Association of Programmed Death Ligand-1 Overexpression with the Grade and Stage of Oral Squamous Cell Carcinoma

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ABSTARCT

Programmed Death Ligand-1(PD-L) expression can be used to predict targeted therapy against PD-L1/PD-1 pathway in oral squamous cell carcinoma (OSCC). Forty-nine patients were included in the study. Out of the total, 42 well differentiated tumor 3 (7.1%) exhibited strong PD-L1 membranous and cytoplasmic staining, 14 (33.3%) weak staining, and 25 (29.5%) negative staining. Moderately differentiated tumor, (6/49), showed strong staining in 1 (16.7%), weak in none, and negative in 5 (83.3%) cases. Poorly differentiated tumor (1/1) showed strong staining (100%) (p = 0.018). Stage I tumors (n = 38) showed strong staining pattern in 4 (10.5%) cases, weak in 10 (26.3%), and negative in 24 (63.2%) cases. Stage II (n = 8) tumors had strong PD-L1 expression in 1 (12.5%), weak in 3 (37.5%), and negative in 4 (50%) cases. Stage III had negative in 2/3 (66.7%), weak in 1/3 (33.3%) cases, (p = 0.929).

Key Word: *Grade, PD-L1, Stage, Squamous cell carcinoma, Oral cracinoma.*

How to cite this article: Qureshi ZM, Qamar S. Association of Programmed Death Ligand-1 Overexpression with the Grade and Stage of Oral Squamous Cell Carcinoma. *J Coll Physicians Surg Pak* 2020; **30(06)**:662-664 https://doi.org/10.29271/jcpsp.2020.06.662.

Oral squamous cell carcinoma (OSCC) of head and neck has significant mortality and morbidity due to high incidence, recurrence, and metastasis.1 SCC is the fifth most common cancer worldwide; and in Pakistan, 8-22% of oral malignancies are SCCs.2 Search for molecular and biomarkers is going on to predict prognosis and guide immunotherapy in SCC.3,4 Programmed cell death protein (PD-1), a member of CD 28 family, is immune checkpoint regulator, and expressed on activated T cells. It can bind to PD-L 1 (Programmed death ligand 1/ B7-H1) and PD-L2 (Programmed death ligand 2/ B7-DC) present on antigen presenting cells. PD-L1 is located within the cell membrane and its main function is to suppress immunity and T cells in pregnancy, grafts and autoimmune diseases.5 PD-L1 is normally expressed in macrophages, salivary gland ducts, placenta and spleen. Carcinomas of colon, skin, endometrium, liver, lung, ovary, stomach, and thyroid show positive staining. Tumors are immunogenic and they need to create immunosuppressive microenvironment to protect themselves from immune attacks. Thus, cancer cells in order to evade host immunity, overexpress PD-L1 on their membranes and result in aggressive behaviour (higher grade and stage).

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Received: June 18, 2019; Revised: August 29, 2019; Accepted: October 14, 2019
DOI: https://doi.org/10.29271/jcpsp.2020.06.662

Immuno-oncology therapies are being developed to block interaction between PD-L1 and PD1, enabling T cells to kill cancer cells, such as durvalumab and avelumab. PD-L1 expression is an independent risk factor in males and smoker cancer patients. Objective response of PD-L1 expressing tumors to anti PD 1 therapy is better than non-expressing tumors.[6]

This study presents frequency of PD-L1 expression in squamous cell carcinoma of oral cavity and its association with grade and stage of cancer. This will highlight the frequency of PD-L1 expression in our cancer patients and evaluate the need of targeted immunotherapy for improvement of survival outcome. Paraffin blocks of diagnosed cases of OSCC were selected from July 2018-December 2018, for immunostain PD-L1, using avidin biotin technique. One hundred cell focus with highest intensity of color was counted and percentage of positive cells was calculated. Intensity score was measured as 0 = negative (<5% cells positive in lower 1/3 of epithelium), 1 = mild/ weak positive (5-50% cells positive in upper 2/3 epithelium, and 3= strong (>50% cells positive in upper 2/3 epithelium). Data was analysed by SPSS 21. Chi-square test was used to determine association between PD- L1 staining, smoking and grade of tumor. P <0.005 was taken as significant. Mean ± SD value was calculated for quantitative variables like age. Frequency and percentage was calculated for qualitative variables like gender, grade of tumor, stage and intensity score of PD-L 1.

Table I: Gender-wise clinico-pathological characteristics of squamous cell carcinoma patients.

Parameter	Male (%)	Female (%)	Total (100%)	p-value
A. Risk factor				0.0001
Paan chewing	5 (62.5%)	3 (37.5%)	8	
Smoking	19 (95%)	1 (5%)	20	
Unknown risk factor	4 (19%)	17 (81%)	21	
B. PD-L1 expression				0.072
PDL-1 negative	21 (70%)	9 (30%)	30	
PDL-1 weak	5 (35.7%)	9 (64.3%)	14	
PDL-1 strong	2 (40%)	3 (60%)	5	
C. Grade of SCC				0.211
Grade I SCC	23 (54.8%)	19 (45.2%)	42	
Grade II SCC	5 (83.3%)	1 (16.7%)	6	
Grade III SCC	0 (0%)	1 (100%)	1	
D. Stage of SCC				0.867
pT1	22 (57.9%)	16 (42.1%)	38	
PT2	4 (50%)	4 (50%)	8	
pT3	2 (66.7%)	1 (33.3%)	3	
E. Site of SCC				0.007
Tongue	6 (40%)	9 (60%)	15	
Cheek	9 (47.4%)	10 (52.6%)	19	
Gingiva	12 (100%)	0 (0%)	12	
Palate	1 (33.3%)	2 (66.7%)	3	

This study enrolled 50 patients of oral squamous cell carcinoma. One patient had insufficient data to stage the tumor which was excluded. A total of 49 patients were included. Mean age was 55.73 ±11.37 years. Youngest patient was of 27 years and oldest was of 80 years. Twenty-eight (57.1%) were males and 21 (42.9%) were females. Risk factors included were betel leaf *(paan)* chewing in 8 (16.3%), smoking 20 (40.8%) and no known factor in 21 (42.9%) patients. Type of biopsy sent to pathology department were 35 (71.4%) Incisional, 7 (14.3%) excisional and 7 (14.3%) neck dissection surgeries. Most common site of cancer was inner side of cheek (n=19, 38.8%), followed by tongue (15, 30.6%), gingival (12, 24.5%) and the least common site was palate (3, 6.1%). Forty-two (85.7%) cases were reported as well differentiated, 6 (12.2%) as moderately differentiated and only 1 (2%) as poorly differentiated squamous cell carcinoma. Tumors were staged according to pathological staging system based on tumor size. Thirty-eight (77.6%) were at stage I, 8 (16.3%) at stage II and 3 (6.1%) at stage III. PD-L1 immunostaining was negative in 30 (61.2%) and positive in 19 (38.7%). Out of the positive cases, 14 (28.6%) were weak positive and 5 (10.2%) were strong positive for PD L-1 (Table I). Out of the 42 well differentiated tumors, 3 (7.1%) exhibited strong PD-L1 membranous and cytoplasmic staining, 14 (33.3%) weak staining and 25 (29.5%) negative staining. Moderately differentiated tumor showed strong staining in 1 (16.7%), weak in none and negative in 5 (83.3%) cases. Poorly differentiated tumor 1/1 showed strong staining (100%, p=0.018, Table I) Stage I tumors (n: 38) showed strong staining pattern in 4 (10.5%) cases, weak in 10 (26.3%) and negative in24 (63.2%) cases. Stage II (n=8) tumors had strong PD-L1 expression in 1 (12.5%), weak in 3 (37.5%) and negative in 4 (50%) cases. Stage III had negative in 2/3 (66.7%), weak in 1/3 (33.3%) cases, (p=0.929). No identifiable risk factor was seen in stage I (16/21, 76.2%), stage II (3/21, 14.3%) and stage III (2/21, 9.5%). Smoking was present in 15/20 (75%) of stage I, 4/20 (20%) of stage II and 1/20 (5%) of stage III patients. Betel leaf *(Paan)* chewing was observed in 7/8 (87.5%) of stage I, 1/8 (12.5%) in stage II patients, (p=0.854). Grade and etiology when compared revealed no definite etiology in 19/21 (90.5%) well, 1/21 (4.8%) each in moderate and poorly differentiated tumors. History of smoking was present in 16/20 (80%) of well differentiated and 4/20 (20%) of moderately differentiated SCC (p=0.492). Strong PD-L1 staining was observed in 3/5 (60%) of unknown etiology, 1/5 (20%) each with history of paan and smoking. Weak PD-L1 was seen in 6/14 (42.85%) cases with unknown etiology and 4/14 (28.5%) each with paan and smoking. Negative PD-L1 was seen in 12/30 (40%) with unknown risk factor, while 15/30 (50%) of smokers and 3/30 (10%) of paan (betel leaf) users, p=0.389.

PDL-1 overexpression is associated with higher grades of oral squamous cell carcinoma, non-smokers and female gender. We could not find any relation between higher stage and over-expression.

This study is a small scale study due to limited resources avai-

lable, so its findings must be interpreted with caution. However antiPD-L1 therapy can prove to improve progression-free survival. Therefore, more studies with larger sample size, focusing on survival outcome, are needed to emphasise upon the prognostic role of PD-L1 expression and anti-PD-1 therapies.

CONFLICT OF INTEREST:
Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
ZMQ: Data collection.
SQ: Data analysis.

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