mm of the resection margin also worth noting as this might predict recurrences (Helliwel and Woolgar, 2013).

Perineural and lympho-vascular invasion status also serve as crucial prognosticators as it determines the risk for local recurrences and lymph node metastasis, which directly influenced the outcome of management of OSCC patients. The presence of these factors has significant impact on survival outcomes in patients with advanced stage tumours (Jardim *et al*., 2015). Tumour site, tumour size, histological grading, lympho-vascular and perineural invasion are also associated with

contralateral metastasis, and poor survival in OSCC (González-García *et al*., 2008; Fan *et al*., 2011). In this study, the overall survival benefit could be the result of the combined treatment modalities rather than the effect of clear surgical margin alone as this most likely addressed any potential micro metastasis of the tumours.

It has been established that HPV positive tumours respond better to treatment thus warrants its routine detection (Ang *et al*., 2010). There are various methods for detection e.g. polymerase chain reaction (PCR), *in situ* hybridization (ISH) and immunohistochemistry (IHC) (Cantley *et al*., 2011). Expression of high-risk (HR) HPV oncogene E6/E7 by PCR is considered as gold standard for detection of HR HPV infection but is not likely practical in clinical settings (Jordan *et al*., 2012).

In this study, 9.8% (n = 4) cases were found to have overexpression of p16. Overexpression of p16 can be used as a surrogate biomarker for HPV-related OSCC and serve as the basis for IHC method of detection. HPV oncoproteins E6/E7 causes the degradation of tumour suppressor protein (p53) and retinoblastoma protein (pRB) which ultimately promotes cell proliferation and overexpression of p16 protein (Hellman *et al*., 2014).

A recent meta-analysis has shown a significant association between the presence of HR HPV infection and p16 overexpression in OSCC (p = 0.001) *(*Smitha *et al*., 2017). Validation of HPV infection in tumour tissue by p16 overexpression has previously been described and it was documented that p16 IHC is a better option as a standalone test for detection of HR HPV infection (Lewis, 2012). A trial validated p16 with high sensitivity (96.8%), lower specificity (83.8%) to oncogene expression of HR HPV as compared to HPV 16 ISH which had lower sensitivity (88%) and higher specificity (94.7%) (Jordan *et al*., 2012).

A recent classification of head and neck tumours by WHO on HPV carcinogenesis stated that 3% of oral cavity OSCC is related to HR HPV infection (Seethala and Stenman, 2017). The prevalence of HR HPV infection varies from 0% (Chen *et al*., 2016) to 100% (Koyama *et al*., 2007) in OSCC worldwide. Antonsson *et al*. (2015) reported 20% of HR HPV infection in a head and neck tumour and prevalence of HR HPV infection in the oral cavity to be 6% while Chandarana *et al*. (2013) reported p16 overexpression in 13% of OSCC.

The variation in reported HR HPV prevalence are more likely due to different detection methods and specimen types. Usage of PCR for detection of HPV 16 infection is considered as gold standard (Kulkarni *et al*., 2011; Jalouli *et al*., 2012; Mondal *et al*., 2013). Kulkarni *et al*. (2011) detected HR HPV infection from the saliva rinse as compared to this study which used formalin-fixed paraffin embedded (FFPE) tissue specimens. It is important to note that HR HPV detection in saliva does not confirm the presence of HR HPV in the OSCC lesions thus the results cannot be used to classify OSCCs into HPV positive or negative tumours.

Detection of HPV infection from tumour specimens is likely to be a standard approach and should be used for comparable findings (Chaudhary *et al*., 2010). Previous studies on Malaysian population has demonstrated HPV 16 E6 seropositivity in 30% of OSCC patients (Wong *et al*., 2014) with another study reported 51.4% HPV-related OSCC and HPV 16 being the most prevalent type (Saini *et al*., 2011).

Detection of HR HPV in OSCC has a pivotal role in the management of OSCC as HPV positive and negative cases have different molecular forms and response to treatment. The preferred treatment for OSCC is surgical excision followed by chemotherapy or radiotherapy. Following HR-HPV status determination, good clinical judgement can be made into consideration for a lower effective radiation dose as well as whether or not chemotherapy is necessary in the management of HPV positive OSCCs. This, in turn, will reduce the possibility of overtreatment, which often results in unnecessary toxicity and reduced quality of life. HPV detection also useful in cases where clinical presentation involved patients with cervical lymph node metastasis of undetermined primary as positive result may point to the oropharynx as the most likely site. With the advancement of molecular technique, HPV-related tumours might provide a corridor for targeted therapeutics and immunotherapy (Taberna *et al*., 2017).

In this study, a statistically significant association between p16 overexpression and Ki-67 status ($p = 0.003$) was found. This finding was consistent with previous study, which indicated that higher expression of Ki-67 with p16 positivity relates to better prognosis (Liu *et al*., 2015). This relates to radio sensitivity of OSCC as proliferating cells are better receptive to ionizing radiation. It was observed that high proliferating oral cavity tumours had better prognosis to radiotherapy as compared to the low proliferating tumours (Freudlsperger *et al*., 2012). The co-expression of Ki-67 and p16 were significantly associated with higher expression in malignant cells as more than 50% cells were found to be positive (Prigge *et al*., 2015). A similar conclusion was noted which indicated Ki-67 expression to be predictive of HPV infection (Mimica *et al*., 2010).

Conclusion

In this study, male gender, alcohol users, late stage (T3, T4), moderately differentiated and poorly differentiated tumours, and no treatments were associated with increased mortality in OSCC patients managed at Hospital USM. Patients' records with incomplete data or missing paraffin-embedded blocks were excluded from investigations and this may have underestimated the findings. More comprehensive studies with larger sample size are warranted for complete assessment of important histopathological prognosticators, to ascertain the prevalence of HPV infection and to provide survival analysis between HPV-positive and HPV-negative OSCCs.

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