Immunopathologia Persa
DOI:10.34172/ipp.2025.43772
http immunopathol.com

Expression of programmed death-ligand 1 and epidermal growth factor receptor in head and neck squamous cell carcinoma; an immunohistochemical and clinicopathological study

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Received 17 Oct. 2024
Revised: 25 Dec. 2024
Accepted 3 May 2025
ePublished 22 Jun. 2025

Keywords: Squamous cell carcinoma, PD-L1 expression, Immunohistochemistry, Prognostic factors, EGFR expression, Clinicopathological study

Citation: Ismail IM, Hassan HA, AlFarabi Ali S, Alkeheli M, Bukhary S, Jazzar A, Alhindi NA, Amin HA, Badr BM, Fathelbab J, Radwan GSI, Radi NA. Expression of programmed death-ligand 1 and epidermal growth factor receptor in head and neck squamous cell carcinoma; an immunohistochemical and clinicopathological study. Immunopathol Persa. 2025;x(x):e43772. DOI:10.34172/ipp.2025.43772.

Abstract

Introduction: The predominant head and neck cancers arise from the mucosal epithelium located in the oral cavity, throat, and larynx. These malignancies are typically termed head and neck squamous cell carcinoma (HNSCC).

Objectives: This study aims to examine the immune-expression of programmed death-ligand 1 (PD-L1) and epidermal growth factor receptor (EGFR) in HNSCC.

Materials and Methods: In this cross-sectional study, we conducted the link between PD-L1and EGFR proteins with the clinicopathological features of the cancer. Our study included 50 patients that have been histologically diagnosed with HNSCC, and patients who were diagnosed with other types of malignancies, especially in the new cases of human papillomavirus and triple-negative breast cancer

Results: This study analyzed a total of 50 instances of HNSCC, which are classified based on their T, N, and TNM stages. Immunohistochemistry analysis showed that 68% of patients exhibited immunopositivity, primarily in the tumor cell membrane and cytoplasm. There was no notable link between the expression of PD-L1 and factors such as gender, age, or tumor site. Additionally, a positive expression of PD-L1 was identified in 87.5% of patients (P = 0.203), across with no significant correlation of PD-L1 expression and tumor differentiation.

Conclusion: This study revealed that more than two-thirds of cases with HNSCC exhibit PD-L1 expression. However, patient characteristics such as age, gender, and tumor site were not shown to have any influence on EGFR expression.

Introduction

Programmed death-ligand 1 (PD-L1), a common expression in solid human cancers, has been identified as an important therapeutic target due to its association with adverse clinicopathological features and poor prognosis in head and neck squamous cell carcinoma (HNSCC) patients (1). On the other hand, epidermal growth factor receptor (EGFR) protein levels have been extensively studied in HNSCC, with research indicating its correlation with patient survival and its upregulation in oral potentially malignant disorders and leukoplakia (2).

The study of PD-L1 and EGFR in HNSCC is motivated by several key factors. A previous study has indicated the significance of EGFR protein levels in HNSCC and its association with patient survival (2). Additionally, the expression of PD-L1 in HNSCC has been found to have prognostic significance, with implications for tumor progression and patient survival (1).

The existing knowledge gaps in understanding the expression patterns, clinical significance, and potential mechanisms of

Key point

In a cross-sectional study on 50 cancer patients, we evaluated the link between programmed death-ligand 1 (PD-L1) and epidermal growth factor receptor (EGFR) with the clinicopathological features of the cancer. Our study showed that, more than two-thirds of cases with head and neck squamous cell carcinoma (HNSCC) exhibit programmed death-ligand1 expression. However, patient characteristics such as age, gender, and tumor site were not shown to have any influence on EGFR expression.

PD-L1 and EGFR in HNSCC highlighted the need for further investigation (3). Moreover, the identification of PD-L1 as an independent predictor for the prognosis of HNSCC patients, along with its tumor-intrinsic functions underscores the importance of studying these biomarkers in the context of HNSCC (4). Therefore, this study aims to contribute to the understanding of the role of PD-L1 and EGFR in HNSCC, with potential implications for clinical management and therapeutic strategies.

The significance of PD-L1 and EGFR in HNSCC is multifaceted and crucial for understanding the disease at various levels. PD-L1, as a common marker in solid human malignancies, emerges as a crucial therapeutic target, with its expression profile and clinical relevance in HNSCC, which warranting particular attention (5). Previous studies indicated that PD-L1 expression in HNSCC tumor tissues is substantially associated with increased clinical progression and reduced patient survival, establishing it as an independent prognostic predictor (6).

Furthermore, functional experiments have demonstrated that PD-L1 can influence the proliferation, migration, and invasion of cancer cells, as well as promote epithelial-mesenchymal transition through the AKT-mTOR signaling pathway (1).

On the other hand, EGFR overexpression in HNSCC has been linked to various risk factors, clinicopathologic parameters, and prognostic indicators, including patient survival. The upregulation of EGFR in oral potentially malignant disorders, such as leukoplakia and submucous fibrosis, further underscores its significance in the disease process (2).

HNSCC encompasses a range of malignancies affecting the oral cavity, oropharynx, larynx, and other anatomical sites within the head and neck region (7). It is the 9th most common malignant tumor globally, accounting for 6% of all cancer cases and up to 2% of cancer-related deaths (8). HNSCC is characterized by its biological diversity and genetic heterogeneity, with traditional risk factors including smoking, betel nut, and alcohol consumption, across with its well understood association with human papillomavirus (HPV) and Epstein Barr virus (EBV) (9). Notably, HNSCC has two main subtypes; HPV-positive and HPV-negative, each with distinct clinical and therapeutic implications (10). The HPV-positive disease, predominantly found in the oropharynx, which is associated with a younger patient population and exhibits increased sensitivity to treatment, leading to more favorable survival rates compared to HPV-negative HNSCC (11). This diversity underscores the necessity for tailored therapeutic strategies based on the specific subtype, highlighting the emerging need for individualized treatment approaches in the management of HNSCC (12).

Objectives

This study aims to examine the immune-expression of PD-L1 and EGFR in head and neck squamous cell cancers.

Materials and Methods

Study design

This cross-sectional investigation involved an analysis of 50 specimens of HNSCC. The analysis focused on several clinical factors such as age, gender, tumor site, differentiation, T stage, N stage, and TNM staging (13). The patients were diagnosed by faculty members of the pathology department, located inside the faculty of medicine. Tissue blocks embedded in paraffin were conducted to obtain sections that were four microns in thickness. These sections were then stained with hematoxylin and eosin using a conventional procedure.

Participants

Inclusion and exclusion criteria

Inclusion criteria include (a) histologically verified diagnosis of HNSCC, and (b) availability of comprehensive clinical and pathological data, encompassing age, sex, tumor location, grade, and lymph node status.

Exclusion criteria include (a) histological diagnoses excluding HNSCC (e.g., adenocarcinoma, melanoma, sarcoma and metastasis), (b) incomplete clinical or pathological information, (c) patients who have undergone previous treatment for HNSCC (e.g., surgery, radiation, chemotherapy) prior to the biopsy or surgical sample collection for diagnosis.

Sampling method

A sequential sampling strategy was employed. All patients with a histologically confirmed diagnosis of HNSCC who satisfy the inclusion criteria and present at the designated time and location for sampling were incorporated into the study until the target sample size of 50 cases is attained.

Time and place of sampling

The data was collected from surgical pathology laboratory of Assiut university hospitals at the period from (2021-2023).

Procedures

The immunohistochemical staining process consisted of removing paraffin from formalin-fixed paraffin-embedded tissue, retrieving the antigen, and washing it with distilled water and phosphate buffer saline. Sections on the slides were treated with primary antibodies (PD-L1 and EGFR)

and left to incubate for 60 minutes in a humidified atmosphere at room temperature. The antibodies PD-L1 and EGFR were titrated to ascertain the optimal dilution, which was determined to be 1/100.

The quality control process involved the use of both positive control specimens and negative control specimens. Positive controls for PD-L1 were obtained from sections of human placental tissue, while EGFR positive controls were obtained from tonsil tissue. During each staining run, extra tissue sections were stained simultaneously, but without using the main antibody and instead using PBS.

The scoring of PD-L1 immunoreactivity was determined based on the combined positive score (CPS), which is calculated by dividing the total number of PD-L1-positive tumor cells, lymphocytes, and macrophages by the total number of viable tumor cells and then multiplying the result by 100 (14). In order for the specimen to be deemed suitable for examination, it is necessary for there to be a minimum of 100 live tumor cells present. The cases were subsequently categorized into three groups based on PD-L1 expression levels; PD-L1-negative (score 0, CPS < 1), moderate expressors (score 1, 1 ≤ CPS < 20), and strong expressors (score 2, CPS ≥ 20) (14).

The EGFR staining slides were assessed by grading their staining intensity and identifying hotspots to determine the percentage of positive cells. The "IRS score" was thereafter compared to the clinicopathological criteria and assessed for its statistical correlation with them (15).

Statistical analysis

Descriptive statistics were employed to summarize the immunohistochemical expression of PD-L1 and EGFR including frequencies and percentages for categorical variables (e.g., PD-L1 positivity defined as tumor proportion score (TPS) ≥1%, EGFR overexpression defined as 3+ staining in ≥10% of cells) (16). Clinicopathological correlations were evaluated using chi-square (χ^2) test, which evaluates the independence or association of the variables.

The statistical analysis of the results was conducted using IBM SPSS statistics version 20 for Windows (IBM Corp., Arm). Additionally, the chi-square test was employed to assess the variations between PD-L1, EGFR expression, and clinicopathological variables. This test was also conducted to examine the differences between PD-L1, EGFR expression and clinicopathological factors. Significance level was set as $P < 0.05$.

Results

This study investigates a total of 50 instances of HNSCC, comprising 70% males and 30% females. The average age is 46 years, with 32% originating from the oral cavity, 16% from the oropharynx, 24% from the hypopharynx, and 28% from the larynx. The tumors are categorized into T, N, and TNM stages, with 26% falling under T1-T2, 74% in T3-T4, 62% falling under N0-N1, and 86% were in the III-IV stages (Table 1).

Immunohistochemistry for PD-L1 was conducted on 50 instances of HNSCC, revealing that 68% of the patients exhibited immunopositivity. The expression of PD-L1 was mostly notable positivity was identified in the cytoplasm and membrane of tumor cells, whereas a distinct subset of infiltrating lymphocytes in tumors also demonstrated strong immunoreactivity. Among the 35 male patients, 71.4% exhibited PD-L1 positive expression, whereas nine out of the 15 female patients showed positive expression. The chi-square test indicated no significant correlation between PD-L1 expression and gender, age, or tumor site. Meanwhile, PD-L1 positive expression was detected in 87.5% of oral cavity cases (14 out of 16 instances), 37.5% of oropharynx cases, 41.7% of hypopharynx cases, and 85.7% of larynx cases. The chi-square test also revealed a statistically significant correlation between the expression of PDL1 and the location of the tumor (Table 2, Figure 1).

Moreover, PD-L1 positive expression was detected in 73% of patients with head and neck squamous cell HNSCC that were well-moderately differentiated, and in 53.8% of cases with carcinoma that were poorly-undifferentiated. Nevertheless, the chi-square test revealed no statistically significant correlation between PD-L1 expression and tumor differentiation in HNSCC (Table 3, Figure 2).

Accordingly, positive expression of PD-L1 was detected

Table 1. Number of cases according to the gender and age and site of tumor

	Number of cases (%)
Gender	
Male	35 (70)
Female	15 (30)
Age (y)	
<60	27 (54)
≥60	23 (46)
Site of the tumor	
Oral cavity	16 (32)
Oropharynx	8 (16)
Hypopharynx	12 (24)
Larynx	14 (28)

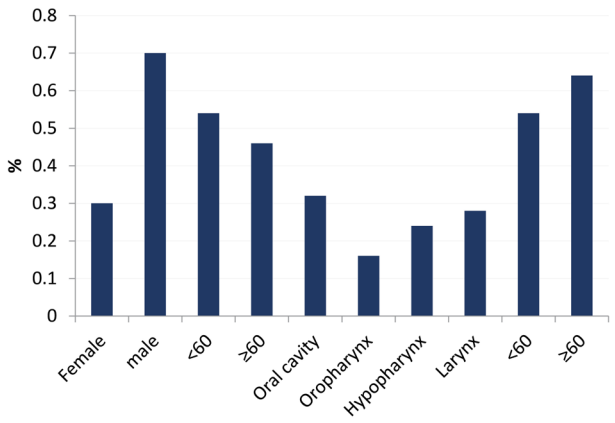

Figure 1. Number of cases according to the gender and age and site of tumor.

Table 2. Relation between PD-L1 expression and (age, gender and tumor site) by chi-square test

		PD-L1 expression		*P* value
		Positive No. (%)	Negative No. (%)	
Sample size		34 (68.0%)	16 (32.0%)	
Age (y)	<60	17 (63.0%)	10 (37.0%)	0.408
	≥60	17 (73.9%)	6 (26.1%)	
Gender	Male	25 (71.4%)	10 (28.6%)	0.427
	Female	9 (60.0%)	6 (40.0%)	
Tumor site	Oral cavity	14 (87.5%)	2 (12.5%)	0.007
	Oropharynx	3 37.5%)	5 (62.5%)	
	Hypopharynx	5 (41.7%)	7 (58.3%)	
	Larynx	12 (85.7%)	2 (14.3%)	

Table 3. Relation between PD-L1 expression and differentiation of the tumor by chi-square test

Variable	Positive No. (%)	Negative No. (%)	*P* value
Sample size	34 (68.0%)	16 (32.0%)	
Age (y)			
<60	17 (63.0%)	10 (37.0%)	0.408
≥60	17 (73.9%)	6 (26.1%)	
Gender			
Male	25 (71.4%)	10 (28.6%)	0.427
Female	9 (60.0%)	6 (40.0%)	
Tumor differentiation			
Well-moderate	27 (73.0%)	10 (27.0%)	0.203
Poor-undifferentiated	7 (53.8%)	6 (46.2%)	
Total			
Total positive	34 (61.8%)		
Total negative		16 (38.2%)	

in 61.5% of cases with stage T1-T2 HNSCC, and in 71.3% of cases with stage T3-T4. There was no notable correlation was observed between the expression of PD-L1 and the T stage of the tumor (chi-square test). Meanwhile, PD-L1 positive expression was detected in 71.4% of cases with stage N0-N1 HNSCC, and in 73.7% of cases with stage N2-N3 HNSCC. There was no notable observed between the expression of PD-L1 and the N stage of the tumor (chi-square test). Finally, positive expression of PD-L1

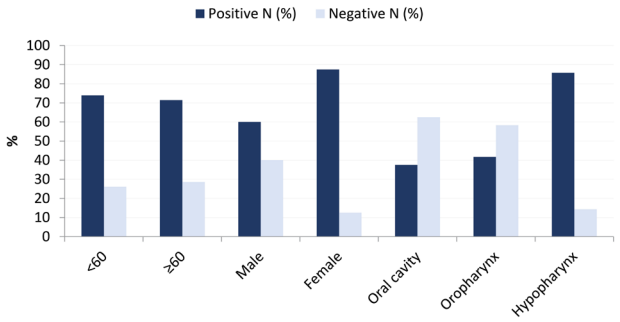

Figure 2. Frequency of PD-L1 expression among patients of different age, gender and tumor site.

Table 4. Relation between PD-L1 expression and stages of the tumor by chi-square test

	PD-L1 expression		*P* value
	Positive No. (%)	Negative No. (%)	
T stage			
T1-T2	8 (61.5%)	5 (38.5%)	0.562
T3-T4	26 (70.3%)	11 (29.7%)	
N stage			
N0-N1	20 (64.5%)	11 (35.5%)	0.500
N2-N3	14 (73.7%)	5 (26.3%)	
TNM stage			
1-11	5 (71.4%)	2 (28.6%)	0.834
111-1v	29 (67.5%)	14 (32.5%)	

was detected in 71.4% of patients with stage I-II HNSCC, and in 67.5% of cases with stage III-IV HNSCC (Table 4, Figure 3).

Study regarding EGFR immunohistochemistry on a total of 50 patients, showed its immunopositivity in 32 cases, accounting for 64% of the total (Figure 4). The expression of EGFR was predominantly observed at the cellular membrane and cytoplasm of tumor cells. Among male patients, 68.6% had positive expression of EGFR, whereas among female patients, 53.3% exhibited positive expression of PD-L1.

The study demonstrates the expression of EGFR by tumor cells in different types of HNSCC. In moderately differentiated HNSCC, tumor cells do not express EGFR, while in moderately differentiated HNSCC, they exhibit mild to moderate reactivity to EGFR. In moderately differentiated HNSCC, tumor cells exhibit mild to moderate reactivity to EGFR, indicating EGFR-positive cells. In moderately differentiated HNSCC, tumor cells exhibit significant reactivity to EGFR, indicating robust

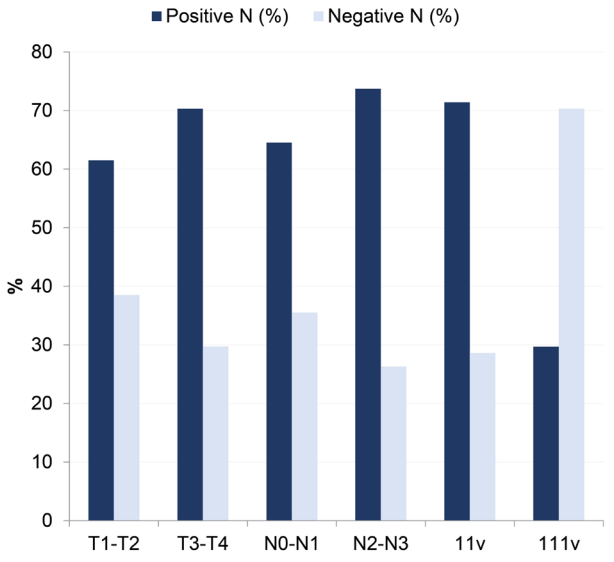

Figure 3. Frequency of PD-L1 in stages of tumor.

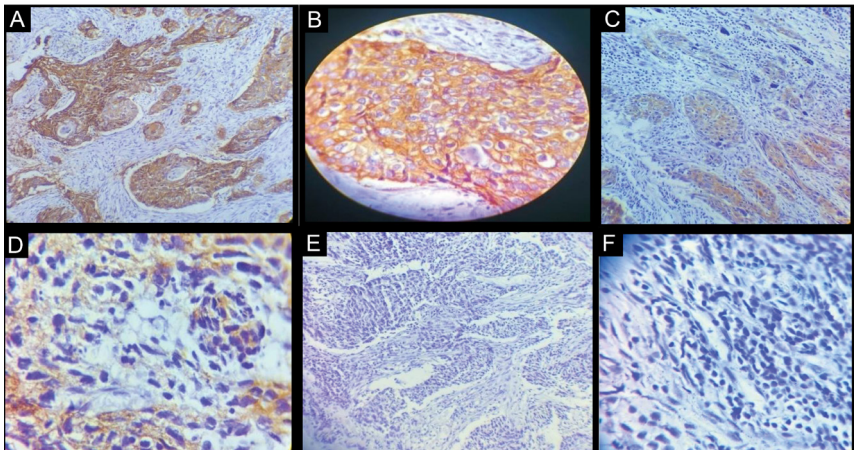

Figure 4. (A) PD-L1 strong positive expression (score2, CPS >20) in moderate differentiated squamous cell carcinoma 200×. (B) PD-L1 strong positive expression (score 2, CPS>20) in moderate differentiated squamous cell carcinoma 400×. (C) PD-L1 intermediate positive expression (score 1, 1 ≤ CPS < 20) in moderate differentiated squamous cell carcinoma 200×. (D) PD-L1 intermediate positive expression (score 1, 1 ≤ CPS < 20) in moderate differentiated squamous cell carcinoma 400×. (E) PD-L1 negative expression (score 0, CPS < 1) in undifferentiated squamous cell carcinoma 200×. (F) PD-L1 negative expression (score 0, CPS < 1) in undifferentiated squamous cell carcinoma 400×.

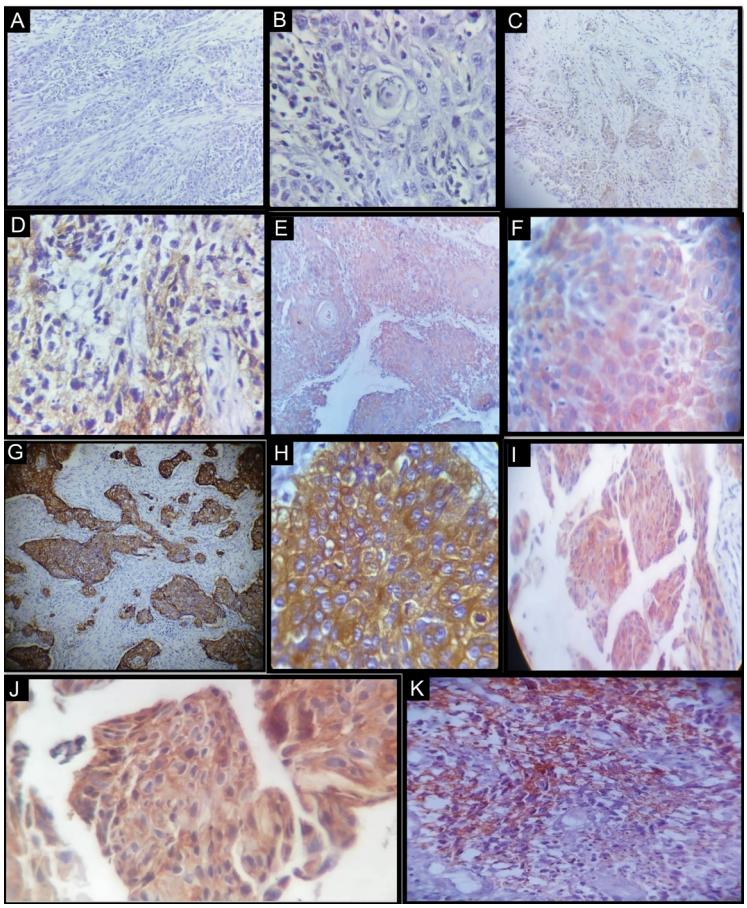

Figure 5. (A) (EGFR; 100×): The tumor cells in moderately differentiated HNSCC do not express EGFR (EGFR-negative tumor cells). (B) (EGFR; 400×): The tumor cells in moderately differentiated HNSCC do not express EGFR (EGFR-negative tumor cells). (C) (EGFR; 100×): The tumor cells exhibit mild to moderate reactivity to EGFR, indicating EGFR-positive tumor cells in moderately differentiated HNSCC. (D) (EGFR; 400×): The tumor cells exhibit mild to moderate reactivity to EGFR (EGFR-positive tumor cells) in moderately differentiated HNSCC. (E) EGFR; 100×): The tumor cells exhibit mild to moderate reactivity to EGFR, indicating EGFR-positive tumor cells in moderately differentiated HNSCC. (F) (EGFR; 400×): The tumor cells exhibit mild to moderate reactivity to EGFR, indicating EGFR-positive tumor cells in moderately differentiated HNSCC. (G) (EGFR; 100×): The tumor cells exhibit significant reactivity to EGFR, indicating a robust EGFR positivity in moderately differentiated HNSCC. (H) (EGFR; 400×): The tumor cells exhibit significant reactivity to EGFR, indicating robust EGFR positivity in moderately differentiated HNSCC. (I) (EGFR; 100×): The tumor cells exhibit significant reactivity to EGFR, indicating a robust EGFR positivity in poorly differentiated HNSCC. (J) (EGFR; 400×): The tumor cells exhibit significant reactivity to EGFR, indicating a robust EGFR positivity in poorly differentiated HNSCC. (K) (EGFR; 200×): The tumor cells exhibit weak to moderate reactivity to EGFR, indicating EGFR-positive tumor cells in undifferentiated HNSCC.

EGFR positivity. In poorly differentiated HNSCC, tumor cells show significant reactivity to EGFR, indicating robust EGFR positivity. In undifferentiated HNSCC, tumor cells show weak to moderate reactivity to EGFR (Figure 5).

The chi-square test revealed no statistically significant correlation between EGFR expression and gender, age, or tumor site. It evaluates the independence or association of the variables. EGFR positive expression was detected in 68.8% of oral cavity cases (11 out of 16 instances), 62.5% of oropharynx cases (5 out of 8 cases), 58.3% of hypopharynx cases, and 64.3% of larynx cases. There was no notable link observed between the expression of EGFR and the location of the tumor. In HNSCC patients, there was also no significant association between the site of the tumor and the expression of EGFR (chi-square test; Table 5).

Our study also showed, EGFR positive expression in 54.1% of cases with well-moderately differentiated HNSCC, and in 92.3% of cases with poorly-undifferentiated cancer. The study of the data using the chi-square test revealed a strong correlation between the expression of EGFR and the differentiation of tumors (Table 6, Figure 6).

There was no significant association between EGFR expression and the T stage of the tumor (chi-square test). EGFR expression was observed in 0.3% of stage N0–N1 HNSCC cases, accounting for 48.4% of all samples. Similarly, no significant correlation was found between EGFR expression and the N stage of the tumor. EGFR was detected in 47.1% of stage I–II HNSCC cases. However, no overall significant association was identified between EGFR expression and the tumor's TNM stage (Table 7). All correlations were assessed using the chi-square test.

This study showed a noteworthy correlation using chi-square test between the expression of PDL1 and EGFR in tumors of HNSCC. Out of the total number of cases, 25 exhibited positive expression of both PD-L1 and EGFR. In contrast, nine samples showed positive PD-L1 expression but negative EGFR, while seven cases showed negative PD-L1 expression but positive EGFR (Table 8, Figure 7).

The logistic regression analysis was conducted to examine the association between PDL1 status (positive or negative) and EGFR status (positive or negative). The results indicate that the odds ratio (OR) for EGFR positivity is 3.57. This suggests that individuals with positive EGFR status are 3.57 times more likely to have positive PDL1 status compared to those with negative EGFR status. The 95% confidence interval (CI) for the OR ranges from 1.03 to 12.43, indicating a statistically significant relationship, as the interval does not include 1. These findings suggest a potential link between PDL1 and EGFR expression, which may have implications for targeted therapies and further research in this domain. A P-value of less than 0.05

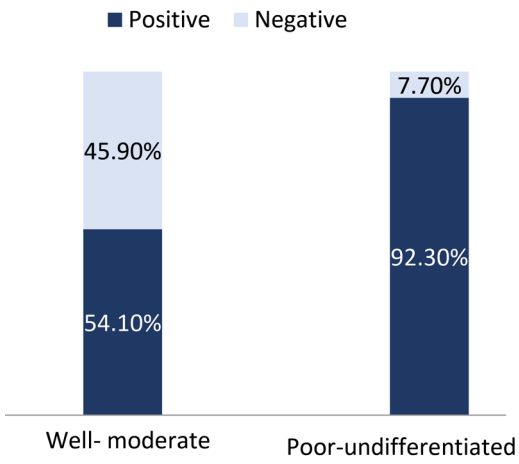

Figure 6. The frequency distribution of tumor differentiation (well-moderate vs. poor-undifferentiated) based on EGFR expression.

Table 5. Correlation between EGFR expression and (age, gender and tumor site) by chi-square test

	EGFR expression		*P* value
	Positive No. (%)	Negative No. (%)	
Sample size	32 (64.0%)	18 (36.0%)	
Age (y)			
<60	20 (74.1%)	7 (25.9%)	0.108
≥60	12 (52.2%)	11 (47.8%)	
Gender			
Male	24 (68.6%)	11 (31.4%)	0.304
Female	8 (53.3%)	7 (46.7%)	
Tumor site			
Oral cavity	11 (68.8%)	5 (31.3%)	
Oropharynx	5 (62.5%)	3 (37.5%)	0.954
Hypopharynx	7 (58.3%)	5 (41.7%)	
Larynx	9 (64.3%)	5 (35.7%)	

Table 6. Correlation between EGFR expression and differentiation of the tumor by chi-square test

		EGFR expression		*P* value
		Positive	Negative	
Differentiation	Well-moderate	20 (54.1)	17 (45.9)	0.033
	Poor-undifferentiated	12 (92.3)	1 (7.7)	

Chi-square value = 4.56, $P = 0.033$, Since $P < 0.05$, there is a statistically significant correlation between the degree of differentiation (well-moderate versus poor-undifferentiated) and expression PDL1, which means that the degree of differentiation may affect the positive expression of PDL1.

To calculate odds ratio (OR), we used the following formula: OR= (A×D)/(B×C).

Where: A = Number of positive cases in the first group (20); B = Number of negative cases in the first group (17); C = Number of positive cases in the second group (12); D = Number of negative cases in the second group (1)

Odds ratio (OR) = 0.098.

95% confidence interval (95% CI) = [0.0115, 0.8333].

The odds ratio is less than 1, indicating that patients with poor-undifferentiated tumors have a lower probability of expressing PDL1 compared to those with well-moderate differentiated tumors. Since the confidence interval does not include 1, this suggests that the result is statistically significant and unlikely due to chance. However, the results should be interpreted with caution given the small sample size. Additionally, EGFR positive expression was detected in 53.8% of cases with stage T1-T2 HNSCC and in 67.6% of those with stage T3-T4. No notable relationship was observed.

Table 7. Correlation between EGFR expression and stages of the tumor by chi-square test

	EGFR expression		*P* value
	Positive No. (%)	Negative No. (%)	
T stage			
T1-T2	7 (53.8%)	6(46.2%)	0.375
T3-T4	25 (67.6%)	12 (32.4%)	
N stage			
N0-N1	15 (48.4%)	16 (51.6%)	0.03
N2-N3	17 (89.5%)	2 (10.5%)	
TNM stage			
1-11	4 (57.1%)	3 (42.9%)	0.684
111-1v	28 (65.1%)	15 (34.9%)	

T stage (T1-T2 vs. T3-T4). P-value = 0.375 (No significant statistical correlation), N stage (N0-N1 vs. N2-N3): OR=(B×C)/(A×D)
Where: A = 15 (positives in N0-N1), B = 16 (negatives in N0-N1), C = 17 (positives in N2-N3), D = 2 (negatives in N2-N3)
OR=0.11
P=0.003 (There is a statistically significant association), TNM stage (I-II versus III-IV): *P*=0.684 (no significant statistical correlation). As shown in Figure 6 and Table 7, the results of the chi-square test showed a statistically significant relationship between the lymph node stage (N stage) and the studied variable, with a probability value of *P*=0.03. Accordingly, chi-square test results showed a statistically significant relationship between lymph node stage (N stage) and EGFR expression, with a *P* value of 0. 03 was performed to calculate the odds ratio. The results showed that patients in stage N2–N3 had a 9.07 times higher probability of belonging to the positive category compared to patients in stage N0–N1. This high odds ratio value, together with statistical significance, indicates a strong association between advanced lymph node staging and an increased likelihood of the outcome being studied. To calculate OR (odds ratio):
OR=B·CA·D
Where: A = 15 (number of positive cases in group 1: N0–N1), B = 16 (number of negative cases in group 1), C = 17 (number of positive cases in group 2: N2–N3)
OR=9.07.

indicates that the relationship between PDL1/EGFR and outcomes is not due to random chance, meaning there is a statistically significant difference.

Discussion

This study aims to examine the immune-expression of PD-L1 and EGFR in HNSCC. Our study is essential for personalized medicine, immunotherapy, targeted therapy, prognosis, and the advancement of novel treatments aimed at PD-L1 and EGFR in HNSCC. Comprehending these expressions can assist in customizing treatment tactics, focusing on PD-L1 for immunotherapy, and formulating novel medicines that target these genes. This discovery could substantially enhance therapy and results for people with HNSCC.

Table 8. Correlation between PDL1 and EGFR expression by chi-square test

PDL1/EGFR status	No. of cases (n=50)	Percent	*P* value
+ve/+ve	25	50%	0.041
+ve/neg	9	18%	
neg/+ve	7	14%	
neg/neg	9	18%	
Total	50	100%	

Head and neck squamous cell carcinoma is a prevalent cancer that arises from the mucosal epithelium in the oral cavity, throat, and larynx. There is a frequent association between it and the misuse of tobacco or alcohol, whereas pharyngeal cancers are more commonly attributed to HPV infection. The majority of patients are diagnosed with advanced-stage HNSCC without a pre-malignant lesion (17).

In 2018, there were 890 000 new instances of HNSCC, which is the sixth most prevalent cancer worldwide. Out of these cases, 450 000 resulted in death. The global cancer observatory predicts that the number of new HNSCC cases will rise by approximately 30% year, reaching 1.08 million by the year 2030 (18).

PD-L1 is an immune checkpoint protein that controls the immune system by interacting with the programmed cell death protein 1 (PD-1) receptor. It is present on many immune cells and is detected in numerous types of cancer. It should remember that, PD-L1 binds to PD-1, which assists tumor cells in evading the immune system by suppressing T cell functions such as activation, proliferation, anergy, exhaustion, and apoptosis. Discontinuing this connection has emerged as a successful therapeutic approach for boosting the body's ability to fight against tumors in

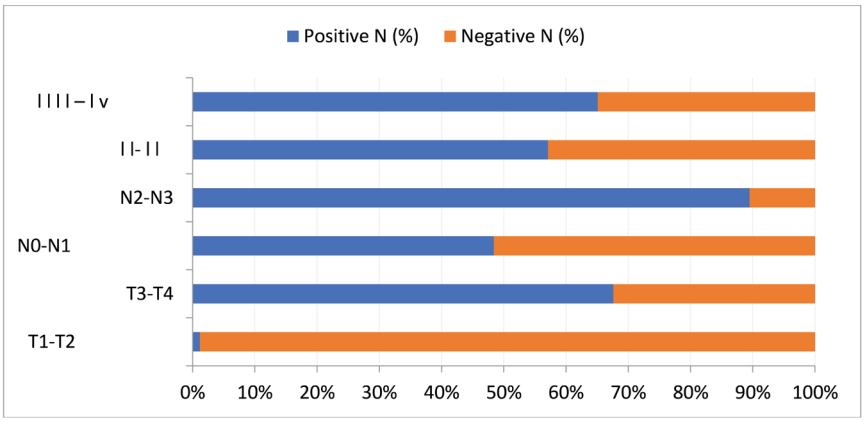

Figure 7. The frequency distribution of stages of the tumor based on EGFR expression.

several types of cancers, including patients with HNSCC (19,20).

The study revealed that 68.0% of HNSCC cases exhibited PD-L1 overexpression, which is consistent with the number reported by Chen et al (21) study. In a cross-sectional observational analysis of 95 patients, the presence of PD-L1 was detected in 67% of the cases. It has been detected that, PD-L1 immunohistochemistry was conducted on 93 cases, and 59 cases (63.4%) showed positive immunoreactivity (22).

The expression of PD-L1 in HNSCC varies considerably, ranging from 39% to 79% (23-26). This variation can be due to several variables, including varied methods, antibody clones, cut-offs, and interobserver variability. Additionally, the use of different antibody clones for immunohistochemistry (IHC) also contributes to the variability (27).

Several clinical and pathological factors were analyzed, including age, gender, tumor site, tumor differentiation grade, T stage, N stage, and TNM stage (23,24,28,29).

A recent study by Lin et al (30) investigated the relationship between PD-L1 expression and clinical factors in HNSCC. Their results showed that higher tumor stage (T3-T4) and distant metastasis were significantly associated with increased PD-L1 expression in a cohort of 305 patients with oral squamous cell carcinoma. However, these findings contrast with some previous studies, highlighting the need for further research on this topic. The recent study by Lin et al, on the relationship between PD-L1 expression and other clinical and tumor-related factors has shown conflicting results. They should higher tumor stage and distant metastasis were strongly related with PD-L1 expression in research comprising 305 patients with HNSCC (31).

Higher PD-L1expression in HNSCC was found to be substantially linked with tumor stage, lymph node status, and regional metastases, according to studies by Theodoraki MN (32). Bossi et al (33) found EGFR activation in HNSCC cell lines and a high percentage of primary HNSCC.

Our study demonstrated EGFR overexpression in 32 (64.0%) HNSCC cases. This percentage is similar to Pandey et al (34). This cross-sectional observation study included 100 HNSCC cases. We found EGFR was 58% positive.

Janecka-Widła et al (35), Numico et al (36), and Owusu-Afriyie et al (37) found that in 34.3%, 35.0%, and 29.4% of 155 HNSCC, 149 HNSCC, and 154 non-oropharyngeal HNSCC patients, respectively, these authors found EGFR overexpression. However, several HNSCC articles reported EGFR positive rates from 45.2% (2) to 100%. The discrepancies may be due to various immunological ratings and cut off points utilized to identify tumors with or without EGFR overexpression. Some scientists divide tumors by EGFR positivity/negativity (34), while others use EGFR overexpression/lack of overexpression (38).

Different cut-off points are used to distinguish EGFR overexpression/under expression. Murray et al (39), and Bernardes et al (40) used week or moderate or strong staining in more than 10% of tumor cells as a cutoff. In turn, Owusu-Afriyie et al (37) used immunoreactive score (multiplication of intensity of staining and percentage of positive staining cells, range from 0 to 12) with cutoff point at 4, and Atkins et al (41) categorized tumors by EGFR expression into four classes: no, weak, moderate and strong.

In our study, EGFR expression was not associated with age ($P=0.108$), gender ($P=0.304$), primary site of the tumor ($P=0.954$), T stage ($P=0.375$) or TNM stage ($P=0.684$) but it was associated with tumor differentiation grade ($P=0.013$) and N stage ($P=0.003$), which was consistent with Sheikh Ali et al (42).

In contrast, Pandey et al (34) found no statistically significant connection between EGFR expression and any clinicopathological variables of HNSCC cases. The association between EGFR expression and epidemiological, clinical and histological characteristics is likewise inconsistent. EGFR expression was not associated with patient age, TN stage or grade.

Hashmi et al (2) found that older patients and T3-T4 malignancies had a larger percentage of EGFR overexpression than younger patients or tumors with lower T stages. Others showed a link between EGFR, expression and grade, with more grade 3 cancers overexpressing EGFR (37). Meanwhile, Brand et al (43) found higher expression in oral tumors than other HNSCC localizations, but we did not corroborate this in this analysis. These discrepancies may be due to tumor distribution, sample size, scoring criteria, and author-used antibodies, and also for HNSCC localization-specific EGFR expression (43).

Recently, Ogiya et al (44) investigated whether PD-L1 and EGFR expression were linked in head-and-neck squamous cell cancer. They found, in HNSCC, PD-L1 expression and EGFR presence were positively associated. Huang et al (45) and Kim et al (26) revealed a positive association between PD-L1 expression and EGFR presence in HNSCC, suggesting that both markers may be linked to disease development and progression.

Our research found a substantial correlation between PD-L1 expression and EGFR in HNSCC. These data suggest that PD-L1 and EGFR may operate synergistically and that immunotherapy targeting both proteins may treat head and neck squamous cell cancer (46).

This finding suggests that PD-L1 and EGFR may be involved in HNSCC beginning and progression (47). However, further research is required to determine this connection's clinical importance and mechanisms. HNSCC may be treated by targeting PD-L1 and EGFR. The precise role of EGFR in modulating PD-L1 expression and its potential impact on immunotherapy and targeted treatments for HNSCC need more study (44-47).

Conclusion

Over two-thirds of HNSCC cases have PD-L1 expression, and the study found a strong association between tumor site and PD-L1 expression. Evidence from this study links EGFR expression to tumor differentiation and N stage. Nevertheless, there was no evidence that patient demographics including age, gender, or tumor site impacted EGFR expression.

Limitations of the study

The study's constrained sample size and retrospective approach may restrict generalizability, as larger samples could enhance statistical power and bolster confidence in conclusions, while the retrospective aspect may add possible biases.

Authors' contribution

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Investigation: Hussein Abdel-moneim Hassan.
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Project administration: Ismail Mohamed Ismail.
Resources: Ismail Mohamed Ismail and Hussein Abdel-moneim Hassan.
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Conflicts of interest

The authors declare no conflict of interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Faculty of Medicine Al-Azhar University, Assiut (Ethical code #AZAST/MD/117/15-SEP-2024). Prior to any intervention, all participants provided written informed consent. The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

Funding/Support

None.

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