RESEARCH ARTICLE

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High *P16INK4A* Expression, Keratinizing Features, and Surgical Margin-Free Status are Associated with Improved Survival in Patients with Oral Squamous Cell Carcinomas

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Abstract

Background: Oral squamous cell carcinoma (OSCC) continues to prevail as a highly prevalent cancer in Southeast Asia and causes a significant health burden. Stratification of patients with high risks of recurrence and mortality is important in the planning of treatment and surveillance. **Methods:** Formalin-fixed paraffin-embedded (FFPE) tissues of OSCC were immuno-stained and analyzed for p16 expression. Risk factors and clinical parameters of OSCC patients were collected and compared to identify factors associated with recurrences and overall survival. **Results:** After a median follow-up of 32 months, OSCC recurrences and mortality were observed in 82% and 78% of patients (N=60), respectively. Larger and more extensive tumors (T3 and T4) were significantly associated with both recurrences and cancer-associated mortality (OR = 3.967, 95% CI = 1.007-15.618 and OR = 5.885, 95% CI = 1.541-22.47, respectively). *P16INK4A* positive staining was found in 31% of tumors. Patients with p16INK4A positive staining were significantly associated with better recurrence-free and overall survivals (medians of recurrence-free survivals were 31.2 vs 19.0 months, P=0.038 and overall survivals were 39.0 vs 28.8 months, P=0.048; respectively). Some other clinical characteristics including early stages, non-keratinizing tumors, negative cervical node, and free-surgical margin were significantly associated with better recurrence-free and overall survivals (log-rank tests, P<0.05). **Conclusion:** *P16INK4A* positive staining, early stages, negative cervical lymph node infiltration, and free-surgical margins are associated with better prognosis in OSCC patients. The study emphasizes the importance of early detection and the potential use of *p16INK4A* and other clinical variables to stratify OSCC patients with high risks of recurrence and worse overall survivals.

Keywords: Squamous cell carcinoma- buccal and tongue- p16- prognosis

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Introduction

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer worldwide, with relatively high recurrence and mortality rates [1]. There has been a significant improvement in the management of oral cancer with the introduction of multimodal treatment of surgery, concomitant chemotherapy-radiotherapy, the addition of taxane-based induction chemotherapy, and the emergence of some novel targeted therapies [2]. The high prevalence in South Asia and South East Asia has been associated with the consumption of specific substances of betel quid, tobacco products, and smokeless tobacco [3, 4]. Smoking and alcohol consumption are two principal predisposing factors for oral squamous cell carcinomas (OSCCs) [4]. Tobacco use is a very common practice among Indonesian men yet alcohol is not regularly consumed [5]. In addition, evidence shows that high-risk human papilloma virus (HPV) infection is involved in oral cancer [6]. Patients with chronic HPV infection often have specific biological mechanisms and clinical consequences [7].

P16 protein acts as a tumor suppressor and it is negatively regulated by protein retinoblastoma (pRB) [8]. In the presence of HPV infection, the virus can express viral oncoproteins, E6 and E7 [9, 8]. E6 protein can degrade p53 causing cell cycle arrest and induction of apoptosis [9]. E7 oncoprotein induces breakdown of retinoblastoma protein (pRb) that is involved in cell cycle regulation . Retinoblastoma protein is a negative regulator of p16 protein, therefore, depletion of pRb can cause *p16* over-expression [9]. HPV-associated oral cancer is usually accompanied by *p16* expression [10]. On the contrary, in tobacco-associated oral cancer, loss of the P16 locus

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occurs in the early steps of carcinogenesis including cell dysplasia and carcinoma in situ [10]. P16 over-expression is commonly used as a surrogate marker of HPV-related oral cancer [10].

Several studies have suggested that *p16* expression can serve as a prognostic marker in oral cancer patients [11-13] particularly for patients receiving radiotherapy. Locoregional recurrences are significantly lower among patients with *p16*-overexpression HR=0.40, 95%CI: 0.27-0.59) [13]. However, in some studies using randomized trials, the association of *p16* overexpression with better outcomes is still unclear [12, 14]. Oral cancer with *p16* overexpression represents a particular molecular pattern, clinical manifestation, and treatment responses [9]. Those with *p16* overexpression might also have a subpopulation with a poor prognosis that needs further investigation, particularly with the interaction of other risk factors including consumption of tobacco and non-tobacco products. In this study, we evaluated *p16* expression among patients in Indonesia with squamous cell carcinoma of the oral cavity and the association with the risk factors, treatment response, and recurrence rates.

Materials and Methods

Study population

Oral squamous cell carcinoma patients admitted to the Division of Surgical Oncology in our hospital were recruited for this study. All patients attained standard clinical management according to the national guidelines for head and neck squamous cell carcinomas. Socio-demographic and clinical variables were categorized and tabulated. Tumor size and lymph node involvement were classified according to the American Joint Committee on Cancer (AJCC) [15]. Histological grades, primary tumor site, smoking status, and margin status were determined according to the medical chart and direct patient interview. Surveillance after therapy completion was performed according to the institutional guidelines. This study was performed in adherence to the ethical principles of the 1964 Declaration of Helsinki. The study protocol has been reviewed and officially approved by our local Medical and Health Research Ethics Committee of Universitas Gadjah Mada (0471/EC/2021).

Expression of p16

Immunohistochemistry staining was performed using formalin-fixed paraffin-embedded (FFPE) tissues of oral squamous cell carcinoma patients. FFPE sections of 3μm-thick were incubated, deparaffinized, and rehydrated before immunostaining. Antigen was retrieved using a BioCare decloaking chamber (NXGen, USA). Monoclonal primary antibody of anti-CDKN2A / *p16INK4a* was used with a dilution of 1:150 in phosphate-buffer saline (Clone 2C5, LSBio, USA). For visualization of positive cells, semiautomatic Intellpath FLX (BioCare Medical, USA) was used according to the manufacturer's protocol. For positive controls, pancreatic carcinoma tissues available from our lab were used for each staining.

Expression of *p16INK4a* was then observed using a light microscope by two independent and experienced pathologists. We used a semi-quantitative German scoring system that has been previously described[16]. Scoring was based on the intensity of the nucleic staining (no stain = 0, weak stain = 1, moderate stain = 2, strong stain = 3) and the extent of stained the tumor cells (0% = 0, 1-10% = 1, 11-50% = 2, 51-80% = 3, and 81-100% = 4). The extent of stained tumor cells was calculated from counting of nuclear positive staining every 500 tumor cells in 5 fields [17, 18]. The expression index (immunoreactive score) was calculated by multiplying the three-tier intensity score and four-tier of the fraction area score. The final score ranged between 0 and 12, a final score of 0-3 was considered negative and 4-12 was considered positive [16]. Representative *p16* staining is shown in Figure 1.

Clinicopathological variables and survivals

Pathological staging (T, N, M) was determined using the American Joint Committee on Cancer (AJCC) [15]. Histological type, grades, and keratinization were categorized using the guideline of World Health Organization (WHO) [19]. Recurrence-free survival (RFS) was defined as the time from the date of histopathological diagnosis of OSCC to the date of any loco-regional recurrences, distant metastases, or death of any cause or to the last follow-up. Overall survival (OS) was calculated from the time from the date of histopathological diagnosis to the date of mortality or the last follow-up. The status of patients to the last follow-up was cross-checked by contacting the patient's family members. Tumor infiltration leucocyte (TIL) was calculated using guidelines from the International Working Group [20]. Smoking status was defined if participants had smoked more than 100 cigarettes in their lifetime [21]. Types of surgery were classified into biopsy and excision with or without neck dissection. Surgical margins were classified into positive and negative according to the pathological report. Receiving chemotherapy was defined for patients who ever received systemic cytostatic agents in the settings of induction therapy, adjuvant, or concomitantly with radiotherapy. All statistical tests were calculated using SPSS 17.0 software (SPSS Inc., Chicago) in two-sided with a P-value <0.05 was considered as a significant difference.

Results

Clinical and pathological characteristics of oral squamous cell carcinomas

From 2017-2019, 60 patients diagnosed with oral squamous cell carcinoma (OSCC) were recruited to this study. The median age of OSCC patients in this study was 58 years. Most patients were male (60%) with tobacco smoking as the common risk factor among them (Table 1). More than 83% of tumors were located at the tongue and 65% of them were diagnosed at T3 or T4 (diameter more than 4 cm or thickness more than 10 mm or with infiltration to the surrounding tissues). More than half patients were initially diagnosed with regional lymph node infiltration with 78% of them at Stage III or IV. Around 90% of tumors were well-differentiated, 28% of them were attributed to

Table 1. Clinical Characteristics

Clinical characteristics	Cathegory	N(%)
Age	<50 years	18 (30)
	≥50 years	42 (70)
Sex	Male	36 (60)
	Female	24 (40)
Tumor site	Tongue	50 (83.3)
	Buccal mucous	10 (16.7)
Tumor size (T)	T1	2 (3.3)
	T2	20 (33.3)
	T3	22 (36.7)
	T4	16 (26.7)
Regional lymph node (N)	Negative (N0)	29 (48.7)
	Positive (N1-3)	31 (51.7)
Clinical Stage	Stage I-II	13 (21.7)
	Stage III-IV	47 (78.3)
Histological differentiation	Grade I	54 (90)
	Grade II-III	6 (10)
Keratinization	Yes	17 (28.3)
	No	43 (71.7)
Vascular invasion	Yes	35 (58.3)
	No	25 (41.7)
Lymphatic invasion	Yes	32 (53.3)
	No	28 (46.7)
Perineural invasion	Yes	27 (45)
	No	33 (55)
TIL	Low	17 (28.3)
	Moderate-High	43 (71.7)
Surgical Margin	Positive	30 (50)
	Negative	30 (50)
P16 staining	Positive	19 (31.7)
	Negative	41 (68.3)
Surgery	Biopsy	9 (15)
	Excsion and Node procedures	51 (85)
Neck dissection	Yes	26 (43.3)
	No	34 (56.7)
Chemotherapy	Yes	47 (78.3)
	No	13 (21.7)
Radiotherapy	Yes	44 (73.3)
	No	16 (26.7)
Smoking	Yes	36 (60)
	No	24 (40)

keratinization, and around half of tumors featured vascular invasion, lymphatic invasion, and perineural invasion. More than 70% of tumors presented with TIL higher than 20%. Most patients were managed with surgical excision (85%) and 43% of them performed neck lymph node dissection. Most patients received radiotherapy (73%) and chemotherapy (either induction, adjuvant, or concurrent chemo-radiotherapy). P16 positive staining was found in 31% of patients.

Clinical and pathological factors associated with progression into recurrences and cancer-associated mortality

After a median follow-up of 32 months, we observed recurrences and mortality in 82% and 78% of patients, respectively. Larger tumor size (T3 and T4) was associated with both recurrences and mortality (OR = 3.967, 95% CI = 1.007-15.618 and OR = 5.885, 95% CI = 1.541-22.47, respectively). Histological characteristics of OSCCs with vascular invasion and lymphatic invasion were associated with higher risk of recurrence (OR = 3.014, 95% CI = 0.774-11.729 and OR = 2.333, 95% CI = 0.603-9.063, respectively) and mortality (OR = 2.824, 95% CI = 0.796-10.013, and OR = 3.316, 95% CI = 0.891-12.338, respectively) although they were not statistically significant. Keratinization was also associated with higher recurrences and mortality rates although it was not statistically significant (OR = 1.985, 95% CI = 0.382-10.319 and OR = 1.414, 95% CI = 0.337-5.931, respectively). Excision surgery with or without neck dissection was associated with a lower risk of recurrences and mortality in comparison to biopsy (P=0.005 and P=0.032, respectively). Patients receiving chemotherapy and radiotherapy were also associated with lower risks of recurrences and mortality (Table 2). Positive staining of *p16INK4a* was also associated with lower recurrences and mortality although it was not statistically significant (OR = 0.480, 95% CI = 0.126-1.831 and OR = 0.446, 95% CI = 0.126-1.479, respectively).

Association of p16INK4a positive staining, staging, and keratinization with recurrence-free and overall survivals of OSCCs

P16INK4a positive immunostaining was associated with longer recurrence-free and overall survivals (medians of recurrence-free survivals were 31.2 vs 19.0 months, P=0.038 and overall survivals were 39.0 vs 28.8 months, P=0.048; respectively) (Figure 2). Other clinical variables including early stages, negative cervical nodes, non-keratinizing tumors, and negative surgical margin status were associated with better recurrence-free and overall survivals. OSCC patients diagnosed at Stage I-II were associated with longer recurrence-free and overall survivals compared to patients diagnosed at Stage III (median survivals were 32.5 vs 21.3 months, P=0.023, and 43.8 vs 28.5 months, P=0.016, respectively). In addition, tumor infiltration in the regional cervical lymph nodes was associated with shorter recurrence-free and overall survivals (median survivals were 18.4 vs 29.3 months, P=0.013 and 25.6 vs 38.8 months, P=0.004, respectively). Tumors with keratinization were also associated with shorter recurrence-free and overall survivals (median survivals were 15.5 vs 26.7 months, P=0.008 and 22.7 vs 35.7 months, P=0.012, respectively). Positive surgical margins were also associated with shorter recurrence-free and overall survivals (median survivals were 20.0 vs 27.3 months, P=0.020 and 27.4 vs 36.9 months, P=0.032, respectively).

Table 2. The Association of Clinico-Pathological Variables of Bone Metastatic Cancers and Skeletal-Related Events at Diagnosis. Odds ratios and 95% confidence intervals were calculated using multivariable binary logistic regression.

Variables	Category	Reference	Recurrence (OR, 95%CI)	Mortality (OR, 95%CI)
Age	≤50 years	>50 years	0.458 (0.089-2.373), P=0.352	1.048 (0.276-3.975), P=0.945
Sex	Male	Female	0.500 (0.118-2.116), P=0.346	0.600 (0.162-2.228), P=0.445
Tumor site	Buccal	Tongue	0.878 (0.159-4.851), P=0.881	1.128 (0.209-6.101), P=0.889
Tumor size	T3T4	T1T2	3.967 (1.007-15.618), P=0.049	5.885 (1.541-22.47), P=0.010
Node status	Positive	Negative	2.148 (0.556-8.296), P=0.268	3.038 (0.818-11.28), P=0.097
Stage	I-II	III-IV	1.463 (0.327-6.540), P=0.619	1.877 (0.470-7.488), P=0.373
Grade	II-III	I	1.136 (0.119-10.826), P=0.911	1.429 (0.152-13.430),P=0.755
Keratinization	Yes	No	1.985 (0.382-10.319), P=0.382	1.414 (0.337-5.931), P=0.636
Vascular invasion	Yes	No	3.014 (0.774-11.729), P=0.112	2.824 (0.796-10.013), P=0.108
Lymphatic invasion	Yes	No	2.333 (0.603-9.023), P=0.220	3.316 (0.891-12.338), P=0.074
Perineural invasion	Yes	No	0.978 (0.263-3.637), P=0.973	1.408 (0.401-4.942), P=0.593
TIL	Low	Moderate-High	2.569 (0.664-9.948), P=0.172	2.805 (0.778-10.115), P=0.115
Surgical margin	Positive	Negative	1.978 (0.513-7.635), P=0.322	2.786 (0.751-10.331), P=0.126
P16 staining	Positive	Negative	0.480 (0.126-1.831), P=0.283	0.446 (0.126-1.579), P=0.211
Surgery	Excision	Biopsy	-P=0.046	-P=0.028
Neck dissection	Yes	No	1.426 (0.369-5.508), P=0.607	1.980 (0.534-7.336), P=0.307
Chemotherapy	Yes	No	- P=0.005	-P=0.032
Radiotherapy	Yes	No	0.073 (0.0158-0.3440), P=0.001	0.083 (0.104-0.323), P<0.001
Smoking	Yes	No	0.500 (0.118-2.116), P=0.346	0.600 (0.162-2.28), P=0.445

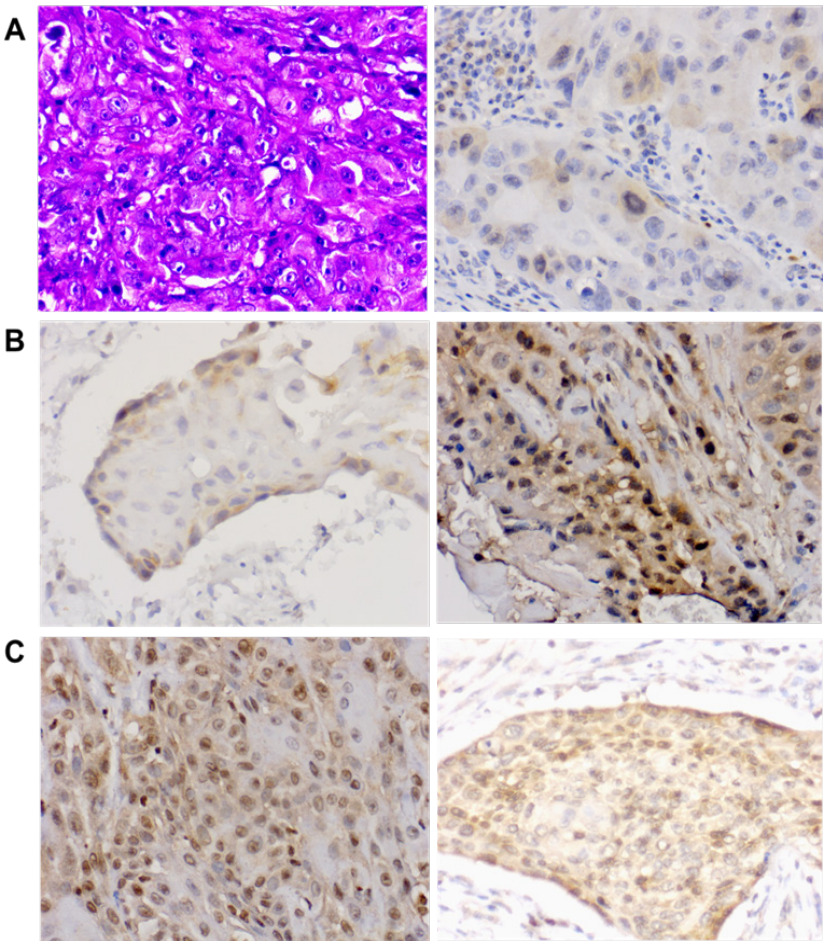

Figure 1. Immunostaining of *p16INK4a* in Oral Squamous Cell Carcinoma (OSCC). (A) Weakly to no nuclear staining intensity, no cytoplasmic staining in 0-10% of cancer cells; (B) Moderately positive nuclear staining intensity in 10-20% of cancer cells, C). Strongly positive nuclear staining in diffusely cancer cells (more than 20% of total cancer cells). The images were taken in high-power, x40.

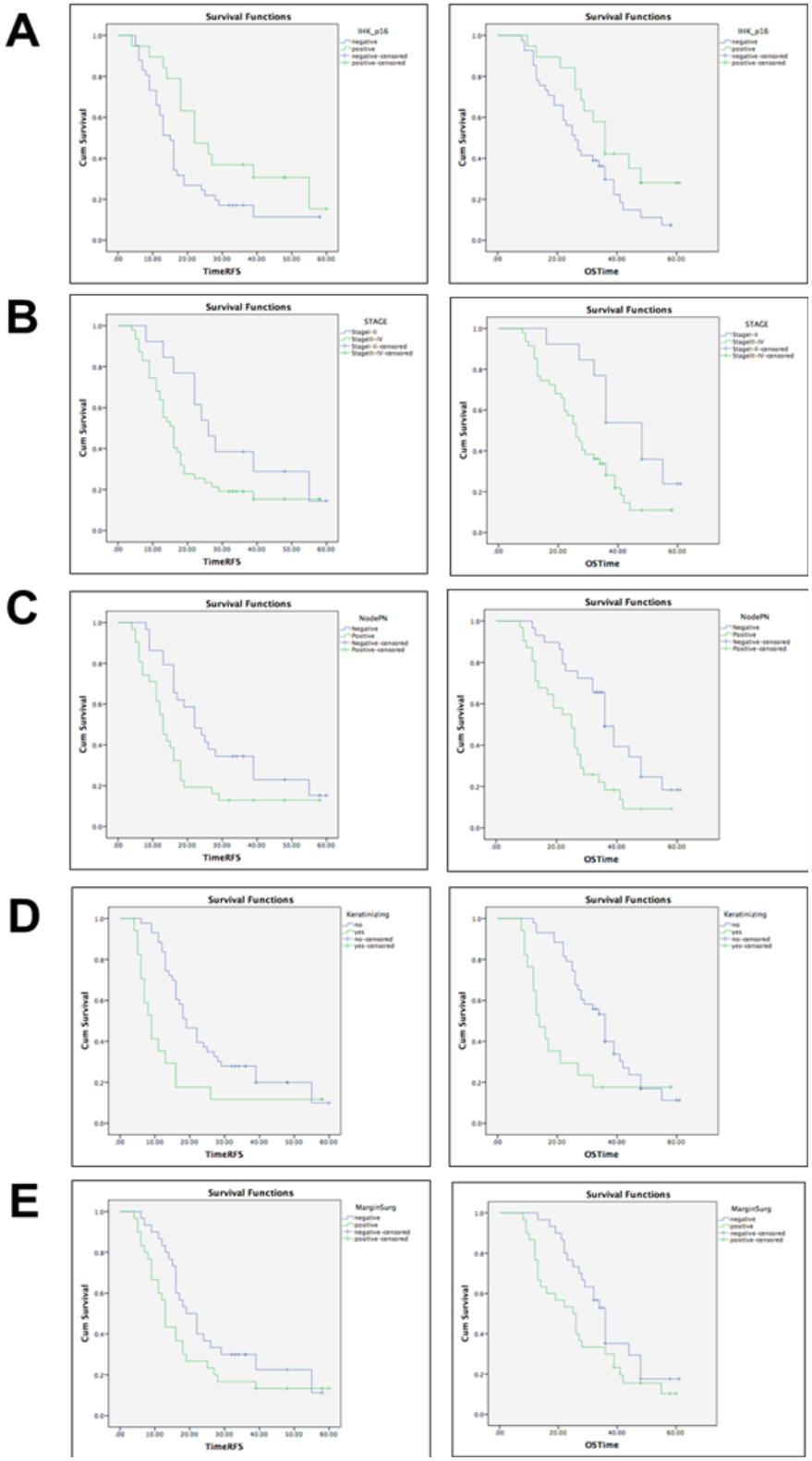

Figure 2. Association of *p16INK4A* Expression, Late Stages, Positive Cervical Lymph Nodes, Keratinization with poor recurrence-free and overall survivals of OSCCs. A) Positive *p16INK4* staining was significantly associated with better recurrence-free survival (medians of recurrence-free survivals were 31.2 vs 19.0 months, P=0.038) and overall survival (medians overall survivals were 39.0 vs 28.8 months, P=0.048), B) Stage I-II were associated with longer recurrence-free (median survivals were 32.5 vs 21.3 months, P=0.023) and overall survivals (medians overall survivals were 43.8 vs 28.5 months, P=0.016) compared to patients diagnosed at Stage III and IV, C. Positive cervical lymph nodes were associated with shorter recurrence-free and overall survivals (median survivals were 18.4 vs 29.3 months, P=0.013 and 25.6 vs 38.8 months, P=0.004, respectively). D. Keratinization was were also associated with shorter recurrence-free and overall survivals (median survivals were 15.5 vs 26.7 months, P=0.008 and 22.7 vs 35.7 months, P=0.012, respectively), E) Positive surgical margins were also associated with shorter recurrence-free (median survivals were 20.0 vs 27.3 months, P=0.020) and poorer overall survivals (median survivals were 27.4 vs 36.9 months, P=0.032)

Discussion

Oral squamous cell carcinomas (OSCCs) are exceptionally more prevalent in South Asia and South-East Asia including Indonesia [9, 22, 23]. The use of tobacco products and betel quid has been associated with the high incidence although systematic studies on these risk factors in these regions are not yet available [4]. In our study, most male patients reported tobacco smoking before the diagnosis of OSCCs. Additionally, 65% of the tumors were found as T3 or T4, more than half patients were found with positive tumor infiltration to the lymph nodes. This indicates that most OSCC patients in our study were diagnosed in the late stages (78% of patients were diagnosed at the Stage III and IV). Patients with larger tumor sizes (T3 and T4) had significantly higher rates of recurrence and mortality. Other studies also show that OSCC patients in low-resource settings are also diagnosed in late stages [23-25]. Advanced OSCC patients often need multimodality treatment with more severe impairments in their functioning and quality of life [2]. In addition, developing countries including Indonesia also experience a shortage of facilities and manpower in cancer treatment and prevention which can add to the health burden [26] [27]. Most clinical failures in the OSCC are caused by loco-regional recurrence [28]. Haring et al. have shown that patients with tumors larger than 4 cm and positive cervical nodes are at higher risks of recurrence and distant metastases [28]. Most of the loco-regional recurrences occur in the marginal and out-of-field [29]. Local OSCC recurrences have also been associated with a significant decline in the patient's functioning including swallowing, chewing, and taste [30]. These findings might emphasize the importance of early detection and facilitation of a referral system to initiate comprehensive treatment for OSCCs.

OSCC patients receiving surgical excision, radiotherapy, and chemotherapy had lower risks of recurrence and death. As most patients in this study were diagnosed in the late stages, multimodal treatment was needed. Curative intent in the OSCC depends on largely the loco-regional control. Extensional surgery with around 1 cm tumor-free margins is commonly considered the mainstay treatment in OSCCs particularly in the early stages [31]. Although free margins were not often achieved in this study (Table 1), surgical excision was associated with lower risks of recurrence and mortality in comparison to biopsy only. Larger and more extensive tumor sizes are not often easily managed with surgery alone. The use of induction treatment, immunotherapy, and targeted therapy will provide a great promise to optimize OSCC treatment to achieve a surgical clear margin in the future.

Our study also showed that advanced stages were associated with shorter recurrence-free and overall survivals (Figure 2). This suggests that the use of AJCC TNM staging in OSCCs is very important in treatment planning and prognostication [15]. The advanced stages are determined particularly from the large and depth of the tumor sizes as well as lymph node infiltration [15]. Early-stage OSCCs are often treated with surgery and/or radioterapy only, while those in advanced stages receive multimodal intensive treatment [32]. Although different treatment modes were allocated, our study showed significant different prognoses according to the staging. Other studies have also revealed that advanced stages are associated with shorter overall survivals in OSCC patients [10] [33] In a larger study from developing countries shows that advanced stages remain the most striking predictor of worse survival in OSCC [10].

Using the Kaplan-Meier curve, our study showed that *p16INK4a* positive immunostaining was associated with longer recurrence-free and overall survivals (Figure 2) suggesting that *p16INK4a* positive staining may serve as a favorable marker for prognosis in OSCC patients. Our study confirmed previous results from different populations that *p16* overexpression displayed as an independent prognostic factor of better clinical outcomes and survival [33, 34]. In the presence of HPV infection, E7 oncoprotein binds to pRB causing E2F release causing transcriptional activation as well as a negative feedback loop from pRb to the overexpression of *p16*. Tumors with *p16* expression have been associated with sensitivity to radiotherapy and a combination of chemo-radiotherapy by modifying the cell-cycle checkpoints, particularly through pRB and p53 inactivation during H2-M and G1-phase transition. Chemotherapy and fractionated radioterapy can work better in transitioned cells in those phases [35] . In Table 2, we also showed that radiotherapy and chemotherapy were associated with lower rates of recurrence and mortality. In our study, 31% of tumors were *p16INK4a* positive. Several reports show p16INK4a positive in 20-50% of OSCCs [11, 29, 34] . P16 marker has been included in the biomarker that should be examined in OSCC tumors [2, 32].

Other clinical parameters including cancer infiltration to the cervical lymph nodes and positive surgical margins were also associated with poorer OSCC outcomes. In a large meta-analysis, positive cervical lymph node is associated with lower 5-year survivals [36]. By our results, more than half of OSCC patients are presented with positive lymph nodes [11, 36]. In addition, tumors with keratinization were also associated with poorer clinical outcomes (Figure 2). In a larger study, Cooper et al. reported that keratinized OSCCs were significantly associated with poor outcomes [37]. Keratinization results from protein overexpression of more differentiated cells that are more likely represented by HPV/*p16*-negative OSCCs [38]. It has also been suggested that keratinization can be combined with staging, *p16*, and other pathological variables to subclassify OSCC patients for the risks of recurrences [39].

OSCCs are considered preventable cancers with the main risk factors being tobacco product consumption and HPV infection [6, 9]. OSCCs also can be potentially cured if they are diagnosed at the early stages [40]. Therefore, coordinated strategies should be implemented to reduce the health burden of OSCCs by eliminating risk factors, such as smoking and HPV vaccination. The prevention and early diagnosis in OSCCs could be aligned with the national cancer control program to cover the area and target population [41-43]. Quit smoking programs and

implementation of tobacco policies will be very beneficial in the prevention of OSCCs. HPV vaccination in both sexes will ensure for reduction HPV-positive OSCCs and public health policy should reinforce the HPV vaccination programs [6].

In conclusion, our study highlights the importance of *p16*-positive immuno-staining, staging, keratinization, and surgical margin status in determining the recurrence-free and overall survivals of patients with OSCCs. Further study and validation of these results could potentially help in developing more accurate prognostic markers and treatment strategies for OSCC patients. Our study also provides valuable insights into the clinical and pathological characteristics of OSCCs in our population. As most OSCC patients in this study are diagnosed in the late or advanced stages with high rates of recurrences, early detection is needed particularly to reduce recurrences and improve outcome. This study also highlights the aggressive nature of OSCCs and might shed light on potential avenues for future personalized treatment approaches. However, our study has several limitations including the retrospective design and single study site. Medical comorbidities that might influence clinical outcomes are also not specifically analyzed in this study. Patients with histological diagnoses from other hospitals are not included for *p16*-staining which might cause selection bias. Future larger and multicenter studies need to confirm our study.

In conclusion, we highlight the significance of *p16INK4a* positive staining in the stratification of OSCC patients with better recurrence-free and overall survivals. In addition, AJCC staging, tumor keratinization, and surgical margin status can also serve as prognostic markers for patients with OSCCs. Our results might also emphasize the importance of early detection and subsequent comprehensive treatment for OSCC patients to reduce the risks of recurrences.

Abbreviations

AJCC: American Joint Committee on Cancer
CI: Confidence interval
EC: Ethical Clearance
FFPE: Formalin-fixed paraffin embedded
HNSCC: Head and Neck Squamous Cell Carcinoma
HPV: Human Papilloma Virus
OR: Odds Ratio
OSCC: Oral Squamous Cell Carcinoma
SD: Standard Deviation
TIL: Tumor Infiltrating Lymphocytes
WHO: World Health Organization

Author Contribution Statement

SLA analyzed the data and drafted the manuscript. EKD and SPL performed *p16INK4a* staining and scoring. RC, SLA, WS, and GDP collected pathological and clinical data. All authors reviewed and approved the final draft of the manuscript.

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Ethics approval and consent

The protocol to perform this study has been reviewed and approved by the local Ethics Committee (Nr. KE/FK/0471/EC/2021). This study has been performed following the ethical principles for research involving human participants of the1964 Declaration of Helsinki. All study participants in this study have provided written informed consent during the admission to the hospital.

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Peer review process

Not commissioned, externally peer reviewed.

Availability of data and materials

The datasets of clinical and pathological variables will be available upon request to the corresponding author.

Competing interest

All authors have declared for no existing conflict of interests.

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