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Immunohistochemical expression of PDL1 in laryngeal carcinoma in relation to P53 and ki67 proliferative index

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Abstract---Squamous cell carcinoma of larynx is one of the most common malignant neoplasms of the head and neck. Carcinoma of the larynx represents 2.2% of all cancers in men and 0.4% in women, with predilection of old aged men of more than 50 years rather than women. Smoking is the main risk factor. Alcohol, nutritional factors and virus as Human papillomavirus (HPV) play a role in the pathogenesis of laryngeal carcinoma. Programmed death-ligand (PDL1) is the new introduced marker that be considered as treatment target nowadays in addition to its prognostic application. Aims of study: This study was designed to assess the predictive the role of PDL1 in laryngeal squamous cell carcinoma in comparison with other prognostic markers (P53 and Ki-67).Results: The current study revealed that PDL1 expression was reported in about 20 % of laryngeal squamous cell carcinoma and 10% in premalignant, while it was negative in benign laryngeal lesions. Others markers; P53 protein was shown to be positive in around 42.5 percent of laryngeal squamous cell carcinomas, 40 percent of premalignant laryngeal lesions, and about 12 percent of benign laryngeal lesions. While looking at the ki67 protein, we discovered that 32.5% of laryngeal carcinomas had high ki67 expression, 40% of premalignant lesions had high ki67 expression, and 20% of benign laryngeal lesions had high ki67 expression.Conclusion: From current study we can conclude that PDL1 expression has an essential role in pathogenesis of laryngeal carcinoma and noticed in both premalignant and malignant laryngeal lesions without statistical significant difference and it is associated and correlated to P53 and Ki-67. This finding

assists the PDL1 target therapy and the screening surveillance of any laryngeal lesion particularly premalignant lesions

Keywords---laryngeal squamous cell carcinoma, PDL-1, P53, ki67, prognosis.

Introduction

Globally, head and neck cancers (HNSCC) are the sixth common cause of morbidity and mortality. Squamous cell carcinoma of nasopharynx, oropharyngeal cavity, nasal sinus, hypopharynx and larynx accounted for more than 90% of cases (1). In males, laryngeal cancer accounts for 2.2 percent of all cancers, whereas in women, it accounts for 0.4 percent of all cancer. Most patients were men above 50 years of age, although some cases were reported in younger patients (1). It has been documented that the incidence of women is increasing in the last decades (2). Smoking is the main risk factor, and excessive drinking can exacerbate this risk (2).

Anatomically, there are three types of laryngeal cancer; glottic, supraglottic and subglottic type. Squamous cell carcinoma (verrucous, basaloid, spindle) is the main histological type. Other types, mucoepidermoid, adenoid cystic, sarcoma (chondrosarcoma, rhabdomyosarcoma), lymphoma, and neuroendocrine tumors. The major risk factors are smoking, alcohol, viral infection (HPV) and nutritional deficiency. In laryngeal cancer, HPV is considered as a etiologic and prognostic factor (3,4). The main predisposing factors of laryngeal carcinoma are precancerous lesions (keratosis and papilloma) (4), this fact was noted in more than 90% of cases. Therefore, early detection of these lesions and treatment is the main step in management in order to prevent progression to invasive form (5).

The WHO (5) has classified the precancerous lesions into three grades as; Grade 1 (simple hyperplasia or keratosis with or without mild dysplasia), Grade2 (moderate dysplasia) and Grade3 (severe dysplasia). Therefore, the progress sequence of these lesions continues and evolves in to a malignant form (6). In contrast, non-neoplastic laryngeal lesion as; laryngeal nodule and amyloidosis have no role in the pathogenesis of laryngeal carcinoma (6).

Programmed death-ligand 1 (PD-L1); also known as 274 differentiation cluster (CD274), is a 40kDa type 1 transmembrane protein that has been speculated to play a major role in suppressing the adaptive arm of the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. In cancer, PDL1 gets more expression and hence more inactivation of the immune system will result in evolution of cancer (7). Nowadays PDL1 is planned to be a target for treatment of many cancers, including laryngeal carcinoma. The tumor suppressor gene P53 and the proliferative marker Ki-67 are well known prognostic markers that has been employed for proper assessment of the biological outcome laryngeal cancer (8). The relationship of PDL1 and these markers has been investigated by many researchers in many cancers, still few evidence of this relation has been found regarding laryngeal carcinoma. In our country, little data has been found about

this evidence and no published paper on PDL1 and its relation to P53 and Ki-67 in laryngeal cancer has been found. So from this point we found an essential need clarify this evidence.

Materials and Methods

This study is a cross sectional retrospective one, conducted at the College of Medicine, Department of Pathology, University of Kufa, during the period from September 2020 till September 2021. A total of 75 samples of formaldehyde fixed and paraffin-embedded tissues (FFPET) were included. Forty cases were Squamous cell carcinoma (SCC), 10 cases were premalignant laryngeal lesions (SIL) and 25 cases were benign laryngeal lesions (papilloma and singer's nodules). For confirmation of diagnosis, cases were resectioned and stained with hemotoxyllin and eosin (H&E). The expression of PD-L1, P53, and ki67 proteins in all malignant, premalignant, and benign laryngeal lesions was assessed by immunohistochemistry. The PD-L1 (clone 28-8) protein expression is measured using the Combined Positive Score (CPS), which is positive if the CPS is ≥ 1 %. Score index was used for P53 (clone) protein expression, which is positive if SI is ≥ 1 %. Score index for Ki67 protein expression was also assessed using a score index with a cutoff of 4%, as well as low and high expression levels.

Statistical analysis

The significance of expression, association, and correlation between these markers were assessed using statistical analysis using Chi-squared (χ^2) test, Fischer test, correlation and regression test.

Results

The current study revealed that PDL1 expression was reported in about 20 % of laryngeal squamous cell carcinoma and 10% in premalignant, while it was negative in benign laryngeal lesions. P53 protein was shown to be positive in around 42.5 percent of laryngeal squamous cell carcinomas, 40 percent of premalignant laryngeal lesions, and about 12 percent of benign laryngeal lesions. While looking at the ki67 protein, we discovered that 32.5% of laryngeal carcinomas had high ki67 expression, 40% of premalignant lesions had high ki67 expression, and 20% of benign laryngeal lesions had high ki67 expression (Tables.1,2,3).

Table.1: PDL1 expression in laryngeal lesions that are benign, premalignant and malignant (N=75)

Types of lesion	PDL1 expression		Total n(%)	P Value
	Positive n(%)	Negative n(%)		
Benign	0 (0)	25 (33.3%)	25 (33.3%)	0.053*
Premalignant	1 (10%)	9 (90%)	10 (13.3%)	
Malignant	8 (20%)	32 (80%)	40 (53.4%)	
Total	9 (12%)	66 (88%)	75 (100)	

*At least 20% of expected frequencies are less than 5

* Fischer test for premalignant and malign lesion is 0.6651 at level of significance of <0.05.

Table.2: P53 expression in laryngeal lesions that are benign, premalignant and malignant (N=75)

Types of lesion	P53 expression		Total n(%)	P Value
	Positive n(%)	Negative n(%)		
Benign	3 (12%)	22 (88%)	25 (33.3%)	0.03
Premalignant	4 (40%)	6 (60%)	10 (13.3%)	
Malignant	17 (42.5%)	23 (57.5%)	40 (53.4%)	
Total	24 (32%)	51 (68%)	75 (100)	

Table.3: KI67 expression in laryngeal lesions that are benign, premalignant and malignant (N=75)

Types of lesion	KI67 expression		Total n(%)	P Value
	High n(%)	Low n(%)		
Benign	5(20%)	20 (80%)	25 (33.3%)	0.41
Premalignant	4 (40%)	6 (60%)	10 (13.3%)	
Malignant	13 (32.5%)	27 (67.5%)	40 (53.4%)	
Total	22 (29.4%)	53 (70.6%)	75 (100)	

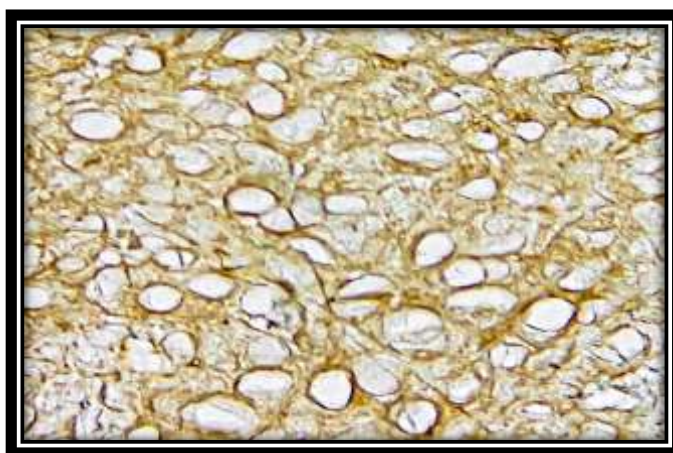


Fig. (1): PDL1 (+3 score), diffuse strong membranous stain in squamous cell carcinoma (10 x 40)

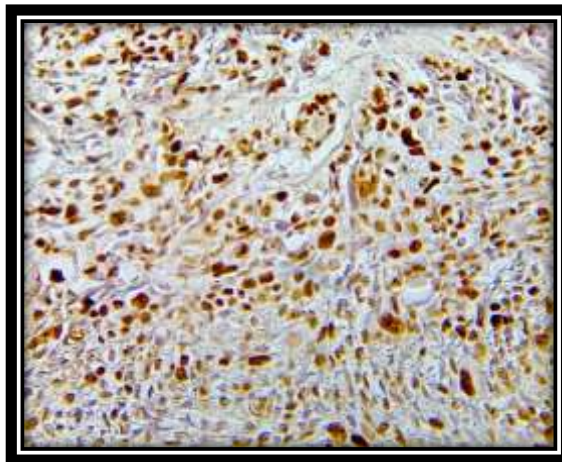


Fig. (2) P53 positive, diffuse strong nuclear stain IHC of squamous cell carcinoma . A10x 4 B 10x 10

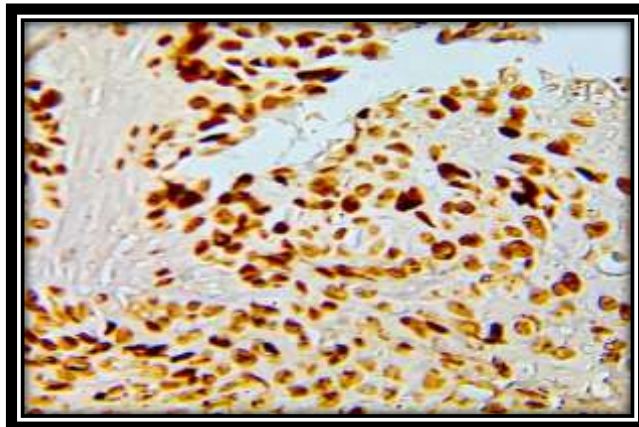


Fig. (3) ki67 high expression ,diffuse nuclear stain IHC of squamous cell carcinoma . 10x 40

Association between PDL1 and P53 in laryngeal carcinoma as follow

This relation explained by our results, in PDL1 positive cases; about five cases (true positive) out of seventeen cases of P53 positive, that associated with PDL1 positive, because p53 was the major surrogate indicator for transcriptionally active PDL1, while in PDL1 negative cases; about twenty cases (true negative) out of twenty-three cases of P53 negative, that associated with PDL1 negative, because p53 was the major surrogate indicator for transcriptionally active PDL1. The ability to be sensitivity (The probability of a true positive test) of PDL1 in relation to P53 = $a / a+c * 100 = 5 / 17 = 29.4\%$. The ability to Specificity (The probability of a true negative test) of PDL1 in relation to P53 = $d / b+d * 100 = 20 / 23 = 87\%$.

So the PDL1test (as preliminary test) has low sensitivity and high specificity in relation to p53 test (as essentially test) that benefit in exclude the false negative (sensitivity) and false positive(specificity), and reduce error rate . Predictive value of PDL1 for positive

and negative test in relation to p53 test. The predictive values values (PPV and NPV, respectively) are the percentage of true positive and true negative outcomes in statistics and diagnostic tests.

$$\text{PPV} = A/A+B * 100 = 5/8 * 100 = 62.5 \%$$

$$\text{NPV} = D/C+D * 100 = 20/32 * 100 = 62.5 \%$$

So thePDL1 is a good test for identify the proportion of those with positive or negative laryngeal carcinoma (table.4.).

Association between PDL1 and KI67 in laryngeal carcinoma as follow

This relation explained by our results, in PDL1 positive cases; about six cases (true positive) out of thirteen cases of KI67 positive, that associated with PDL1 positive, because KI67 was the major surrogate indicator for transcriptionally active PDL1, while in PDL1 negative cases; about twenty-five cases (true negative) out of twenty-seven cases of KI67 negative, that associated with PDL1 negative, because KI67 was the major surrogate indicator for transcriptionally active PDL1.

Sensitivity of PDL1 in relation to KI67 = $a / a+C * 100 = 6 / 13 = 46.15\%$.

Specificity of PDL1 in relation to KI67 = $d / b+d * 100 = 25 / 27 = 92.5\%$.

So the PDL1test (as preliminary test) has low sensitivity and very high specificity in relation to KI67 test (as essentially test) that benefit in exclude the false negative (sensitivity) and false positive(specificity) , and reduce error rate .

Predictive value of PDL1 for positive and negative test in relation to KI67 test

$$\text{PPV} = A/A+B * 100 = 6/8 * 100 = 75\%$$

$$\text{NPV} = D/C+D * 100 = 20/32 * 100 = 78\%$$

So thePDL1 is a good test for identify the proportion of those with positive or negative laryngeal carcinoma (table.5).

Table .4: association of both PDL1 and P53 in laryngeal carcinoma

		P53 as essentially test		
PDL1 as Preliminary Test		P53 +	P53 -	Total (%)
	PDL1 +	True + (A) 5 *	False + (B) 3	8 (20)
	PDL1 -	False – (C) 12	True – (D) 20 **	32(80)
	Total(%)	17 (42.5)	23 (57.5)	40 (100)

Table.5: association of both PDL1 and P53 in laryngeal carcinoma

		KI67 as essentially test		
PDL1 as Preliminary Test		KI67 +	KI67 -	Total (%)
	PDL1 +	True + (A) 6 *	False + (B) 2	8 (20%)
	PDL1 -	False - (C) 7	True - (D) 25 **	32 (80)
	Total (%)	13(32.5)	27 (67.5)	40 (100)

The Correlation between PDL1 expression and P53 in malignant lesions is clarified to be positive correlated; $R = 0.30$ (fig.4).

The Correlation between PDL1 expression and KI67 in malignant lesions is clarified positive correlated, $R = 0.28$ (fig.5).

The Correlation between P53 expression and KI67 in malignant lesions is clarified well correlated, $R = 0.44$ (fig.6).

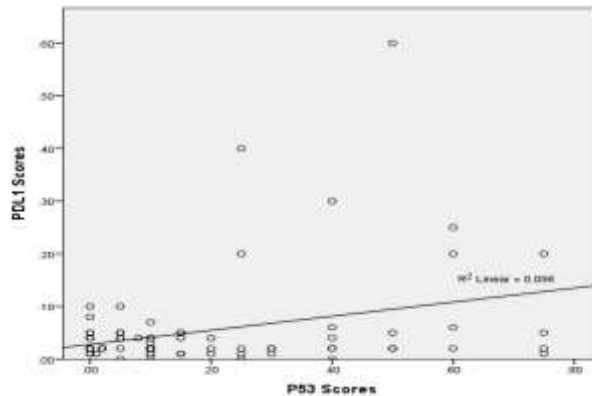


Fig. (4) : Linear correlation test between PDL1 expression and P53 expression in laryngeal carcinoma

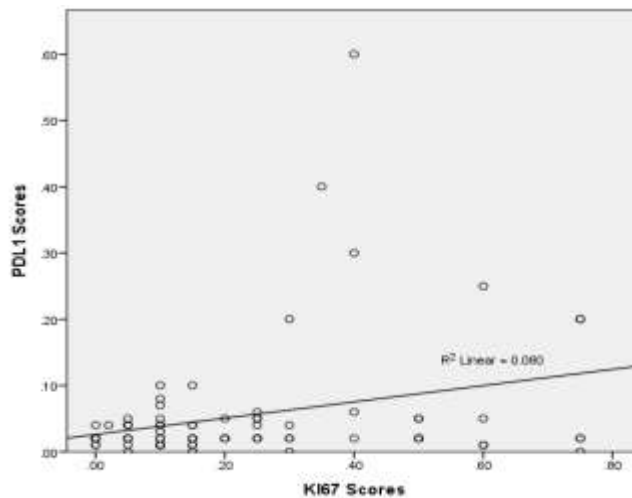


Fig. (5) : Linear correlation test between PDL1 expression and KI67 expression in laryngeal carcinoma

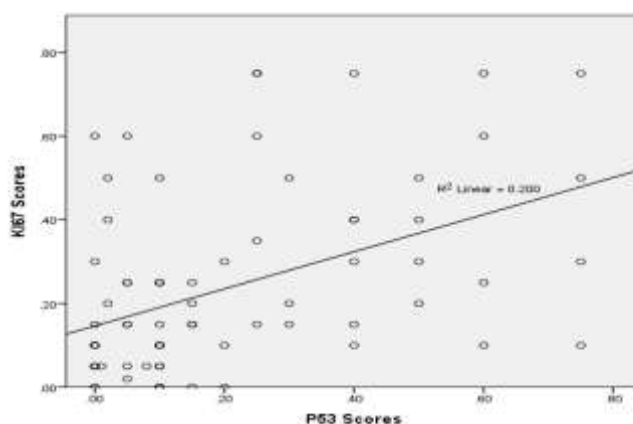


Fig. (6) : Linear correlation test between KI67 expression and P53 expression in laryngeal carcinoma

Conclusion

We can conclude from the current study that PDL1 expression has an essential role in pathogenesis of laryngeal carcinoma and noticed in both premalignant and malignant laryngeal lesions without statistical significant difference and it is associated and correlated to P53 and Ki-67. This discovery aids PDL1 target treatment as well as screening monitoring of any laryngeal lesion, including premalignant lesions.

Discussion

PDL1 expression in laryngeal carcinoma

In this study, it has reported that PDL1 immunoexpression was detected in 20% of malignant laryngeal tumors (squamous cell carcinoma). In comparison with other studies, there was some variation in the rate of PDL1 expression from one to other. Previous studies on upper aropharyngeal carcinomas in Japan (2019), have revealed that PD-L1 expression has a wide range from 18% to 87%, depending on the site of tumor (9,10). In one study done in Taipei Veterans General Hospital, in 2017, 32.1% of laryngeal carcinoma was exhibited PD-L1 expression(9). Similarly, other study done in Istanbul –Turkey, revealed the same result for PDL1 expression (32.7%) (11).

The variation in PDL1 expression may be related to race, size of study and some technical factors. Up to our knowledge, no local published study was reported. Furthermore, PDL1 expression was reported in 10% of premalignant lesions. In comparison with malignant lesions, there was no significant difference in PDL1 expression in both groups ($p > 0.05$), however, less rate of expression was noticed in these lesion. This is a vital and important point in checking and screening all premalignant lesions as the possibility and risk of malignant changes is high, and this is agreed with other study obtained by. Aló, P. L. et al 2021 that documented PDL1 expression in premalignant lesions was less frequent as malignant lesion (12).

P53 expression in laryngeal carcinoma

The outcomes of the present study showed that P53 expression was Positive detected in 42.5% of malignant laryngeal tumors (squamous cell carcinoma) with significant difference with a premalignant laryngeal lesion, this agrees with study in Spain obtained by Raquel V. et al 2003, that documented p53 was detected in 41.9% of carcinomas (13), and this percentage was slightly lower than other study in Spain don by Jose Lera et al 1998, which was documented the expression of p53 was found in 32 (56 percent) of 57 instances, were(14).

KI67 expression in laryngeal carcinoma

The outcomes of the present study showed that ki6n expression was positive detected in 32.5% of malignant laryngeal tumors (squamous cell carcinoma), this percentage of expression is proximal to other study in Spain done by Jose Lera et al 1998, that found Ki-67 positive cells was reported in 23.7% (The mean percentage of positive cells in three groups was range, 2.6%-53.5%) (14).

Association between PDL1, p53 and KI67 among malignant tumor

To ensure the validity (sensitivity and specificity) of PDL1 expression in laryngeal carcinoma we need compare PDL1 IHC test (as preliminary test) with other considered as golden test (like PCR), or compare P53 IHC test and KI67 IHC test (that consider in our study as essential test) with other golden test. Sensitivity of PDL1 in relation to P53 was 29.4%. Specificity of PDL1 in relation to P53 was 87%, The PPV (positive predictive value) was 62.5 percent. The NPV (negative predictive value) was 62.5 percent. Sensitivity of PDL1 in relation to KI67 was 46.15%. Specificity of PDL1 in relation to KI67 was 92.5%. PPV was 75% .NPV was 78%.

This result was not discussed in the previous studies, but in one study done by Lars Hagmeyer et al 2020; The immunocytochemistry evaluation of pleural effusion was compared to the immunohistochemistry evaluation of pleural tissue in non-small cell lung cancer, where PDL1 evaluation was suggested. They found that the sensitivity and specificity of PD-L1 detection (PD-L1 expression positive) with a TPS $\geq 50\%$ were 71% and 71% . The positive predictive value was 42% and the negative predictive value was 89%, sensitivity and specificity of PD-L1 detection (PD-L1 expression positive) with a TPS $\geq 1\%$ were 71% and 64%. The negative predictive value (NPP) was 64%, whereas the positive predictive value (PPV) was 71% (15). Also other study obtained in China done by Ying-mei Zheng et al 2022, that compare the patients who underwent immunohistochemical examination of PD-L1 expression in HNSCC with CECT-image-based radiomics (16).

The Correlation between the expression of PDL1, P53 and ki67 proteins

In our study, the correlation between PDL1 expression and P53 in malignant lesions is clarified to be positively correlated ($R = 0.30$). This finding explains the high expression rate of PDL1 in P53 positive cases (it looks to be increasing as p53 expression increasing), this mean that PDL1 expression is considered to be

a prognostic marker, well correlated to the outcome of the disease. This finding has been supported by study done by Rasmussen et al 2020, that documented significant correlations between IHC biomarkers p53 and PD-L1 (17). Also other study obtained by Hui Chen et al 2021 that refer to this correlation (18).

The Correlation between PDL1 and KI67 expression in malignant lesions is clarified positive correlated ($R=0.28$). This agree with study done by Rasmussen et al 2020 that documented The significant correlations between the IHC biomarkers Ki-67 and PD-L1(17) (120). This is agreed with study obtained by Chen et al 2018 that revealed PD-L1 expression was shown to be positively connected with Ki-67 ($p = 0.003$), indicating that tumor PD-L1 expression is linked to a higher Ki-67 proliferation index.(19).

The Correlation between P53 expression and KI67 in malignant lesions is clarified well correlated ($R= 0.44$). This agree with study done by WOJCIECH PASTUSZEWSKI et al 2007 As a result, Ki-67 antigen expression was strongly connected with p53 protein expression ($r=0.477$; p less than 0.05), and expression of either marker was favorably correlated with grading of malignancy ($r=0.47$, p less than 0.05; $r=0.31$, p less than 0.05; for Ki-67 and p53, respectively) (20).

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