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Immunohistochemical Expression of Programmed Death-Ligand-1 (PDL-1) in Non-Melanoma Epidermal Skin Cancer with Clinicopathological Correlation

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ABSTRACT

Background: Blockade of the immune checkpoint pathway involving programmed death receptor ligand-1 (PD-L1) and programmed death-1 (PD-1) has demonstrated significant clinical efficacy across a broad spectrum of malignancies. Immunohistochemical (IHC) assessment of PD-L1 protein expression is increasingly recognized as a predictive biomarker for therapeutic response to these agents.

Aim of the study: This retrospective cross-sectional cohort study evaluated PD-L1 expression in non-melanoma skin cancers (NMSC), with a primary focus on squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). Also, to evaluate the correlation between PD-L1 expression in tumor cells (TCs) and tumor-infiltrating lymphocytes (TIL) with various clinicopathological parameters in the examined cases of BCC and SCC.

Patients and Methods: sixty specimens of wedge & excision from SCC& BCC cases were collected. Paraffin blocks in excision biopsy& Tissue microarray (TMA) blocks were prepared from paraffin blocks in excision biopsy. Slides were immunostained with anti-PDL1 antibody& PDL1 expression on TC & TIL was scored using intensity & percentage separately. The intensity of PD-L1 staining was graded as follows: 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). PD-L1 expression levels were determined based on the proportion of positive TCs or TILs of any staining intensity. For TCs, the categories were defined as 0 (negative), <10% positive, and >10% positive. For TILs, the classification was 0 (negative), <10% positive, and >10% positive. Statistical analysis was conducted using the SPSS software, Windows version 24 (Standard edition).

Results: Among the 60 NMSCs, 35 were BCC& 25 were SCC. In SCC, significant positive relationship was observed between PDL-1 expression on TC &TIL and many pathological risk factors as subtype, grade, thickness, depth of invasion, lymphovascular invasion (LVI), and perineural invasion (PNI), but no significant correlation was observed with clinical parameters except for correlation between PDL-1 intensity in TILs & case age. In BCC, no detectable significant relation was found between clinicopathological characteristics& intensity as well as percentage of PDL-1 expression on TC and TILs, but there is significant negative relationship was observed between PDL-1 percentage on TC, TIL & high-risk pathological subtypes. Also, significant negative relationship was observed between PDL-1 intensity on TC& high-risk pathological subtypes.

Conclusion: PDL-1 expression on TC& TIL in SCC was significantly correlated with many tumor risk parameters such as size, pathological subtype, grade, thickness, depth of invasion, LVI and PNI. In BCC, PDL-1 expression on TC& TIL shows significantly negative correlation with age of the case & pathological subtype of the tumor.

Keyword: Basal Cell Carcinoma, Cutaneous Squamous Cell Carcinoma; PD-L1.

INTRODUCTION

Non-melanoma skin cancer (NMSC) represents the most frequently diagnosed malignant neoplasm among white populations, including Caucasians, and is highly prevalent in Germany. In contrast, its occurrence is uncommon in African and Asian populations, largely due to the substantial photoprotective effect conferred by increased skin pigmentation [1].

NMSCs carry a lower likelihood of progression to advanced disease compared with cutaneous melanoma. For cases presenting with advanced NMSC, recent advances in immunotherapy—particularly immune checkpoint inhibition using anti-PD-1 and anti-CTLA-4 monoclonal antibodies—have provided substantial therapeutic benefit [2].

Basal cell carcinoma (BCC) constitutes the most prevalent form of skin cancer. The majority of cases can be effectively managed with locally directed therapeutic approaches. However, a small subset of cases may progress to surgically unresectable, locally advanced, or metastatic disease [3].

The management of advanced BCC presents considerable clinical challenges and warrants evaluation within a multidisciplinary framework. Alongside surgical intervention, therapeutic strategies may include radiation therapy and systemic treatment options [4].

Cutaneous squamous cell carcinoma (SCC) is recognized as the second most frequently occurring variant of non-melanoma skin cancer. Locally advanced cases often demonstrate limited responsiveness to surgical or radiotherapeutic interventions; nonetheless, systemic therapy offers a viable alternative for cases ineligible for local treatment modalities. Recently, the FDA has approved PD-1 inhibitors for the management of cutaneous SCC, showing superior therapeutic efficacy [5].

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Activation of PD-L1 and programmed cell death-1 (PD-1) pathways plays a pivotal role in enabling tumors to evade immune surveillance. The most reliable approach for assessing a tumor's PD-L1 status is the evaluation of PD-L1 expression through IHC analysis [6].

The advent of antibodies targeting the PD-1 receptor and its ligand (anti-PD-1/PD-L1) has markedly transformed therapeutic strategies for advanced and metastatic malignancies. By inhibiting the PD-1/PD-L1 interaction, these agents counteract tumor-induced immune exhaustion, thereby restoring antitumor immune activity. Furthermore, in comparison with conventional cytotoxic chemotherapy administered in advanced-stage cancer, anti-PD-1/PD-L1 therapies typically yield more sustained responses and are associated with a more favorable toxicity profile [7].

A tumor represents more than a mere aggregation of malignant cells, it constitutes a heterogeneous assembly comprising infiltrating and resident host cells, soluble mediators, and extracellular matrix components. Through intricate interactions, TCs induce profound molecular, cellular, and biophysical alterations within surrounding host tissues, thereby fostering tumor development and progression. While the precise composition of the tumor microenvironment differs among tumor types, its defining elements typically encompass immune cells, stromal cells, vascular structures, and extracellular matrix [8].

MATERIALS AND METHODS

1- Study design and data collection:

This retrospective cross-sectional cohort study was performed on 60 cases, comprising 35 BCC and 25 SCC. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks obtained from wedge and excision biopsies were utilized. For excision biopsy cases, TMA blocks were constructed from pre-existing paraffin blocks. All specimens were retrieved from the archives of the surgical pathology laboratory at the Oncology Center and the Pathology Department, Mansoura University, covering the period from July 2020 to December 2022.

Inclusion criteria:

- Cases not previously treated by radiotherapy or chemotherapy.
- Cases with full clinicopathological data& available paraffin blocks.

Exclusion criteria:

- Cases with unavailable clinicopathological data& unavailable paraffin blocks

Clinical parameters, including age, gender, tumor location and size, Histopathological assessment was based on hematoxylin and eosin (H&E) stained slides reviewed for tumor differentiation, depth of invasion, thickness, LVI, PNI. Histological subtype classification was done according to the 2022 WHO criteria [9].

2- Immunohistochemical staining:

Immunohistochemical (IHC) analysis was conducted on paraffin and TMA sections, each 4 μm in thickness, utilizing automated staining with Anti-PD-L1 (QR001) Rabbit Monoclonal primary antibody (Quartett, Berlin, Germany; dilution 1:100, ready to use). Tonsillar tissue sections were utilized as the positive control, while the negative control was generated by excluding the primary antibody.

3-Interpretation of IHC staining:

Slides were examined at 400× magnification, and PDL1 scoring of both TCs and tumor infiltrating lymphocytes (TILs) present in the immediate extratumoral stroma was done. Membranous staining for TC of TCs was only considered positive, but membranous &cytoplasmic staining for TILs [10].

PD-L1 staining intensity was graded as follows: 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The extent of PD-L1 expression was determined based on the percentage of positive TCs or TILs of any staining intensity, categorized as: 0 (TC-negative), TCs <10% positive, and TCs >10% positive; and 0 (TIL-negative), TILs <10% positive, and TILs >10% positive. Evaluation was performed only when each analyzable array core section contained a minimum of 100 viable cancer cells [10].

Ethical considerations:

Archived material from paraffin-embedded tissue blocks stored in the pathology laboratory was used for this work. The study protocol was approved by the Ethics Committee at the Faculty of Medicine, Mansoura University (proposal code "MS.20.04.111 "). Case confidentiality was rigorously preserved by assigning code numbers in place of personal identifiers. No supplementary medical interventions were carried out on the cases in connection with this study. Upon completion of the analyses, the donor tissue blocks were returned to the archive for any future clinical or investigative purposes.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software for Windows, Standard Version 24, was employed for data analysis. Continuous variables demonstrating a normal distribution were summarized as mean ± SD, with comparisons between two groups performed using the independent t-test, and those among more than two groups assessed via analysis of variance (ANOVA). The normality of data distribution was verified using the one-sample Kolmogorov–Smirnov test.

Qualitative variables were expressed as frequencies and percentages, and associations between categorical variables were evaluated using the Chi-square test; when the expected cell frequency was fewer than five, Fisher's exact test or the Monte Carlo test was applied.

Non-parametric data were expressed as median (minimum–maximum), with comparisons between two groups performed using the Mann–Whitney test and comparisons among more than two groups analyzed using the Kruskal–Wallis test. Spearman's correlation coefficient was applied to assess relationships between quantitative variables. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

This study included 60 cases previously diagnosed as BCC&SCC. Their age ranged between 45 -80 years old for SCC & between 10-80 years old for BCC. The mean age ± SD in SCC cases was 54.72±16.35 years while in BCC cases it reached 53.68±20.61years. Males predominated in both groups. Head & neck regions were mainly affected in all cases. Other clinical features are illustrated in **table 1**.

Table (1): Demographic &clinical data among SCC and BCC groups

Demographic data & clinical characteristics	SCC group (n=25)	BCC group (n=35)
Age (years)		
Mean ± SD	54.72±16.35	53.68±20.61
Min-Max	45.00-80.00	10.00-80.00
Sex		
Male	15 (60%)	23 (65.7%)
Female	10 (40%)	12 (34.3%)
Number		
Single	21 (84%)	27 (77.1%)
Multiple	4 (16%)	8 (22.9%)
Site		
Head & neck	13 (52%)	27 (77.1%)
Other sites	12 (48%)	8 (22.9%)
Size		
<2 cm	4 (16.0%)	6 (17.1%)
2-4 cm	12 (48.0%)	16 (45.7%)
>4 cm	9 (36.0%)	13 (37.1%)

Independent t test, Chi square test and Mann Whitney test were used.

Pathological features of SCC &BCC cases:

The median tumor thickness in the SCC group was 8.5 mm (range: 4–12 mm). The median depth of invasion was higher in the SCC group at 7.00 mm (range: 2–13 mm), compared to 4 mm (range: 2–10 mm) in the BCC group. Regarding LVI, 28% in the SCC group were positive for LVI; in contrast, all 35 cases in the BCC group were negative. PNI was observed in 24% of the SCC group, while 8.6% were PNI positive in the BCC group.

Table (2): Pathological types and grades of SCC cases

Pathological types and grade	SCC group (n=25)
Pathological types	
Spindle cell	8 (32%)
Acantholytic	8 (32%)
SCC with sarcomatoid differentiation	3 (12%)
Lymphoepithelial	3 (12%)
Verrucous	2 (8%)
Clear	1 (4%)
Grades	
Grade I	5 (20%)
Grade II	9 (36%)
Grade III	4 (16%)
Grade IV	7 (28%)

Based on WHO 2022 classification, most cases of SCC in the present study were classified as spindle cell and acantholytic (32% for each). Clear cell type was the rarest variant, observed in only one case (4%). Grade II represented the most prevalent histological grade (36%), whereas grade III was the least frequent (16%). **Table 3**

Pathological types of BCC cases are shown in table [3].

In BCC, the nodular subtype was the most frequently observed, accounting for 31.4% of cases. The least frequent subtypes were BCC with adnexal differentiation and pigmented BCC, each comprising 5.7% of cases.

Table (3): Pathological types and grade of BCC cases

Pathological type	BCC group (n=35)
Nodular	11 (31.4%)
Micronodular	5 (14.3%)
Basosquamous	3 (8.6%)
BCC with adnexal differentiation	2 (5.7%)
Fibroepithelial	4 (11.4%)
Pigmented	2 (5.7%)
Morphoeic	4 (11.4%)
Superficial spreading	4 (11.4%)

Immunohistochemical results of PDL-1 of SCC cases: Figure (3&4).

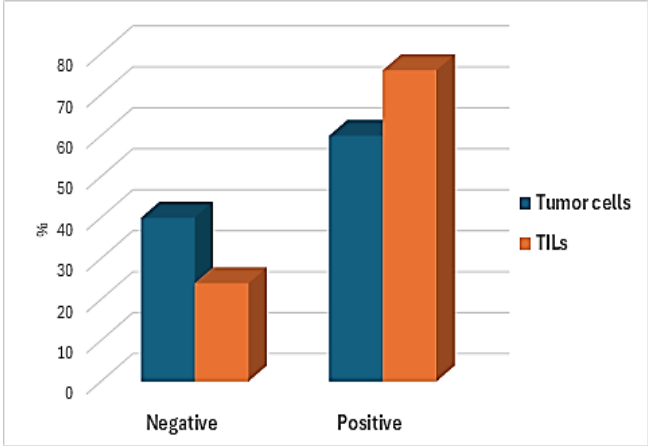

Figure (1): **PDL-I intensity expression in SCC group**

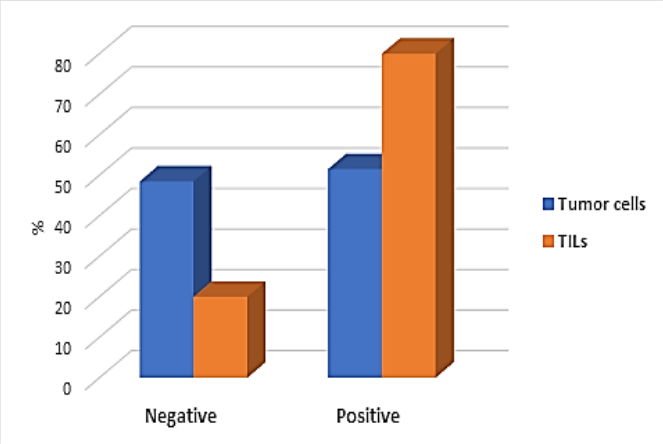

Figure (2): **PDL-I intensity expression in BCC group**

Among 25 cases of SCC: 60% show PDL-1 positivity in tumor cells & 79% were positive in TILs with varying percentage (figure 1)**.**

In 35 BCC cases, 18 (51.5%) showed PD-L1 positivity in tumor cells & 28 (80%) showed PD-L1 positivity in TILs with varying degrees (figure 2).

Table (4): Association between tumor cell percentage of PDL-1 and clinical characteristics in SCC group

Clinical characteristics	SCC group (n=25)			P- value
	Negative	<10%	>10%	
Age Mean ± SD	48.55±23.97	56.71±10.02	59.33±9.04	0.365
Sex				
Male	6 (60%)	3 (50%)	6 (66.7%)	0.883
Female	4 (40%)	3 (50%)	3 (33.3%)	
Number				
Single	8 (80.0%)	5 (83.3%)	8 (88.9%)	1.0
Multiple	2 (20.0%)	1 (16.7%)	1 (11.1%)	
Site				
Head & neck	6 (60%)	2 (33.3%)	5 (55.6%)	0.612
Other sites	4 (40%)	4 (66.7%)	4 (44.4%)	
Size				
<2 cm	4 (40.0%)	0 (0%)	0 (0%)	**0.049***
2-4 cm	4 (40.0%)	2 (33.3%)	6 (66.7%)	
>4 cm	2 (20.0%)	4 (66.7%)	3 (33.3%)	

ANOVA test, kruskil wallis test & Monte carlo test were used.

Table (5): Association between tumor cell intensity of PDL-1 in SCC and pathological characteristics of the tumor

pathological characteristics	SCC group (n=25)				P- value
	Negative	Mild	Moderate	Strong	
Pathological type					
Spindle cell	0 (0%)	0 (0%)	5(50%)	3(100%)	**0.025***
Acantholytic	5 (50%)	1 (50%)	2(20%)	0(0%)	
SCC with sarcomatoid differentiation	0 (0%)	0 (0%)	3(30%)	0(0%)	
Lymphoepithelial	3 (30%)	0 (0%)	0(0%)	0(0%)	
Verrucous	1 (10%)	1 (50%)	0(0%)	0(0%)	
Clear	1 (10%)	0 (0%)	0(0%)	0(0%)	
Grade					
Grade I	4(40%)	1(50%)	0(0%)	**0(0%)**	**≤0.001***
Grade II	6(60%)	1(50%)	2(20%)	**0(0%)**	
Grade III	0(0%)	0(0%)	1(10%)	**3(100%)**	
Grade IV	0(0%)	0(0%)	7(70%)	**0(0%)**	
Tumor thickness (mm) Median (Min-Max)	6(4-16)	5(3-7)	12(9-15)	**12(6-15)**	**0.022***
Depth of invasion (mm) Median (Min-Max)	4(2-10)	3.5(2-5)	8.5(6-12)	**8(8-13)**	**0.007***
LVI					
Negative	10(100%)	2(100%)	5(50%)	**1(33.3%)**	**0.028***
Positive	0(0%)	0(0%)	5(50%)	**2(66.7%)**	
PNI					
Negative	10(100%)	2(100%)	6(60%)	**1(33.3%)**	**0.047***
Positive	0(0%)	0(0%)	4(40%)	**2(66.7%)**	

kruskil wallis test & Monte carlo test were used, *significant p≤0.05.

Table (6) Association between tumor cell PDL-1 percentage and pathological characteristics among SCC group

pathological characteristics	SCC group (n=25)			P- value
	Negative	PDL-1 <10%	PDL-1 >10%	
Pathological type				
Spindle cell	0 (0%)	4 66.7%)	4 (44.4%)	**0.03***
Acantholytic	5 (50.0%)	0 (0%)	3 (33.3%)	
SCC with sarcomatoid differentiation	0 (0%)	1 (16.7%)	2 (22.2%)	
Lymphoepithelial	3 (30.0%)	0 (0%)	0 (0%)	
Verrucous	1 (10.0%)	1 (16.7%)	0 (0%)	
Clear	1 (10.0%)	0 (0%)	0 (0%)	
Grade				
Grade I	4 (40.0%)	1 (16.7%)	0 (0%)	**0.014***
Grade II	6 (60.0%)	1 (16.7%)	2 (22.2%)	
Grade III	0 (0%)	2 (33.3%)	2 (22.2%)	
Grade IV	0 (0%)	2 (33.3%)	5 (55.6%)	
Tumor thickness (mm) Median (Min-Max)	6(4-16)	10(3-15)	12(6-15)	0.204
Depth of invasion (mm) Median (Min-Max)	4(2-10)	8(2-13)	8(5-12)	0.106
LVI				
Negative	10(100%)	3 (50.0%)	5 (55.6%)	**0.028***
Positive	0 (0%)	3 (50.0%)	4 (44.4%)	
PNI				
Negative	10 (100%)	2 (33.3%)	7 (77.8%)	**0.005***
Positive	0 (0%)	4 (66.7%)	2 (22.2%)	

kruskil wallis test & Monte carlo test were used, *significant p≤0.05

In SCC cases; we did not find any substantial correlation between PDL-1 intensity on TCs & clinical parameters. Meanwhile, we found an inverse significant association between PDL-1 expression intensity on TILs & age of the case (P=0.03). Also, we found statistically significant correlation of PDL1 intensity as well as percentage with LVI (P=0.28& P=0.028 in respective manner), and PNI (P=0.047 & P=0.005 respectively). Also, we found statistically significant correlation of PDL1 intensity with other pathological risk parameters including pathological subtype (P=0.25), tumor grade (P=0≤ 0.001), tumor thickness (P=0.022) & depth of invasion (P=0.007) (table 6&7).

On analysis of PDL-1 intensity on TIL in SCC, no substantial correlation was observed between PD-L1 expression and case clinical characteristics except for the size of the lesion (P=0.049). Also, statistically substantial inverse correlation was detected between TIL PDL-1 intensity & pathological high-risk factors as pathological subtype≤ (P=0.001), tumor grade (P=0.005), tumor thickness (P=0.016), depth of invasion (P=0.05), and PNI (P=0.04). As regard correlation between TIL PDL-1 percentage, the only positive correlation was present with pathological grade only (P=0.013).

In BCC cases, there was no substantial correlation between PDL-1 expression on TC as well as TILs &clinical parameters, significant negative correlation was present only between TILs PDL-1 percentage &size of the tumor. We encountered significant correlation between PDL-1 percentage & intensity scoring on TC & high-risk pathological subtypes (micronodular, basosquamous& morphea) (P=0.019& P=0.008 respectively), but no significant correlation was found with other pathological parameters.

We also reported a significant negative correlation between percentage of PDL-1 on TILs and high-risk pathological subtypes. Also, negative correlation was reported between PDL-1 percentage on TIL & high-risk pathological subtypes as well as the size of the tumor (P=0.05) (table 7 & 8).

Table (7): Association between tumor cell percentage of PDL-1 and pathological characteristics in BCC group

Tumor characteristics	BCC group (n=35) TC PDL-1 percentage			P- value
	Negative	<10%	>10%	
Pathological type				
Nodular	9 (52.9%)	2 (25.0%)	0 (0%)	
Micronodular	2 (11.8%)	0 (0%)	3 (30%)	
Basosquamous	0 (0%)	2 (25.0%)	1 (10%)	
Fibroepithelial	3 (17.6%)	1 (12.5%)	0 (0%)	**0.008***
Morphea	0 (0%)	1 (12.5%)	3 (30%)	
Superficial spreading	2 (11.8%)	2 (25.0%)	0 (0%)	
Pigmented	0 (0%)	0 (0%)	2 (20%)	
BCC with adenexal differentiation	1 (5.9%)	0 (0%)	1 (10%)	
Depth of invasion (mm), Median (Min-Max)	4 (2-10)	3 (2-8)	6 (2-8)	0.503
PNI Negative	16 (94.1%)	7 (87.5%)	9 (90%)	1.0
Positive	1 (5.9%)	1 (12.5%)	1 (10%)	

Monte carlo test & kruskil wallis test were used, *significant p≤0.05.

Table (8): Association between TIL PDL-1 intensity and pathological characteristics in BCC group:

Pathological characteristics	BCC group (n=35) TILs PDL-1 intensity				P-value
	Negative	Mild	Moderate	Strong	
Pathological type					
Nodular	0 (0%)	1 (25%)	6 (37.5%)	4 (50%)	
Micronodular	1(14.3%)	2 (50%)	2 (12.5%)	0 (0%)	
Basosquamous	1 (14.3%)	0 0%)	2 (12.5%)	0 (0%)	
BCC with adnexal differentiation	1 (14.3%)	1 (25%)	0 (0%)	0 (0%)	0.067
Fibroepithelial	0 (0%)	0 (0%)	2 (12.5%)	2 (25%)	
Pigmented	2 (28.6%)	0 (0%)	0 (0%)	0 (0%)	
Morphea	2(28.6%)	0(0%)	2 (12.5%)	0 (0%)	
Superficial spreading	0(0%)	0 (0%)	2 (12.5%)	2 (25%)	
Depth of invasion (mm), Median (Min-Max)	7 (3-8)	4.5 (3-7)	4 (2-10)	3 (2-7)	0.213
PNI Negative	6(85.7%)	4(100%)	15(93.8%)	7(87.5%)	1.0
Positive	1(14.3%)	0(0%)	1(6.2%)	1(12.5%)	

Monte carlo test & kruskil wallis test were used, *significant p≤0.05.

This study demonstrated a substantial inverse relationship between tumor cell intensity and TILs intensity across the analyzed cases, evident in both the SCC and BCC groups.

There is a positive correlation between tumor cell percentage and TILs percentage in BCC group which is significant. In SCC group, there is a negative correlation between tumor cell percentage and TILs percentage which is non-significant.

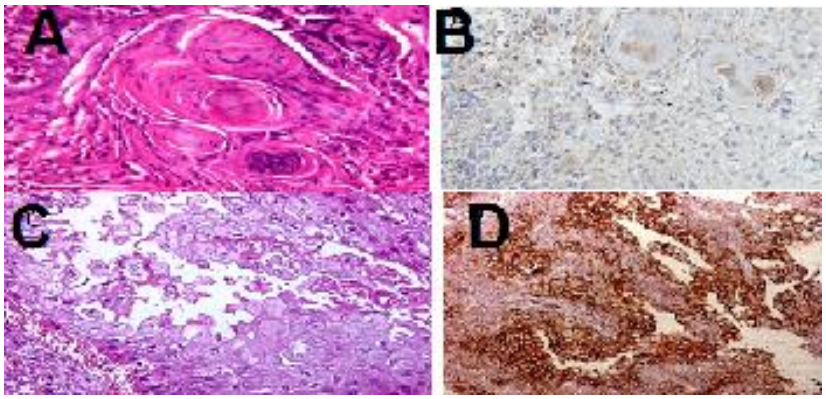

Figure [3]: A case of **SCC**. (A): H&E-stained slide of classic type GI (IHC, magnification x200). (B): PDL1 IHC shows negative staining TC &mild staining in <10% of TIL (IHC: DAB, magnification x200). (C): Acantholytic SCC showing central space contains atypical cells with loss of cohesion between them (H&E, magnification 200) (D): PDL-1 IHC shows positive membranous staining in >10% of TC (IHC: DAB, magnification x200).

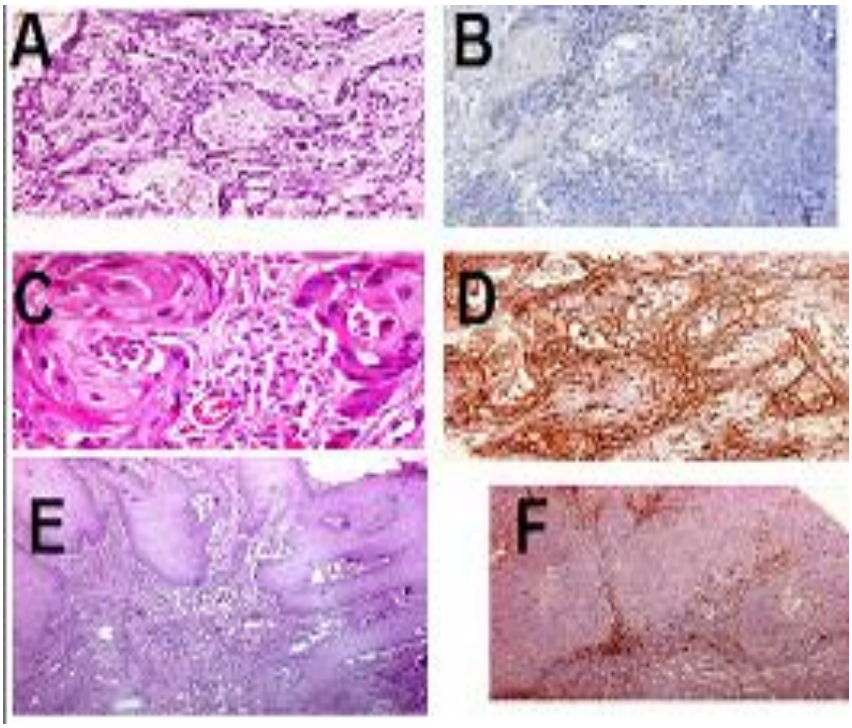

Figure [4]: SCC. (A): H&E-stained slide: spindle cell SCC formed of atypical spindle cells (H&E, magnification x200) (B): PDL1 IHC shows focal moderate membranous staining of <10% of TC &negative TIL (IHC: DAB, magnification x200). (C): Moderately differentiated SCC shows groups of atypical squamous cells with moderate amount of keratin (H&E, magnification x400). (D): moderately differentiated SCC showing strong membranous staining in >10% of TC (IHC: DAB, magnification x400). (E): verrucous carcinoma show acanthosis with broad, bulbous, projections of rete ridges with pushing border (H&E, magnification x200). (F): verrucous carcinoma showing negative staining of TC &strong membranous staining of TIL (IHC: DAB, magnification x200).

Immunohistochemical results of PDL-1 of BCC cases: Figure (5&6).

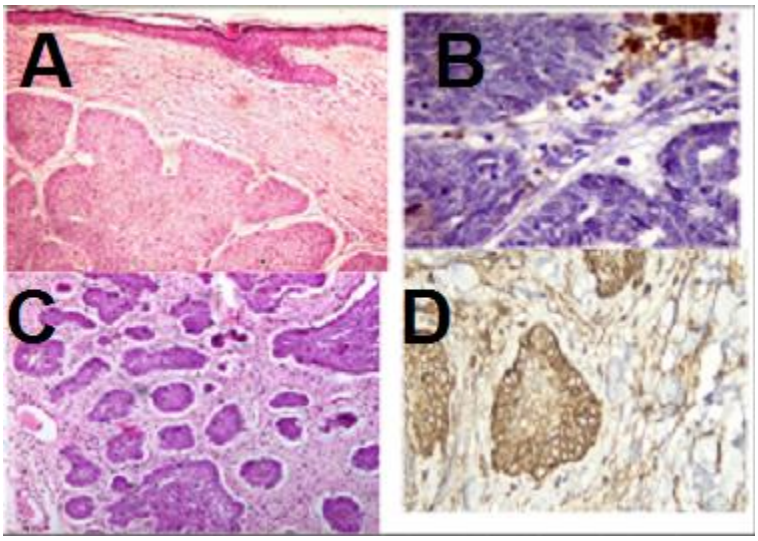

Figure [5]: A case of BCC. (A): Nodular BCC (H&E, magnification x200) (B): Nodular BCC showing moderate membranous staining in >10% TIL (red arrow) & negative TC (IHC: DAB x400). (C): Micronodular BCCshowing small discrete nodules (H&E, magnification x200) (D): Micronodular BCC showing strong membranous staining of TC&TIL (IHC: DAB x200)

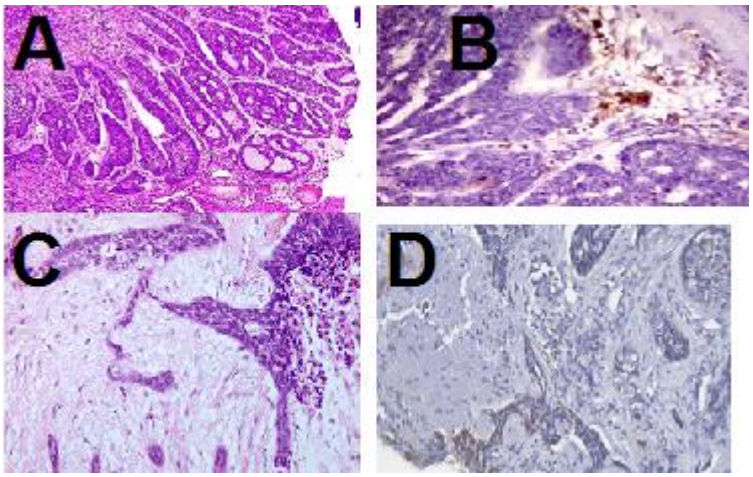

Figure [6]: BCC. (A): Adenoid cystic BCC with nests of tumor cells in cribriform pattern (H&E, magnification x100).(B): Adenoid cystic BCC shows strong membranous staining of >10 of TILs (red arrow) (IHC: DAB x200).(C): BCC -Morphoeic type with thin strands of compressed basaloid cells in abundant dense stroma (H&E, magnification x200) (D): BCC morphoeic type showing negative staining of TC & TILs (IHC: DAB x200).

DISCUSSION

PD-L1 is an apoptosis-associated protein with a pivotal function in modulating immune responses. Upon engagement of T lymphocytes with their PD-1 receptor, PD-L1 triggers inhibitory signaling pathways that can result in T-cell apoptosis, thereby contributing to the regulation of peripheral T-cell immune tolerance. Consequently, it serves as a critical mediator in the body's anti-tumor immune defense mechanisms [11].

NMSCs, including BCC and SCC, constitute a substantial and increasing global health burden. Owing to their inherent immunogenicity, NMSCs represent promising candidates for immunotherapeutic approaches, and consequently, clinical trials in this field are actively underway [12].

The prognostic significance of PD-L1 remains a subject of debate. Several studies have demonstrated that elevated PD-L1 expression is frequently associated with poor clinical outcomes in gastric cancer, hepatocellular carcinoma, and renal cell carcinoma. In contrast, in Merkel cell carcinoma and breast carcinoma, PD-L1 expression has been identified as a marker of more favorable prognosis [13].

In the present study, we used NMSC case to characterize the PD-L1 expression in cancer cells correlated with peritumoral lymphocytes infiltrates focusing our efforts on common tumors such as BCC and SCC and conducting studies to investigate their efficacy as a therapeutic alternative for some cases.

This is a retrospective cross-sectional study conducted on 60 cases. FFPE tissue blocks of wedge & excision biopsies were used. Between July 2020 and December 2022, pre-existing paraffin blocks from the archives of the Surgical Pathology Laboratory at the Oncology Center and the Pathology Department of Mansoura University were utilized to prepare TMA blocks.

The age of NMSC cases in this work ranged from 45-80 years old for SCC, with a mean age ± SD of 54.72±16.35 years. In BCC, the age ranges from 10-80 years old with a mean age ± SD of 53.62±20.61 years. This finding is consistent with the cases reported by **Fekri *et al.*** [14] who observed BCC in younger individuals. This was explained by the notion that BCC development is predominantly driven by intense ultraviolet (UV) exposure during adolescence and childhood, whereas SCC development is more strongly associated with prolonged, cumulative UV exposure over several decades. Regarding age distribution, the present study showed slight male predominance 60% for SCC & 65.7% for BCC. This was in agreement with **Rembielak *et al.*** [15] who reported that 57% of their cases were males. Also, **Caini *et al.*** [16] revealed that about 60.1% of their cases were males. However, **Moser *et al.*** [17] stated that BCC incidence rates in women were more frequent than cSCC, whereas in men this ratio was approximately equal. These were explained by hormonal effect in young females which were not present in the present work.

In the present study, head & neck areas were the main anatomic sites where the majority of the NMSC especially BCC were present, but no statistically significant difference was found; this can be explained as number of cases in our work was relatively small. These results coincided with that reported by **Cochicho *et al.*** [18] who noticed that head& neck were the primary anatomical sites where the majority of the NMSC (94%), as well as that reported by **Bowers *et al.*** [19] who explained the role of immunosuppressive effect of UV light. Grade II represented the most frequently encountered histological grade of SCC, accounting for 36% of cases. The least common grade is grade III (16%) These results were very close to **Szewczyk** [20]**.** This may be due to late detection of low-grade tumors and lack of general knowledge & screening programs for skin tumors.

On evaluation of histological subtypes of SCC cases, we observed that the most common pathological types were spindle cell and acantholytic (8 cases for each: 32%). The least common type was clear cell (only one case: 4%). This aligns with the observations of **Parekh and Seykora** [21], who reported that clear-cell SCC is an uncommon variant occurring in the head and neck region, predominantly among elderly males with a history of extensive sun exposure. They further noted the absence of glycogen or mucin within the TCs, attributing the clear-cell morphology instead of pronounced hydropic degeneration of the neoplastic cells.

The higher incidence of spindle & acantholytic types in the present study may be attributed to the differences in number of collected cases of this subtype during the period of study & this coincides with that stated by **Matsuo *et al.*** [22]**]** who reported that acantholytic SCC was prevalent in sun-exposed areas of the skin, particularly in head and neck of elderly men which can be explained by the common association of acantholytic SCC with areas of follicular epithelium.

As regards pathological subtypes of BCC cases, the most common type was nodular (31.4%) & the least common types were BCC with adnexal differentiation and pigmentation (5.7% for each). This finding was consistent with that recorded by **Cameron *et al.*** [23] who stated that nodular type was about 50% of cases, but they noticed that superficial spreading was about 10-30% & morphea subtype type was <30%. These may be due to different sample size during the period of study.

On analysis of SCC, we noticed (60%) were PD-L1 positive in TCs & 79% were positive in TILs in SCC cases. Other studies reported that 44% of cases were detected as PD-L1 positive on TC which is not coincides with our results [24]**.**

Slater and Googe [25] reported PD-L1 positivity in 20% of low-risk SCC cases and in 70% of high-risk tumors. Their findings demonstrated a substantial association between PD-L1 expression and histopathological parameters indicative of elevated metastatic potential, including larger tumor diameter, higher histologic grade, and greater tumor thickness. In the present study, no substantial relationship was detected between PD-L1 staining intensity in TCs and the evaluated clinical parameters. Conversely, other investigations have documented strong PD-L1 staining in SCC of the head and neck region, a discrepancy that may be attributable to differences in sample size [26]**.**

In the current study, we found statistically significant positive correlation between PDL-1 TC intensity many prognostic pathological parameters including pathological subtype, tumor grade and thickness, depth of invasion, LV, and PNI. In agreement with study done by **Garcia-Diez *et al.*** [27] who reported an increase in the risk of metastasis in cSCCs with higher PD-L1 expression on TC. Conversely, **Yülek *et al.*** [24] reported no significant correlation with any prognostic pathological parameters of cSCC. These differences were attributed to their studying their cases outside the head

and neck region. Also, we found statistically significant positive correlation between percentage of PDL-1 & LVI as well as PNI, but as regard tumor thickness and depth of invasion we reported no significant association. In contrast to the study, a work done by **Vasilev *et al.*** [13] who found association between cSCCs &greater depth of invasion. This may be due to antibody clone or different scoring method.

On analysis of PDL-1 on TILs in SCC, no substantial correlation was observed between PD-L1 expression and case clinical characteristics. But there is significant negative correlation between TILs PDL-1 intensity & age of the case, in agreement with **Schaper *et al.*** [28] who noticed the same results.

In our study, statistically significant inverse correlation was observed between TIL PDL-1 intensity scoring & pathological high-risk factors as pathological subtype, tumor grade, tumor thickness, depth of invasion, and PNI. **Malik *et al.*** [29] **& Stravodimou *et al.*** [30] reported that PD-L1 positively on TILs was inversely correlate only with PNI in cSCC. Our findings aligned with observations of TIL infiltration accompanied by PD-L1 expression in other malignancies, including breast carcinoma, as reviewed by **Dieci *et al.*** [31]**.**

On analysis of BCC, in the present investigation; 51.5% were PD-L1 positive on TCs & 80% were positive in TILs. These coincides with that reported by **Stonesifer *et al.*** [32]**,** where expression on TC ranges from 22% to 89.9% & on TIL ranges from 80% to 94.9%.

On analysis of PDL-1 on TC of BCC, using both intensity & percentage scores, no significant correlation was found with any of clinical parameters. But we encountered significant positive correlation between tumor cell PDL-1 positivity & high-risk subtypes (micronodular, Basosquamous, morphea) but no significant correlation was found with other pathological parameters. **Slater and Googe** [25] initially hypothesized that 'high-risk' histological subtypes of BCC would demonstrate higher tumor cell PD-L1 expression. In contrast, **Gompertz-Mattar *et al.*** [33] reported the opposite trend, noting greater expression of both intra-tumoral and stromal PD-L1 in the nodular BCC subtype ('low-risk') compared with the morpheaform subtype ('high-risk'). The observation of distinct PD-L1 expression patterns among specific BCC subtypes represents a noteworthy finding. Such discrepancies may be attributable to variations in the antibody clones employed for staining, differences in preanalytical conditions, or divergent criteria for defining positivity (membranous versus cytoplasmic staining) [34] **.**

Regarding pathological evaluation, analysis based on percentage scoring revealed a significant inverse correlation between PD-L1 positivity in TILs and high-risk subtypes, with nodular BCC demonstrating higher PD-L1 expression than other variants. Low-risk BCC subtypes (nodular and superficial) displayed greater PD-L1 expression within stromal immune infiltrates compared with high-risk subtypes, consistent with the findings of **Gompertz-Mattar *et al.*** [33]**.**The highest levels of PD-L1 expression were observed in nodular BCC, indicating a potentially targetable avenue for the management of this most prevalent BCC subtype. No statistically significant differences were detected for depth of invasion, LVI, or PNI.

In conclusion, our study demonstrated a statistically significant negative correlation between TC staining intensity and TIL intensity in both SCC and BCC groups. When the percentage score was applied, a significant positive correlation was observed in the BCC group between TC and TIL percentages. In contrast, the SCC group exhibited a negative, statistically non-significant, correlation between these parameters. Our observations concur with the findings of **Chen *et al.*** [35] in urothelial carcinoma, where PD-L1 expression in TCs was significantly associated with increased TIL infiltration. Therefore, the combined evaluation of PD-L1 expression in both TCs and TILs may serve as a relevant predictive marker for disease progression in BCC cases.

LIMITATIONS

This study is constrained by a relatively small sample size and an unequal distribution of cases. Furthermore, the use of non-automated quantification introduces operator dependency, leading to potential intra-observer and inter-observer variability; therefore, the application of image analysis techniques is recommended. Such heterogeneity could be addressed in future research through the inclusion of larger, more representative cohorts. In addition, subsequent studies should investigate the prognostic and therapeutic relevance of PD-L1 stromal expression, as well as its potential as a therapeutic target in both BCC and SCC.

CONCLUSION

This study suggests the utility of immunohistochemistry as a high PD-L1 expression is strongly related to elevated tumor proliferation, aggressiveness, and progression. Significant correlations were found between PDL1 expression& and pathological risk factors, reinforcing the importance of integrated pathological and IHC evaluation for risk assessment.

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