RESEARCH ARTICLE

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The Expression of *PDL-1* and *PD1* in the Microenvironment of Oral Squamous Cell Carcinoma

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Abstract

Objective: Immune checkpoint proteins, especially *PD-1*/*PD-L1*, play a vital role in controlling the intensity and duration of the immune response. However, cancer cells often over-expression *PDL-1* on their surfaces, which leads to the permanent activation of the *PD-1*/*PDL-1* pathway and exhaustion of T cells and creates a resistant tumor microenvironment. This study aimed to analyze *PD-1*, *PD-L1* expression in tumor cells (*PDL-1*/TC) and in Tumor-Infiltrating Lymphocytes (*PDL-1*/TILS) of OSCC patients and associated with and to correlate it with histologic grade of malignancy and clinicopathologic parameters. **Methods:** The sample consisted of 43 archived specimens of 43 patients of OSCC with Clinical features (gender, age, smoking, clinical stage) collected from medical records between 2014-2021. The intensity of *PD-1*, *PDL-1*/TC, *PDL-1*/TILS, positive cells were assessed by immunohistochemistry. **Result:** *PDL-1*/TILS and *PDL-1*/TC were observed in all specimens except two cases in well-differentiated were negative. *PDL-1*/TILS was significant between histological grades($P=0.004<0.05$). There was no significant between *PDL-1*/TC and *PDL-1*/TILS and between poorly differentiated and moderately differentiated groups' ROC P values ($P=0.133$, $0.340>0.05$) respectively. There is a difference between *PDL-1*/TC and *PDL-1*/TILS between poorly differentiated and well-differentiated groups ROC P value (0.005, 0.028), Sensitivity (0.857, 0.857), specificity (0.765, 0.824) respectively and there is a difference between and *PDL-1*/TILS moderate differentiated and well-differentiated groups ROC P value (0.133,) Sensitivity (0.737), specificity (0.765). No differences between *PDL-1*/TC and moderate and well-differentiated groups P value (0.173). There is a significant correlation between *PDL-1*/TC and *PDL-1*/TILS with an age $P>65$ value (0.032) in the well-differentiated group. *PDL-1*/TC and *PDL-1*/TILS with the depth of invasion (DOI) in the well-differentiated group. No significant correlation was obtained between *PDL-1*/TC and *PDL-1*/TILS and smoking, clinical stage, and gender. No significant correlation was obtained between PD1 and the histological grades or clinicopathologic characteristics. **Conclusion:** *PDL-1*/TILS and *PDL-1*/TC are independent prognostic factors in OSCC and PDL1-/TILS has an important role.

Keywords: *PD1- PDL-1*- TILS- OSCC- Tumor-Infiltrating Lymphocytes- Immune checkpoints

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Introduction

Oral Squamous Cell Carcinoma accounts for 90% of all oral malignancies [1]. It was estimated that about 300,000 new cases were diagnosed as OSCC and about 150,000 cases have died from OSCC in2020 [2, 3]. OSCC predominantly occurs in males who are in their fifth to seventh decade of life [4]. Recent studies also found an association of squamous cell carcinoma with females under the age of 45with new cases in young patients [5, 6].

Tumor microenvironment plays a key role in tumor growth and progression [7], The TME contains many components including immune cells (Tumor-Infiltrating Lymphocytes [TILs], macrophages, and dendritic cells); cancer-associated fibroblasts, and endothelial cells, fibronectin and collagen fibers soluble factors [8, 9]. It has been shown that immune checkpoints play an important role in the tumor microenvironment and can be manipulated as a mechanism for tumor immune evasion [10]. the most recent studied - is *PD-1/PD-L1* pathway. which control The duration and intensity of the immune response and prevents autoimmune diseases [11, 12]. However, in the tumor microenvironment (TME), the *PD-1/PD-L1* axis is involved in cancer cells immune escape [13]. And the binding of *PD-1* to programmed death ligand 1 (PDL1),negatively regulates T-cell-mediated immune responses, T cell activation [14] and can be manipulated as a mechanism for tumor immune evasion [15].

PD-1(CD279) a type I transmembrane protein composed of 288 amino acid and belong to the immunoglobulin CD28 family [16]. It was first described in the early 1990s given its expression during induction of apoptosis in a T-cell hybridoma [17] *PD-1* is expressed

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in many immune cells, including peripherally activated T cells, B cells, monocytes, natural killer (NK) cells, and certain DCs [18]. and the recent studies showed that *PD1* is expressed in tumor cells in some cancers, such as melanoma and liver cancers [19, 20] while its ligands *PD-L1* and *PD-L2* are mainly expressed in antigen-presenting cells and tumor cells [21].

programmed death ligand 1 (PDL1/CD274) is a 290-amino-acid type I transmembrane glycoprotein belonging to B7-CD28 family of the immunoglobulin superfamily [22]. It is expressed in tumor cells and (APCs) various types of immune cells, including activated B cells and T cells, macrophages, and dendritic cells [18, 20] and the engagement of PD-L1 with *PD-1* of T cell creates T cell exhaustion, , and interleukin-10 (IL-10) production [24] .Another interacting molecule such as B7-1 (CD80), a protein expressed on activated T cells and APCs, interacts with the PD-L1 of tumor cells causing the negative regulation of activated T-cell [25].

The interaction between tumor cells and T cells and immune checkpoint is complex in tumor microenvironment of OSCC. We aim in this study to evaluate the correlation between the expression of *PD1*/*PDL-1* pathway and T cells in OSCC microenvironment.

Materials and Methods

The sample

43 resection specimens of 43 patients of OSCC treated from 2014 to 2021 were taken from the archives of Damascus hospital. clinical data from medical records of patients were obtained (e.g., gender, age, smoking, clinical stage,....). Tumor stage and clinical stage were classified as initial (I and II) or advanced (III and IV). (Table 1). Histologic grades were classified according to WHO criteria into three groups well- differentiated, moderately differentiated, and poorly differentiated tumors [26]. clinical UICC-stage (I–IV) and TNM classification of OSCC were documented in the histologic reports according to the guidelines of the most recent World Health Organization classification of tumors of the head and neck and the International Union Against Cancer [27].

Immunohistochemistry Stain

Paraffin-embedded specimens were cut into 4 µm thickness and mounted on immunological slides and then dried by autoclave. Sections were deparaffinized in xylene for 3 minutes followed by rehydrated with an alcohol gradient (100%, 95%, ,70%) then washed with water.

For antigen retrieval, and according to manufacturer's instruction of the Ventana company ®the sections were boiled in Immune DNA Retriever by microwave for 30 minutes followed by washing with (wash buffer) for 5mints followed with blocked by incubation with Poly Detector Peroxidase Blocker for 10 mints. then tissue sections were washed in TBS (wash buffer)for 5 minutes 3 times followed by incubation with the primary antibodies *PDL-1*(VENTANA, SP263) *PD1* VENTANA, for 1h at 4 °C overnight and then washed by TBS(Tris -Buffered Saline) for 15 mints After that the sections were incubation with the secondary antibody, Poly Detector plus link for 15 mints followed by washing with TBS and then incubation with Poly Detector HRP label for 15 mints followed by washing with TBS(Tris-buffered saline)for 15 mints followed by (DAB) for 15 min and then washed with H_2O. Finally the samples were counterstained with Meyer's hematoxylin and mounted.

Table 1. Clinicopathologic Features of Oral Squamous Cell Carcinoma (n = 43)

Clinicopathologic	Number	Percentage
Features	n=(43)	%
Age	43-82	64.7
Gender		
Male	24	55.8
Female	19	44.2
Smoking		
Yes	19	44.2
No	24	55.8
Clinical stage		
I/II	21	48.8
III/IV	22	51.2
Lymph node		
N0	27	62.8
N1	9	20.9
N2	7	16.3
Metastasis		
M0	0 (0)	0
M1	0 (0)	0
Location		
Toung	22	51.2
Other sites	21	48.8

Evaluation of PD1 and PDL-1

For each slide, five representative fields were selected for counted the numbers of *PD1*and *PDL-1* at high power (400 ×) The final density of each section was calculated as the average number of five high-power fields (HPFs). Only membranous *PD-L1* expression was considered positive in tumor cells, whereas cytoplasmic and/or membranous reactions were considered positive in TILs the cutoff is *PD-L1* ≥5 [28, 29]. for *PD1* cytoplasmic and/or membranous any staining considered positive in TILs. All slides were evaluated by two investigators blinded to the clinical data.

Statistical analysis

One-Way ANOVA test was used to associate expression of *PDL-1*/TC and *PDL-1*/TILS with histologic grade of malignancy. and Student's t-test was used for associations expression of *PDL-1*/TC and *PDL-1*/TILS clinicopathological parameters. operating characteristic (ROC) and area under curve (AUC) analyses were used to estimate the predictive value of *PDL-1*/TC and *PDL-1*/TILS + in the prognosis of OSCC. Sensitivity and specificity were estimated. Analyses were conducted with SPSS 13.0

Results

Patient Characteristics

The patients were 24 men (55.8) and 19 women (44.2) and ranged in age from 43 to 82 year (mean 64.7, standard deviation 9.4). The tumor sub site was classified as the tongue (n =22= 51.2%), and other sites of oral cavity (n =21= 48.8) is reported in 19 (44.2)., 21 (51.2) had stage I/ II, 22 22 (51.2%) had stage III /IV. table (1), OSCC grades were17(39.5%) well-differentiated, 19(44.2%) moderately and 7 (16.3%) poorly-differentiated (Table 1).

Associations between PD1, PDL-1/TC and PDL-1/TILS density and clinicopathologic features

No significant correlation was obtained between the expression of (*PDL-1*/TC, *PDL-1*/TILS and *PD-1*) with smoking, T stage and gender. There is a significant correlation between *PDL-1*/TC and *PDL-1*/TILS with ages≥65 p value (0.032) in well differentiated group (Table 2). And *PDL-1*/TC with the depth of invasion (DOI) in well differentiated group (Table 3).

Associations between PD1, PDL-1/TC and PDL-1/TILS and histological grade of OSCC

The expression of *PD1*, *PDL-1*/TILS and *PDL-1*/TC were observed in all specimens. Figures (1, 2) except two cases in well differentiated were negative. There was a significant difference between *PDL-1*/TILS and the histological grades(P=0.004<0.05) Table 4. we noticed that *PD1* was expressed in some tumor cells Figure (1). And most expression was in poor- differentiated group with no significant differences with histological grades.

The prognostic value of PDL-1/TILS and PDL-1/TC for poorly-differentiated OSCC between the three groups

There was no significant between *PD1*, *PDL-1*/TC and *PDL-1*/TILS and between poorly differentiated and moderate differentiated groups ROC P value (P=0.236,0.133, 0.340>0.05) respectively. there is a difference between *PD1*, *PDL-1*/TC and *PDL-1*/TILS between poorly differentiated and well differentiated groups ROC P value (0.024,0.005, 0.028) Sensitivity (0.857,0.857, 0.857), specificity (0.765,0.765, 0.824)

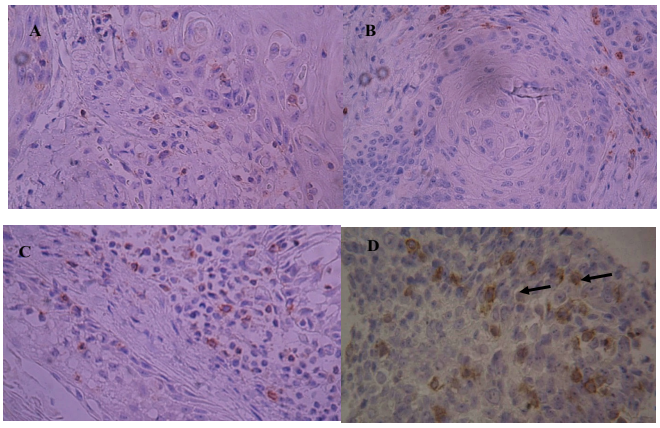

Figure 1. *PD1* Expression in OSCC A: well-differentiated samples, B: Moderately-differentiated samples, and C: poorly-differentiated samples. D: *PD1* expression in some tumor cells (the arrows)

Table 2. Average Numbers of *PD1, PDL-1*/TILS and *PDL-1*/TC and coleration with clinicopathologic features of OSCC according to histological grades of OSCC. P and r indicate P-value and correlation coefficient from T student test Asterisk (*) indicates P-value and correlation coefficient from Pearson test.

Parameter	OSCC grades	*PDL-1/TILS*		*PDL-1/TC*		*PD1*	
		P-value	The difference between the two averages	P-value	The difference between the two averages	P-value	The difference between the two averages
Sex	well-differentiated	3.86	0.559	6.18	0.693	4.85	0.264
	Moderately-differentiated	4.61	0.524	10.61	0.426	6.19	0.293
	poorly-differentiated	14.5	0.246	0.9	0.96	0.58	0.58
							0.58
Age≥65	well-differentiated	16.29	0.023	41.33	0.013	9.23	0.06
P-value	Moderately-differentiated	0.89	0.905	7.18	0.601	6.68	0.267
	poorly-differentiated	9.58	0.418	2.97	0.824	6.67	0.552
Smoking	well-differentiated	5.21	0.448	5.87	0.305	1.89	0.682
P-value	Moderately-differentiated	5.87	0.411	16.52	0.92	2.51	0.671
	poorly-differentiated	9.58	0.418	2.97	0.824	6.67	0.552
stage I/II AND III/IV	well-differentiated	4.4	0.505	20.97	0.168	2.24	0.612
	Moderately-differentiated	5.58	0.294	4.01	0.771	0.11	0.986
	poorly-differentiated	24.33	0.107	1	0.966	4.5	0.78

Table 3. The Result of Pearson Test, which Present the Correlation of *PDL-1/TILS, PDL-1/TC* and *PD1* with Depth of Invasion(DOI).

Parameter	OSCC grades	*PDL-1/TILS*		*PDL-1/TC*		*PD1*	
		P-value*	Correlation coefficient value	P-value*	Correlation coefficient value	P-value*	Correlation coefficient value
Depth of invasion(DOI) *	well-differentiated	0.172	0.347	0.043	0.495	0.297	0.269
	Moderately-differentiated	0.606	0.126	0.601	4.01	0.687	0.099
	poorly-differentiated	0.95	0.029	0.07	0.716	0.199	0.552

respectively and there is a significant difference between *PDL-1*/TILS between moderately- differentiated and well- differentiated groups ROC P value (0.0103,). Specificity (0.765) Sensitivity (0.737) Table 5, Figure 3.

The value of Sensitivity and specificity of PDL-1/TILS and PDL-1/TC as a prognostic for poorly-differentiated OSCC in the group of well-differentiated and poorly differentiated

The best value between sensitivity and specificity was at the value of *PD1*, *PDL-1*/TILS and *PDL-1*/TC 40 /74 where the sensitivity value was equal to 0.714,0.857and the specificity value was equal to 0.941, 0.824and therefore we conclude that the value 40 /74 can be determined as a standard value for *PDL-1*/TILS and *PDL-1*/TC rate as a predictor of moderately-differentiated OSCC in a sample search. Table 6, Figure 4.

The value of Sensitivity and specificity of *PDL-1*/TILS and *PDL-1*/TC as a prognostic for poorly-differentiated OSCC in the group of Well-differentiated and Moderately differentiated. The best value between sensitivity and specificity was at the value of *PDL-1*/TILS 32.5 where the sensitivity value was equal to 0.737 and the specificity value was equal to 0.765 and therefore we conclude that the value 32.5 can be determined as a standard value for *PDL-1*/TILS rate as a predictor of moderately-differentiated OSCC in a sample search Table (6). The correlation of *PDL-1*/TILS and *PDL-1*/TC and the expression of T cells (*CD4*, *CD8*). From our previous study. (Wahbi and Mandili , 2022) we found a correlation between *PDL-1*/ TILS with the immune expression of *CD4* Table (7)

Discussion

Cancer cells express high levels of *PD-L1* witch activate the *PD-L1*/*PD1* pathway as an immune escape mechanism, that increases their survival rate [30]. The overexpressed of *PD-L1* on tumor cells binds to the *PD-1* on tumor-infiltrating lymphocytes (TILs), which counteracts the TCR-signaling cascade through

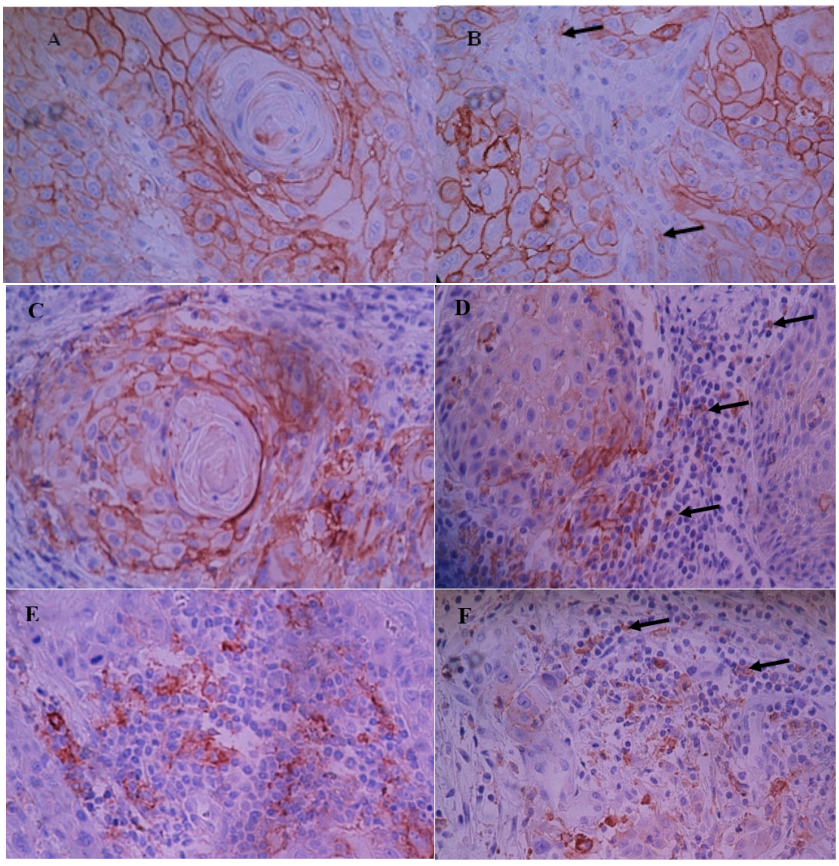

Figure 2. *PDL-1* and Expression in Tumor Cells and TILS (the arrows) OSCC A, B well-differentiated samples, C, D Moderately-differentiated samples, and E, F poorly-differentiated samples.

Table4. Average Numbers of *PD-1, PDL-1/TILS, PD-L1/TC* According to Histological Grades of OSCC

OSCC grades	Number of patients	*PD1*		*PDL-1/TILS*		*PDL-1/TC*	
		Mean± SD	P-value	Mean± SD	P-value	Mean± SD	P-value
			0.056		0.004		0.102
Well-differentiated	17	12.06± 8.69	F-value	21.71± 13.03	F-value	50.35± 30.79	F-value
Moderately- differentiated	19	18.21± 12.34	3.098	34.42± 15.02	6.429	64.89± 27.75	2.415
Poorly-differentiated	7	23.86± 12.99		42.14± 13.96		75.86± 18.77	

Table 5. The Results of (ROC) and Area under Curve (AUC) Test

Parameter	poorly-differentiated & Moderately-differentiated		well-differentiated & poorly-differentiated		well-differentiated & Moderately -differentiated	
	(ROC) and area under curve (AUC)	P.value	(ROC) and area under curve (AUC)	P.value	(ROC) and area under curve (AUC)	P.value
PDL-1/TILS	0.305	0.133	0.803	0.005	0.751	0.0103
PDL-1/TC	0.376	0.34	0.374	0.028	0.633	0.173
PD-1	0.376	0.34	0.79	0.028	0.644	0.141

Table 6. The Sensitivity and Specificity Values of *PD1, PDL-1/TILS* and *PDL-1*/TC between the Groups of Well-Differentiated and Poorly-Differentiated and the Groups of Well-Differentiated and Moderately –differentiated

well-differentiated and poorly-differentiated				well-differentiated and Moderately –differentiated
	PDL-1/TILS	PDL-1/TC	PD-1	PDL-1/TILS
Sensitivity	0.714	0.857	0.857	0.737
specificity	0.941	0.824		0.765
Ratio is equal to or greater than	40	74	16.5	32.5

phosphorylating SHP-2 [32], and represents the main inhibitory signal on T cell activity [10, 11].This study is a part of our research for Master's degree and we evaluated the expression of *CD4+* ,*CD8* in the same sample of this study [33] In this study we evaluated the expression of *PD1* in TILS only, we noticed some positive tumor cells, indicating that tumor cells may express *PD1*; most studies on *PD-1* expression have focused on immune cells, making its expression and functions in cancer cells unclear [31, 32]. Recent studies have shown that cancer cells can express *PD1*; such as the study of Yao et al, [19], wich included a re-analysis of *PD-1* expression in different cancer types. Immunohistochemical staining for *PD-1* within this study also showed that hepatocellular carcinoma, renal carcinoma, urothelial carcinoma, testicular cancer, and melanoma have subsets of tumor cells staining positive for *PD-1* [18]. Other studies have reported the presence of *PD-1* in melanoma [34, 35], breast cancer [36] and liver cancer [37] and lung cancer [38]. However, the mechanism of *PD-1* expression in cancer cells is unknown, and may be explained by several mechanisms such as gene copy number modifications, epigenetic modifications, microenvironment, etc. According to the CCLE dataset, *PD-1* is expressed in some cancer cell lines, with potential effects on *PD-1* mRNA expression. In addition, cytokines and immune

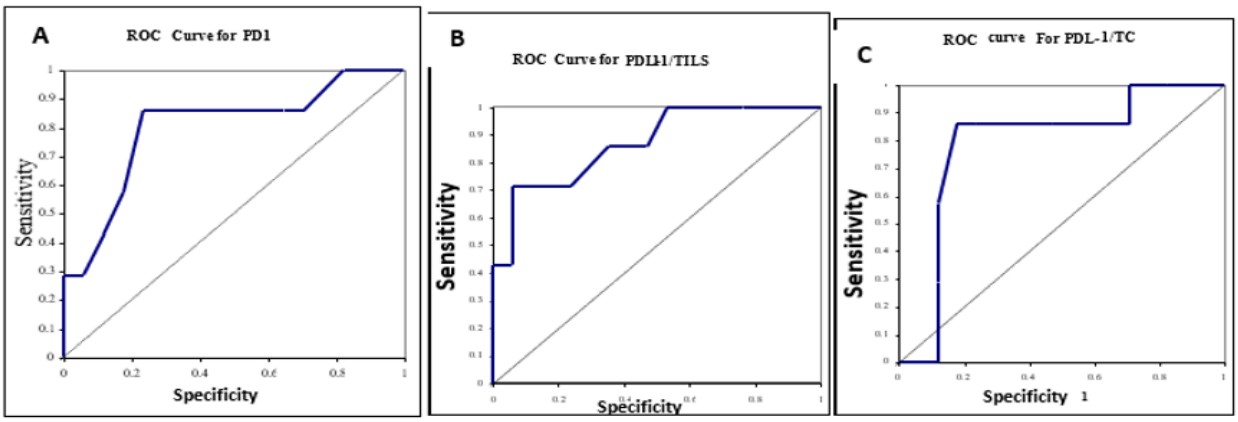

Figure 3. The ROC Curve between the Groups of Well-Differentiated and Poorly-Differentiated. (A) represent the ROC Curve for PD1, (B) represent the ROC Curve for *PDL-1*/TILS and C represent the ROC Curve for *PDL-1*/TC

Table 7. The Correlation of *PDL-1/TILS* and *PDL-1*/TC and the Expression of T Cells (*CD4, CD8*)

Parameter	Histological grade	*PDL-1/TC*		*PDL-1/TILS*	
		Correlation coefficient value	P. value	Correlation coefficient value	P. value
CD4	Well -differentiated	0.302	0.239	0.279	0.277
	Moderately -differentiated	0.093	0.706	0.289	0.231
	poorly -differentiated	0.49	0.316	0.003	0.436
CD8	Well-differentiated	-0.353	0.164	0.416	0.097
	Moderately -differentiated	0	1	0.09	0.715
	Poorly -differentiated	0.094	0.841	0.61	0.146

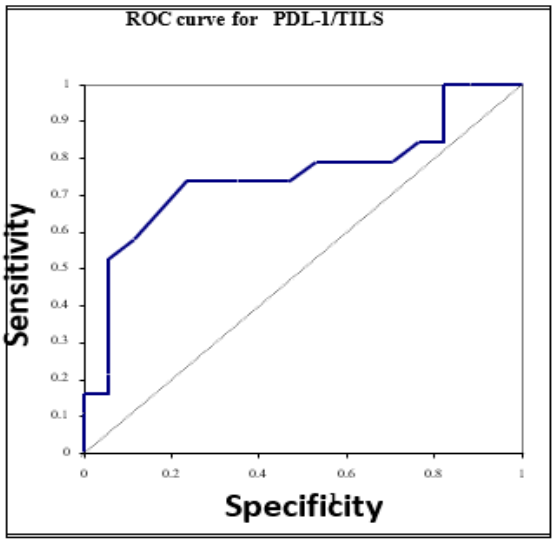

Figure 4. The ROC Curve for *PDL-1*/TILS between the Groups of Well-Differentiated and Moderately -Differentiated

cells present in the tumor microenvironment may also be involved in stimulating tumor *PD-1* expression [29] According to what was mentioned above, head and neck cancers did not express *PD1* in cancer cells, and our study can be considered the first study to indicate the presence of *PD1* in OSCC cells, but in our study we did not include *PD1* expression within the statistical analysis. We noticed that in well-differentiated cancer samples, *PD1* expression in TILS was positive, with two samples were having negative staining. This is considered a high expression rate compared to the study of Maruse et al. [39] that showed *PD-1* expression in 97 oral squamous cell carcinoma samples was positive in (61.9%) of cases.In our study, the highest expression was in poorly differentiated group, with no statistically significant differences in histological grades. We found a prognostic value between well-differentiated and poorly differentiated groups, the presence of negative samples in well-differentiated group can be explained by the presence of an effective immune response, while the increase in expression in poorly differentiated group indicates that cancer cells use the *PD1* pathway as an immune escape mechanism, and Creates an immune-resistant environment [15].The expression of *PD-L1* in cells Cancer in OSCC ranged from 10 to 90% of cases [40, 41]; In our study, the tumor cells showed positive expression for *PDL-1* in the all samples, between 1 and 98% in various histological grades, except two negative cases in the well-differentiated group without any statistically significant differences with the histological grades. The expression was heterogeneous. For *PDL-1*/TILS expression, we found statistically significant differences, where the expression was highest in the poorly differentiated group. We may explain that T cells during tumor growth are constantly stimulated by tumor antigens which lead to T cell exhaustion [42], which express immunosuppressive receptors such as *PD1* and its ligand *PDL-1* [43]. The study of Lechner [44]showed increased expression of *PD-L1* in tumor-infiltrating lymphocytes in OSCC. These results indicate that *PD-L1* that the expression of *PD-L1*in immune cells may be of equal or even greater biological importance than the expression in tumor cells, and this was suggested by a study by Gibbons Johnson [45]. In the study of Kim [46] on 402 patients with head and neck cancers, 204 of them OSCC. That study found that the *PD-L1* expression in TILS, not in tumor cells, has a better prognosis. Adamski [47] studied *PDL-1* expression in 159 patients with OSCC in the tongue and floor of the mouth. The expression of *PD-L1*/TC or *PDL-1*/TILS was not associated with age, gender, clinical stage, histological grade, or smoking and alcohol habits [1]. This is similar to our results in this study; that we found that the expression of *PD1*, *PD-L1* /TILS and *PDL-1*/TC was not associated with gender, clinical stage, or smoking, except for the association of *PD-L1* /TILS with poorly differentiated cancer, and the association of expression of *PD-L1* /TILS and *PDL-1*/TC with ages older than 65 years, and we explain this by weak host immunity in the elderly, which may be affected by chronic diseases, with the presence of chronic infections, which may activate immunosuppressive signals, most notably *PD-L1*, and this contradicts the Mortin [48]; He compared the expression of *PD-L1* in 175 cases of OSCC ,There was an association between *PD-L1* expression with clinical stage, and age. In the study of Ahn, which was conducted on 68 patients with OSCC, he found no correlation between *PD-L1* expression *PD-L1* expression and clinical characteristics, while *PD1*+ expression was associated with older patients, and with advanced clinical stage, as well as in the study of Kouketsu ; He compared the expression of *PD1* and *PDL-1* between patients with OSCC (106 samples) and premalignant lesions (79 samples), and found that positive expression of *PDL-1* in OSCC was higher without differences with histological grades. the expression of *PDL-1* and *PD1* were associated

with tumor size, advanced clinical stage, lymphocytic infiltration, and depth of invasion [49]. and we agreed with his study only with the association of *PDL-1* expression and the depth of invasion.we explain our results by tumor development as a result of overexpressing *PDL-1* in tumor cells, thus suppressing immunity, and promoting tumor growth.

In our study, the expression of *PDL-1*/TC and the expression of *PDL-1*/TILS was not associated with gender, and this is consistent with the study of admaski [47]. In contrast, some studies found a correlation between *PDL-1* expression with females, such as the Hanna study. [50], as well as Satgunaseelan found an association of *PD-L1* expression with females and these studies explained their results to higher estrogen levels after menopause [6]. In contrast, Lin found an association of *PD-L1* expression in males and smokers [51].

The association of *PDL-1* expression in tumor cells with T cell expression has been evaluated in several studies such as Sales de Sá's study [9]; The expression of *PDL-1* was associated with the expression of CD3+ and *CD8*+, and was not associated with the expression of *CD4*+. Takahashi also found that patients with high expression of *PD-L1* combined with high infiltration of *CD4*+ had better outcomes than cases in which infiltration of *CD4*+ was low [52] . however, in the study of Lequerica and her colleagues, they showed that high infiltration of *CD4*+ and *CD8* + and *CD8+/FOXP3*+ were associated with higher positive expression of *PD-L1* on cancer cells [40]. In a study by Cho [53] *PDL-1* expression was associated with low *CD8*+ expression. but in the study of Mattox [54] they found no correlation between the expression of *PDL-1* and the expression of *CD4*, and they found a correlation with the expression of *CD8*, Conversely in our study there was no correlation between the expression of *PDL-1* in tumor cells and the expression of *CD8*+and *CD4* while we found a correlation between the expression of *PDL-1* in TILS and the expression of *CD4+*. This can be explained by the fact that *CD4* T cells are composed of heterogeneous subsets that perform functions. Promoting or anti-tumor depending on the secreted cytokines [55, 56]. And the expression of *PDL-1*/ TILS was associated with poorly differentiated group, we infer from this the predominance of the regulatory pattern of T cells [57]. We also note that all previous studies focused on the expression of PDL- 1 in tumor cells with T cells without mentioning its expression in TILS, so there are not enough studies for comparison.

In conclusion, we conclude that *PDL-1*/TILS, *PDL-1*/TC are independent prognostic factors in OSCC and PDL1-/TILS has an important role ,since it was associated to the histological grade and *CD4* infiltration.

Author Contribution Statement

Collecting data, research and writing the manuscript: Hanan Wahbi. Manuscript revision: Ahmad AL Manadili.

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Approval

It was approved by scientific committee, Faculty of dentistry, Damascus university Study Registration: number 2650 on the date of 2/8/2021 ,Scientific Research Committee /Damascus university

Ethical Declaration

The authors declare that they have no conflict of interest

Conflict of Interest

All patients have consented to all clinical data included in this study according to the ethical approval of the research (number 2650 on the date of 2/8/2021 ,Scientific Research Committee /Damascus university)

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