

# Immunohistochemical Expression of Programmed Death Ligand1(PDL1) in Head and Neck Tumors Among Group of Iraqi Patients

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## ABSTRACT

**Background:** Most head & neck tumors are of squamous cell carcinoma type, with a high tendency for recurrence; Upregulation of programmed death ligand-1 (PDL-1) in some cancers associated with poor prognosis. Immunotherapy by using PDL-1 monoclonal antibodies to block this receptor on tumor cells is mainly used for metastasized, surgically unresectable, or recurrent cancers.

**Aim:** To assess the immunohistochemical expression of PDL-1 in head and neck tumors by the aid of H-score, and its relation to clinicopathological parameters and the staining pattern.

**Methods:** Thirty cases of head and neck tumors were collected randomly from March 2018 till July 2018 for Immunohistochemical staining of (PDL-1). The staining was assessed as either membranous or non-specific cytoplasmic/ membranous. Variable staining patterns were detected between normal squamous cells and tumor cells, besides; variable staining patterns between different areas within the tumor were also noticed. The t-test was performed for statistical analysis.

**Results:** There was a significant statistical difference between the mean percentage of squamous cell carcinoma cells for the non-specific staining patterns ( $p=0.022$ ) and for the calculated H- index ( $p=0.004$ ) with the tumor grade. When comparing the H-index of stained neoplastic cells and stained normal squamous cells, a significant statistical difference was noticed ( $p\text{-value}= 0.0019$ ).

**Conclusion:** Immunohistochemical method for detection of PDL1 in head and neck squamous cell carcinoma shows variable results, with a significant relation between stained tumor cells and tumor grade, which can be used as a predictor to apply immune therapy.

**Keywords:** Head and neck tumors, PDL1 expression, staining patterns

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## INTRODUCTION

Head and neck tumors include tumors of the nasal cavity, Para nasal sinuses, oral cavity, larynx, and salivary glands; most of the tumors are squamous cell carcinomas which are associated with tobacco smoking, HPV infection, and mechanical irritation, alcohol, and sepsis as known risk factors <sup>(1-3)</sup>.

The multiplicity of the tumor is common, and the possibility of developing a second primary tumor in the area is high. Programmed death ligand1 (PD-L1) also known as CD274 is a transmembrane protein, thought to play a major role in suppressing the immune system during certain conditions as pregnancy, tissue allografts, and autoimmune diseases <sup>(4)</sup>. Upregulation of PD-L1 in some cancers allows tumor cells to evade the immune system and increase its expression which may be associated with poor prognosis <sup>(5)</sup>. Immunotherapy by using PD-1 monoclonal antibodies (e.g. pembrolizumab, Nivolumab ) to block the PD-1 receptor on tumor cells that allows the activation of T cells against the tumor is mainly used for metastasized, surgically unresectable, or recurrent cancers <sup>(4-7)</sup>.

The aim of this pilot study is to make an assessment to the immunohistochemical expression of PDL-1 in head and neck tumors, comparing between normal cells and tumor cells staining patterns, trying to apply the H-score and to see if there is a significant association with the clinicopathological parameters and the staining pattern.

## MATERIALS AND METHODS

A retrospective study was held in histopathology lab / Gazi-Al- Harriry hospital for surgical specialties- Baghdad from 12th of march 2018 till 4th of July 2018, where thirty specimens of head and neck tumors were collected randomly, 23 of the cases were males and 7 cases were females, with the age range between 27 years to 90 years with a mean age (58.5 years). Specimens were tissues that had been fixed in formalin, processed, embedded in paraffin, and routinely stained by H&E method<sup>7</sup>.

In order to investigate the expression of (PDL1) within the examined cases, these specimens were stained by immunohistochemical method; immunohistochemical materials from (Bio SB products) were used, 4  $\mu$ m of representative tissue sections were taken from each paraffin block, and fixed on positively charged slides, dried for 24 hours at 37°C, followed by deparaffinization and rehydration, finally, the cases were stained by (DAP HRP) immunohistochemical technique {LSAB+} according to manufacture instruction for immune staining as in provided leaflet<sup>8</sup>.

Slides with H&E staining and IHC stain were examined by three pathologists.

## RESULTS

Most of the examined cases were from the larynx (10 of 30), followed by oral mucosa and lips, others include

tongue, maxilla, mandible, ear, and face. Histological examination revealed 25 cases with squamous cell carcinoma (12 well-differentiated, 11 moderately differentiated, and 2 poorly differentiated), 2 case of nasopharyngeal carcinoma, and three cases carcinoma in-situ.

The immunohistochemical staining for PDL-1 was either membranous or non-specific cytoplasmic/membranous. Variable staining patterns were noticed between normal squamous cells and tumor cells besides variable staining patterns between different areas within the tumor were also noticed. Increase the infiltration of positively stained lymphocytes is noticed in 14 cases. The interpretation of PDL1 staining score was estimated as ( 0, 1+,2+,3+ ) where zero indicate negative stain, 1+ : for cells with lower staining than the control cells, 2+ :cells stain same intensity with control, 3+ : cells stains more intense than control. About 100 cells in three random fields were examined for each score; 48 % of neoplastic squamous cells were negative (score 0); 27% score I, 12% score II, and 1.2 % score III (Fig. 1, 2); then the H- index calculated as in equation:

$$[1 \times (\% \text{ Cells } 1+) + 2 \times (\% \text{ Cells } 2+) + 3 \times (\% \text{ Cells } 3+)]^{(10,11)}$$

Also, tumor cells were evaluated for membranous versus non-specific staining pattern; 10.5% of tumor cells show membranous staining pattern.

The staining pattern for the normal squamous epithelium included within the biopsy also was evaluated. (50% score zero, 25% score I, 5% score II, and zero% for score III).

The statistical analysis was performed by applying the T-test. There was a significant statistical difference between the mean percentage of tumor cells for the non-specific staining patterns and for the calculated H- index (table 1) with the tumor grade (only squamous cell carcinoma cases included).

A significant statistical difference was noticed (p-value 0.0019) when comparing the H-index of stained neoplastic cells and stained normal squamous cells (table 1). Non-significant statistical relationship was found between membranous staining patterns for neoplastic cells and tumor grade (table1)

Fig.1 immunohistochemical stain of PDL1 in squamous cell carcinoma; A- score I ( negative); B- score II ( weak); C- score III ( positive non specific); C- score III (specific membranous stain ).

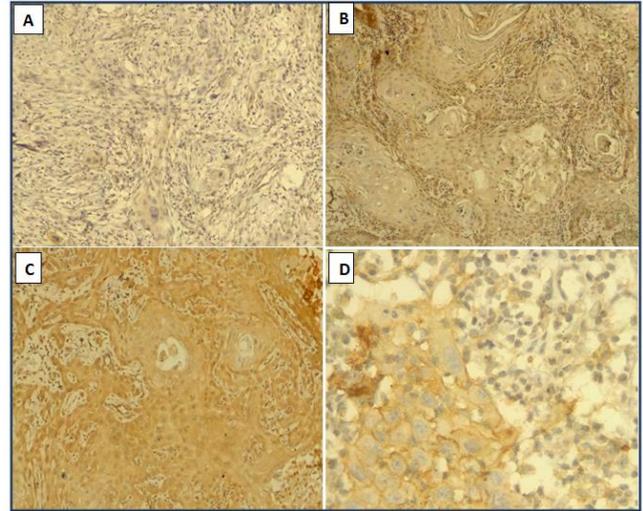


Fig 2: tumor cells with PDL1 expression score IV= strong positive

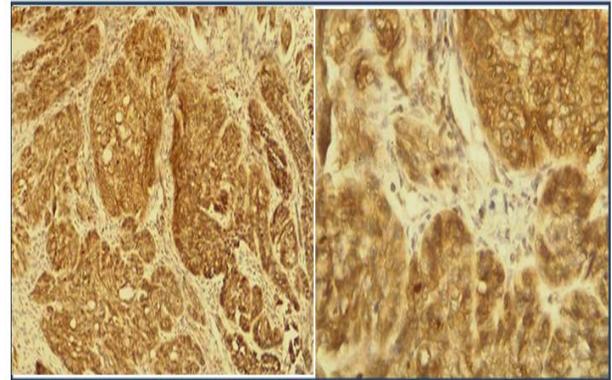


Table 1: Statistic analysis of PDL1 immune stain in relation to tumor grade, membranous stain and normal squamous epithelium

	Tumor grade	Mean percentage± SD	Un- paired T- test	Degree of freedom	SE of difference	95% Confidence Interval	p- value
Mean percentages of stained neoplastic cells for squamous cell carcinoma according to grade of tumor cells	Well differentiated (n=12)	53.33 ± 19.23	2.48	21	8.14	From 3.23 to 37.07	0.022*
	Moderately differentiated (n=11)	33.18 ± 19.78					
Mean percentages of stained membranes for squamous cell carcinoma according to grade of tumor cells	Well differentiated (n=12)	12.5 ± 16.03	1.41	21	5.95	From - 20.78 to 3.96	0.172
	Moderately differentiated	4.09 ± 12.0					

	(n=11)						
H- index of stained neoplastic cells for squamous cell carcinoma according to grade of tumor cells	Well differentiated (n=12)	1.92 ± 0.29	3.26	21	0.17	From 0.2 to 0.91	0.004*
	Moderately differentiated (n=11)	1.36 ± 0.5					
H- index of stained neoplastic cells and normal squamous tissue cells	Well differentiated (n=12)	1.92 ± 0.29	3.26	21	0.17	From 0.2 to 0.91	0.004*
	Moderately differentiated (n=11)	1.36 ± 0.5					

\* Significant

## DISCUSSION

Tumors of the head and neck are mostly of squamous cell carcinoma type, an aggressive and difficult to treat type of tumor, with surgical and radiological therapy usually applied; affect about 4% of people in the USA; it affects men more than women, with age range 25-70 years. The genetic aberrations including abnormalities in many genes<sup>2</sup>. Researches focused on identifying new biomarkers to develop more effective targeted therapies<sup>12-17</sup>.

The higher PDL1 expression is considered a bad prognostic factor in many types of tumors. The immunohistochemical method for evaluation was first applied for non-small cell carcinoma of the lung and for melanoma; where it was helpful in making the decision to apply immunotherapy in positive tumors<sup>12,16-20</sup>.

In Iraq, there was an increase in registered cases, for only oral squamous cell carcinoma; 1787 new cases were recorded from 2000 -2008, with male predominance, and affecting mostly the tongue, lip, oropharynx, nasopharynx, the floor of the mouth, other areas, and larynx<sup>14,18</sup>.

Regarding the clinical data, most of the cases were squamous cell carcinoma, with male predominance, and mean age more than 50 years, which is in agreement with other studies<sup>5,15</sup>. Regarding the location of the tumor; most cases were from the larynx (10/30) agreeing with the higher incidence registered by ICR, but different from OS Museedi<sup>(14)</sup> as tongue was the most common site, which may be due to type of cases collected. In this study tumors from head and neck area collected randomly, while in the other study oral tumors were included only.

The variable immunohistochemical expression for PDL1 was noted as cytoplasmic and membranous for both tumor and normal squamous cells in agreement with to Jacob H. Rasmussen et al<sup>21</sup> and Tim mular et al<sup>5</sup>. The positive PDL1 expression (i.e. score 2+ and 3+) was noted in 25% of tumor cells, and negative expression (score zero, and 1+) was noted in 75% of tumor cells similar to Tim mular et al<sup>5</sup>.

There was a significant statistical difference between tumor grade and the mean percentage of stained tumor cells, also between tumor grade and H- index (p-value= 0.022 and 0.004, respectively). This disagrees with Tim Mular et al<sup>5</sup>. No significant relationship found between PDL1 expression and clinicopathological parameters; which may be due to the small sample size in our study and exclusion of other tumors (only SQ cell CA include in statistics), also, most of our cases were moderately differentiated.

In the current study, statistical analysis was not performed with some clinicopathological parameters (e.g. staging) because of deficiency of related clinical data in patients' reports, as the current study was retrospective. Also, there was a significant association between the H-index of neoplastic cells in comparison with the normal squamous tissue ( P-value 0.0019), this result is in an agreement with the Vassilakopoulou study<sup>(22)</sup> where pdl1 mRNA levels were up-regulated in tumor cells in comparison with normal squamous cells.

## CONCLUSION

Immunohistochemical method for detection of PDL1 in head and neck squamous cell carcinoma shows variable results, with a significant relation between stained tumor cells and tumor grade, which can be used as a predictor to apply immune therapy.

**Ethical statement:** The current study was approved by Research Ethical Committee, College of Medicine, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq.

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**Conflict of Interest:** No conflict of interest

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