Expression of PD-L1 is HPV/P16-independent in oral squamous cell carcinoma

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ARTICLE INFO

Keywords: Human papillomavirus Oral squamous cell carcinoma PD-L1 p16

ABSTRACT

Objectives: This study aimed to investigate the expression of programmed cell death-ligand 1 (PD-L1) and its associations with human papillomavirus (HPV) 16/18 DNA status, p16 expression, demographic, clinicopathologic and risk parameters in patients with oral squamous cell carcinoma (OSCC).

Study design: A total of 85 formalin-fixed, paraffin-embedded OSCC specimens were collected. HPV16/18 DNA was detected by polymerase chain reaction. PD-L1 and p16 expressions were assessed using immunohistochemical technique. The immunostaining scores were calculated by combined positive score (CPS), previously described. The positive scoring value was determined at CPS ≥1, recommended by FDA. The associations between PD-L1 expression and HPV16/18 DNA status, p16 expression, demographic, clinicopathologic, and risk parameters were analyzed by Chi-square, Fisher's exact tests, and multivariate logistic regression.

Results: PD-L1 expression was detected in 22 out of 85 cases of OSCC (25.9%). 16.5% of all cases were HPV 16/18-positive and 62.4% were p16-positive. Statistically, there were no significant associations between PD-L1 expression in OSCC and HPV16/18 DNA status, p16 expression, demographic and, clinicopathologic parameters or risk behaviors.

Conclusion: Approximately one-fourth of OSCC cases were PD-L1-positive, suggesting candidacy for anti-PD-L1 immunotherapy. Furthermore, HPV infection and p16 expression were not involved with PD-L1 expression. Further clinical trials warrant the benefits of immunotherapy in patients with PD-L1-positive OSCC.

1. Introduction

Despite advancements in diagnosis and treatment, oral squamous cell carcinoma (OSCC) remains a devastating disease with high mortality and morbidity rates. The GLOBOCAN database in 2020 revealed that globally, approximately 380,000 new cases of oral cavity and lip cancers represented 2% of all cancer cases and caused approximately 178,000 deaths (1.8% of all cancer deaths) [1]. In certain parts of the world for example Thailand, the rate of OSCC has dramatically increased in the last two decades [2, 3]. The classic risk factors associated with OSCC include tobacco use, alcohol consumption, and betel quid chewing [4]. Recently, human papillomavirus (HPV) infection has been recognized as an emergent risk [5]. High-risk HPV particularly types 16 and 18 are the most common HPV genotypes detected in OSCC. It is believed that oncogenic E6 and E7 proteins in high-risk HPV are key driven factors for OSCC carcinogenesis [6, 7, 8]. In general, HPV-positive oropharyngeal carcinoma shows a strong correlation with p16 expression, given that E7 oncoprotein can inhibit function of retinoblastoma protein (pRb), resulting in p16 overexpression [9]. The associations between HPV and p16 expression in OSCC, however, remains unconclusive [10].

Evasion of immunosurveillance is an early event in carcinogenesis. One of the pivotal mechanisms of evasion of immunosurveillance is overexpression of programmed cell death-ligand 1 (PD-L1) of the tumor cells. Under normal circumstances, T cells express programmed death 1 (PD-1) receptors on the cell surfaces that bind PD-L1 located on the cell surfaces of antigen-presenting cells and resting lymphocytes to...
regulate immune homeostasis [11, 12]. In the microenvironment of the neoplastic stroma, the tumor cells can also express PD-L1 and bind those T cells, causing T cell apoptosis and weakening of the immune system. To further induction of PD-L1 expression in the tumor cells, activated T cells produce many cytokines including interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10), and interleukin-6 (IL-6). These cytokines then activate the tumor cells and signalize the production of PD-L1 [13]. T cells become exhausted and no longer regulate immune homeostasis, leading to evasion of immune surveillance of the host [11, 14, 15]. Evidently, overexpression of PD-L1 has been detected in various malignant neoplasms, including lung, ovary, colon, brain, kidney, esophageal, stomach, and breast cancers. PD-L1 overexpression in these malignancies was found to be associated with negative prognostic values [16, 17, 18, 19, 20, 21]. Although a wide range of PD-L1 expression (7%-87%) in OSCC has been reported [22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33], previous systematic reviews and meta-analyses have shown conflicting results in PD-L1 expression in OSCC and prognostic values [34, 35, 36]. Some studies were in agreement with the findings of other malignancies, while the others revealed no correlation with adverse prognostic values [33].

Previous studies have revealed that PD-L1 might play a role in persistent infection of HPV and evasion of immune surveillance during malignant progression [37, 38]. For example, in cervical cancers, E5 and E6/E7 oncoproteins of high-risk HPV can regulate PD-L1 expression of the tumor cells by various signaling pathways, resulting in compromised immunity of the host [39]. In oropharyngeal cancers, HPV-positive cases were strongly associated with PD-L1 expression [37]. In OSCC, although positive association between HPV infection and PD-L1 expression has been found in some studies [38, 39, 40, 41, 42], the others have shown no association [29, 30, 43, 44, 45].

The objectives of the present study were to investigate (1) the expression of PD-L1 in OSCC by a means of immunohistochemistry (IHC) using 85 paraffin-embedded formalin-fixed specimens, collected from two oral pathology laboratories during 2011–2019 and (2) the associations between PD-L1 expression and HPV-16/18 status, p16 expression, demographic and clinicopathologic parameters or risk behaviors in patients with OSCC. The HPV-16/18 status of OSCC studied by polymerase chain reaction (PCR) and p16 expression in OSCC investigated by IHC were reported in our previous study. The associations between PD-L1 expression and all parameters were analyzed by Chi-square, Fisher's exact tests, and multivariate logistic regression. The experimental settings were performed at the Faculty of Medicine, Chiang Mai University, Thailand. The outcome of the present study demonstrated that PD-L1 expressed in a significant proportion of patients with OSCC but PD-L1 expression was not statistically associated with all parameters studied.

2. Materials and methods

2.1. Specimen collection

A total of 85 formalin-fixed, paraffin-embedded (FFPE) OSCC specimens were collected from the archives of two oral pathology laboratories: the Faculty of Dentistry, Chiang Mai University (CMU) (n = 45) and the Faculty of Dentistry, Prince of Songkhla University (PSU) (n = 40) during 2011–2019. All specimens were of incisional biopsy. The following ethical approvals were obtained from both institutions; CMU (no. 4/2021) and PSU (EC6016-31-L-LR). All of the specimens were primary tumors, histologically diagnosed as conventional OSCC (75 cases), microinvasive OSCC (6 cases) or oral verruccous carcinoma (OVC) (4 cases). The demographic and clinicopathologic features were collected from the laboratory records including age at diagnosis, gender, site of tumor, clinical tumor size, histologic grade and the history of risk behaviors (tobacco smoking, alcohol consumption, and betel quid chewing). The histopathologic diagnosis and grading of all specimens were re-evaluated by an experienced pathologist using the World Health Organization classification [46].

2.2. HPV DNA status and p16 expression

This study was a continual research from our previous investigation [10], where HPV16 and 18 DNA status were detected by PCR. The amplification of HPV16 and 18 DNA were performed using the forward and reverse primers specific to the E6 gene of HPV16 and 18. The PCR components were the same as those of the β-actin gene. Positive controls for HPV16 and 18 were obtained from DNA isolated from SiHa and HeLa cell lines, respectively. A sterile water with negative control was also included. The PCR conditions were 94°C for 5 min, 45 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 7 min. These PCR procedures were tested for sensitivity using serially diluted DNA extracted from SiHa and HeLa cell lines.

The expression of p16 was assessed by a means of IHC using the CINtec™ p16 antibody Histology Kit (clone E6H4, MTM/Roche laboratories AG, Heidelberg, Germany) and the Ventana Benchmark ULTRA autostainer (Ventana Medical Systems, Tucson, AZ, USA). A cervical carcinoma with high p16 expression was used as a positive control in each batch. Specimens with any staining above the background in the invasive parts of the tumor were considered p16-positive.

2.3. PD-L1 immunohistochemistry

Each specimen was sectioned with a thickness of 3-μm. Subsequently, the sections were deparaffinized in xylene and graded alcohols, and rehydrated. The target retrieval procedure was accomplished using EnVision FLEX Target Retrieval Solution (Dako, Carpinteria, California, USA), at low pH 6.0 for 53 min at room temperature. PD-L1 IHC was performed with the Ventana Benchmark ULTRA Autostainer (Ventana Medical Systems, Tucson, AZ, USA) using a mouse monoclonal PD-L1 antibody (clone 22C3, 1:200 dilution, Dako, Carpinteria, California, USA) for 60 min at room temperature. Sections were counterstained with hematoxylin and observed under a microscope at a magnification of 200×. PD-L1 immunostaining of each specimen was evaluated by two examiners. PD-L1 status was assessed using a combined positive score (CPS), previously reported [47, 48]. The CPS was analyzed as the number of PD-L1-positive tumor cells, lymphocytes, and macrophages divided by the total number of tumor cells, multiplied by 100. Any convincing partial or complete linear membrane staining of tumor cells (at any intensity) was considered as positive PD-L1 staining and was included in the scoring. Any membrane and/or cytoplasmic staining of monoclonal inflammatory cells (MICs) within tumor nests and adjacent supporting stroma was also considered positive PD-L1 staining and was included in the scoring. Neutrophils, eosinophils, plasma cells, and other MICs associated with benign structures or ulcers were excluded from the CPS score [49]. PD-L1 expression of all specimens was divided into two groups scoring, including negative PD-L1 expression (CPS <1) and positive PD-L1 expression (CPS ≥1). CPS of each case was scored by consensus of two examiners. The positive control was PD-L1-positive tonsillar carcinoma. The sections omitted the secondary antibody were used as negative control.

2.4. Statistical analysis

The statistical analysis was performed using the IBM® SPSS® Statistics version 23 (Armonk, NY, USA). The Student’s t-test was used to compare the mean age at diagnosis of the patients with positive and negative PD-L1 status. The Chi-square or Fisher's exact test was used to analyze the associations between PD-L1 expression and the categorical variables including age, sex, site of the tumor, clinical tumor size, histopathologic variant and grade, risk behaviors, HPV-16/18 infection, and p16 expression. The results with p-value less than .05 were considered statistically significant.

Multivariate logistic regression was performed to investigate the association between PD-L1 expression and independent variables. Impu-
conducted using the multiple imputation (MI) with a total of 31 imputed datasets, based on the rule of thumb. Independent variables including age, sex, site, histologic variant/grade, p16 expression, HPV 16/18 DNA status were selected to predict the missing values, using logistic regression. Any variables with more than 50% missing data were not included in the analysis.

3. Results

According to our previous data, 53 out of 85 cases (62.4%) were positive for p16 and 14 cases (16.5%) were positive for HPV16/18 DNA [10]. 25.9% (22 out of 85 cases) of all cases were PD-L1-positive (CPS ≥1). PD-L1 expression on the tumor and inflammatory cells is displayed in Figure 1. OSCC without PD-L1 expression is shown in Figure 2. The associations between PD-L1 expression and the demographic and clinicopathologic parameters are shown in Table 1. The associations between PD-L1 expression and risk factors including tobacco smoking, alcohol consumption, and betel quid chewing are shown in Table 2. The associations between PD-L1 expression and p16 expression and HPV16/18 DNA status are shown in Table 3. Essentially, there were no significant associations between PD-L1 expression and all parameters studied. Further, the results of a multivariate logistic regression used to analyze the associations between PD-L1 expression and any parameters studied are shown in Table 4. Again, there were no significant associations between PD-L1 expression and any parameters studied. The positive and negative control sections were appropriately stained.

4. Discussion

PD-L1, located on cell surfaces of hematopoietic cells, normally functions as a key regulator for immune homeostasis [11, 13]. As neoplasms progress, the tumor cells are overexpressed with PD-L1. Upon binding the specific receptors, PD-1, resided on T cells, T cells are signalized into the programmed cell death pathway, resulting in T cell apoptosis. These eventually lead to weakening of the immune defenses against the tumor cells, so that the tumor cells are now able to evade the immune surveillance. Previous investigations have demonstrated overexpression of PD-L1 in various neoplasms including OSCC and subsequently develop immunotherapy to target both PD-L1 and PD-1, aiming to control tumor growth [14].

In the present study, we found that PD-L1 expression was positive in 25.9% of all cases at CPS ≥1, implying that this subpopulation of patients is eligible to immunotherapy targeting PD-L1, recommended by FDA [47]. Prior studies have revealed a variable range of PD-L1 expression (7%–87%) in OSCC [22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32]. These discrepant results may be due to using different clones of the antibody, different scoring techniques for immunostaining, different cutoff-point values, different demographic-clinicopathologic-risk data, and heterogeneity of the tumors studied. A recent review study in head and neck cancer suggested that PD-L1 expression is dynamic and subject to temporal variations and spatial heterogeneity [50]. PD-L1 expression can be altered from stages of tumor detection to tumor recurrences or progression, and can even show different expressions between the primary and co-existing secondary lesions. These findings suggest that PD-L1 expression at different stages of tumors should be interpreted with caution.

Various PD-L1 antibody clones used in IHC may influence the levels of PD-L1 expression and the application for targeted therapies. The antibody clone 22C3 pharmDx (Dako) is one of the most widely utilized anti-PD-L1 antibodies, approved by FDA since 2015 as a companion diagnostic test for pembrolizumab [51]. Other companions or complementary diagnostic tests have also been established and used for PD-L1 expression assessment such as antibody clones PD-L1 28-8, SP142, SP263, and 73-10, coupled with nivolumab, atezolizumab, durvalumab, and avelumab, respectively [52]. A recent study in head and neck squamous cell carcinoma (HNSCC) revealed that performance of different diagnostic anti-PD-L1 antibody clones is less robust and interchangeable compared to reported data of other malignancies. It is, therefore, recommended that determination of PD-L1 expression for therapeutic decision making in HNSCC may be aided by back-to-back testing of different anti-PD-L1 antibody clones [53].

Another aspect that may impact the levels of PD-L1 expression is the scoring assessment. Currently, tumor proportion score (TPS), evaluating PD-L1 in the tumor cells and CPS, evaluating PD-L1 in both the tumor and immune cells are the most common procedures for scoring PD-L1 expression in many tumor systems [54]. A recent study in gastric cancer reported that PD-L1 expression on both the tumor and immune cells (CPS) are a better predictive marker when compared to the expression of PD-L1 on the tumor cells alone (TPS) [55]. PD-L1 expression on the immune cells in the tumor microenvironment could also predict responses to PD-L1/PD-L1 immunotherapies [56]. Notably, a study in HNSCC revealed that CPS ≥50 may be used interchangeably with TPS 50% to determine PD-L1 status in patients but CPS is more sensitive than TPS at lower cutoffs [57]. Thus, CPS appears to be more advantageous over TPS and has currently used to predict pembrolizumab responses in HNSCC.

Some prior studies have revealed that the overexpression of PD-L1 was significantly associated with female patients [34, 35, 36, 58],...
older age [43], advanced histologic grading (poorly/moderately differentiated), lymph node metastasis [34, 58], or classic risk behaviors (non-smoking and non-drinking) [59, 60, 61]. However, the other studies, as well as our study, have found no associations between PD-L1 expression and demographic, clinicopathologic, or risk parameters in patients with OSCC [32, 45, 62]. Interestingly, studies on oropharyngeal carcinoma have also shown discordant results, positive associations and negative associations between PD-L1 expression and demographic, clinicopathologic, or risk parameters [38, 63]. These discrepant results remain unclear.

High-risk HPV particularly types 16 and 18 have long been known to cause cervical cancer [7]. Since then, high-risk HPV has also been detected in malignancies of many organ systems for example anogenitalia and head and neck cancer including OSCC [64]. Previously, we have

### Table 1. The associations between PD-L1 expression and demographic and clinicopathologic parameters.

| Parameters                                           | PD-L1 Positive | PD-L1 Negative | p-value |
|------------------------------------------------------|----------------|----------------|---------|
| Mean age at diagnosis (SD) (Year, n = 85)            |                |                |         |
| Age group (n = 85) <50 years old                     | 5 (33.3%)      | 10 (66.7%)     | .521b   |
| ≥50 years old                                        | 17 (24.3%)     | 53 (75.7%)     |         |
| Sex (n = 85)                                         |                |                |         |
| Male                                                 | 11 (27.5%)     | 29 (72.5%)     | .748e   |
| Female                                               | 11 (24.4%)     | 34 (75.6%)     |         |
| Site (n = 85)                                        |                |                |         |
| Tongue                                               | 6 (23.1%)      | 20 (76.9%)     | .410b   |
| Gingiva/alveolar mucosa                              | 12 (35.3%)     | 22 (64.7%)     |         |
| Floor of the mouth                                   | 1 (33.3%)      | 2 (66.7%)      |         |
| Buccal/labial mucosa                                 | 2 (25.0%)      | 6 (75.0%)      |         |
| Retromolar area                                       | 1 (33.3%)      | 2 (66.7%)      |         |
| Lip                                                   | 0 (0.0%)       | 3 (100.0%)     |         |
| Palate                                                | 0 (0.0%)       | 8 (100.0%)     |         |
| Tumor size in greatest dimension (n = 58, missing data, 27 cases (31.8%)) ≤2 cm | 7 (22.6%) | 24 (77.4%) | .377b |
| >2 cm and ≤4 cm                                      | 7 (31.8%)      | 15 (68.2%)     |         |
| >4 cm                                                 | 0 (0.0%)       | 5 (100.0%)     |         |
| Histologic variant/grade (n = 85)                    |                |                |         |
| Microinvasive SCC                                     | 0 (0.0%)       | 6 (100.0%)     | .165b   |
| Well differentiated SCC                               | 15 (23.8%)     | 48 (76.2%)     |         |
| Moderate differentiated SCC                           | 5 (50.0%)      | 5 (50.0%)      |         |
| Poorly differentiated SCC                             | 1 (50.0%)      | 1 (50.0%)      |         |
| Verrucous carcinoma                                  | 1 (25.0%)      | 3 (75.0%)      |         |

SD: standard deviation, SCC: squamous cell carcinoma, * Student’s t-test, b Fisher’s exact test, c Chi-square test, p-value <.05 is considered significant.

### Table 2. The associations between PD-L1 expression and risk behaviors.

| Parameters                                      | PD-L1 Positive Case (%) | PD-L1 Negative Case (%) | p-value |
|-------------------------------------------------|-------------------------|-------------------------|---------|
| History of tobacco smoking (n = 25, missing data, 60 cases (70.6%)) |                |                        |         |
| Ever                                            | 5 (29.4%)               | 12 (70.6%)              |         |
| Never                                           | 3 (37.5%)               | 5 (62.5%)               |         |
| History of alcohol consumption (n = 14, missing data, 71 cases (83.5%)) |                |                        |         |
| Ever                                            | 3 (48.9%)               | 4 (51.1%)               |         |
| Never                                           | 3 (48.9%)               | 4 (51.1%)               |         |
| History of betel quid chewing (n = 12, missing data, 73 cases (85.9%)) |                |                        | .545b |
| Ever                                            | 1 (16.7%)               | 5 (83.3%)               |         |
| Never                                           | 3 (50.0%)               | 3 (50.0%)               |         |

b Fisher’s exact test. c Chi-square test, p-value <.05 is considered significant.

### Table 3. The associations between PD-L1 expression and p16 expression and HPV16/18 DNA status.

| Parameters                                      | PD-L1 positive Case (%) | PD-L1 negative Case (%) | p-value |
|-------------------------------------------------|-------------------------|-------------------------|---------|
| p16 expression                                   |                         |                         |         |
| Positive                                        | 15 (28.3%)              | 38 (71.7%)              | .512b   |
| Negative                                        | 7 (21.9%)               | 25 (78.1%)              |         |
| HPV 16/18 DNA status                            |                         |                         |         |
| Positive                                        | 0 (0.0%)                | 2 (100.0%)              | .999b   |
| Negative                                        | 2 (25.0%)               | 6 (75.0%)               |         |
| Positive                                        | 1 (25.0%)               | 3 (75.0%)               |         |
| Negative                                        | 19 (26.8%)              | 52 (73.2%)              |         |

b Fisher’s exact test. c Chi-square test, p-value <.05 is considered significant.

### Table 4. Analysis by multivariate logistic regression of independent variables of PD-L1 expression.

| Parameters                                      | Odds ratio | 95% CI  | p-value |
|-------------------------------------------------|------------|---------|---------|
| Age                                             |            |         |         |
| ≥50 years old                                   | 1 (reference) |        |         |
| <50 years old                                   | 1.51       | 0.38–6.25 | .555   |
| Sex                                             |            |         |         |
| Female                                          | 1 (reference) |        |         |
| Male                                            | 1.01       | 0.35–2.85 | .998   |
| Site                                            |            |         |         |
| Others                                          | 1 (reference) |        |         |
| Tongue                                          | 1.63       | 0.34–3.22 | .546   |
| Gingiva/alveolar mucosa                          | 3.14       | 0.86–11.19 | .286   |
| Tumor size in greatest dimension                 |            |         |         |
| ≥2 cm                                           | 1 (reference) |        |         |
| ≤2 cm and ≤4 cm                                 | 1.08       | 0.31–3.84 | .901   |
| >4 cm                                           | 1.08       | 0.31–3.84 | .901   |
| Histologic variant/grade                         |            |         |         |
| Microinvasive SCC/Verrucous carcinoma            | 4.27       | 0.46–38.94 | .198   |
| Invasive SCC                                     | 4.27       | 0.46–38.94 | .198   |
| Positive                                        | 1 (reference) |        |         |
| Negative                                        | 1 (reference) |        |         |
| Positive                                        | 1.69       | 0.54–5.26 | .364   |
| Negative                                        | 1.54       | 0.33–6.99 | .576   |

p-value <.05 is considered significant.
detected HPV 16/18 with a range of 0%–20% in patients with OSCC from different populations in Thailand [10,65]. It was suggested that high-risk HPV may induce an overexpression of PD-L1 through E5- and E6/7-induced pathways [39]. Although some studies have found an association between HPV infection and PD-L1 expression in OSCC and oropharyngeal carcinoma [34,37,38,41], the others including our study have observed no association [29,30,44,45].

In HPV-related cervical and head and neck cancers, p16, a tumor suppressor protein, is aberrantly overexpressed and believed to occur through E7-induced pathway [7,66]. These suggest that p16 be used as a surrogate marker for cervical and head and neck cancers. Our previous results have demonstrated overexpression of p16 ranging from 24.4%–62.8% in OSCC cases [10,65]. However, the present study showed no association between p16 and PD-L1 expression in patients with OSCC. Again, previous studies have shown positive association [63,67,68] and no association between p16 and PD-L1 expression [43,69]. Collectively, these conflicting findings may indicate that high-risk HPV may be an alternative mechanism to induce PD-L1 expression in OSCC.

As PD-L1 is shown to be overexpressed in many malignancies, particularly non-small cell lung cancer, skin melanoma, urothelial cancer, and lymphoma, targeted therapy for blocking PD-1/PD-L1 has been developed and found to improve significant survival and treatment outcomes [70]. Immunotherapies for blocking PD-1/PD-L1 including pembrolizumab and nivolumab have been approved by FDA since 2019 for the treatment of advanced-stage head and neck cancer as an alternative treatment and the treatment of unresectable recurrent/metastatic head and neck cancer as the first-line treatment [47]. The KEYNOTE-012 and KEYNOTE-040 studies have reported that patients with PD-L1-expressed head and neck cancer determined by CPS >1 showed a dramatic increase in overall survival (OS) with treatment with pembrolizumab [71,72]. Interestingly, when using CPS ≥20, a significantly better OS than those with CPS 1≥ was observed [73]. Taken together, selection of CPS cutoff points may also be an important factor for considering the use of immunotherapies at different socio-economic healthcare settings in order to justify cost-effectiveness of this adjuvant treatment.

While the role of PD-L1 has been well established in OSCC, the role of PD-L1 in oral premalignant lesions/conditions or potentially malignant disorders (PMDs), for example leukoplakia, erythroplakia, and oral lichen planus in OSCC carcinogenesis remained an issue for further explorations. Previous studies have revealed a wide range of PD-L1 positivity from 0%–100% in oral PMDs [22,74,75,76,77,78,79]. Interestingly, a systematic review and meta-analysis demonstrated that PD-L1 expression appears to increase in more severe disorders. The highest expression of PD-L1 was found in invasive OSCC, followed by oral PMDs, while normal mucosa showed no or low expression [80]. Moreover, a recent study revealed that there is a significant association between PD-L1 expression in oral PMDs and malignant transformation, emphasizing the importance of PD-L1 in immune checkpoint and suggesting that PD-L1 be used as a biomarker for malignant transformation [74,75].

The limitations of our study were essentially a lack of certain clinical data of patients including clinical staging (tumor size, lymph node involvement, metastasis), treatment modalities and outcomes, and survival. This missing information occurred mainly due to the fact that the present investigation was retrospective in nature and the specimens used were of incisional biopsy performed at the Faculty of Dentistry, hence those clinical data mentioned above were not recorded and unable to be retrieved. Future prospective studies should warrant more informative results, particularly the associations between PD-L1 expression and prognostic values, treatment modalities and outcomes, and survival of patients with OSCC. In addition, the measurement of PD-L1 expression in biopsy material may provide disadvantageous results when compared to resection specimens. Due to the fact that the biopsy specimens are generally smaller with limited malignant tissue quantity, the interpretation of PD-L1 expression from biopsy material may lead to false negative or under-estimated levels of the expression. Thus, CPS scoring in biopsy material should be performed and interpreted with cautions [81,82].

In conclusion, 25.9% of all OSCC cases were PD-L1-positive, suggesting that this subpopulation of patients be candidates for anti-PD-1/PD-L1 immunotherapies. There were no significant associations between PD-L1 expression and all parameters investigated, suggesting that anti-PD-1/PD-L1 immunotherapies may be universally used for this group of patients with OSCC, regardless of HPV/p16 status, demographic, clinicopathological, and patients' risk data. Studies on the clinical trials using anti-PD-1/PD-L1 immunotherapies for patients with PD-L1-positive OSCC are needed in order to further explore treatment outcomes and prognosis of those patients. In addition, the use of PD-L1 expression as a predictive marker for treatment responses warrants further investigation.
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