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Oral squamous cell carcinoma Immunohistochemical Expression of PD-L1 in Oral Epithelial Dysplasia

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ABSTRACT
Background:Epithelial dysplasia is the preferred term for the histopathological changes in the oral epithelium that indicate a risk of malignant transformation.Oral epithelial dysplasia (OED) is a serious condition as the affected mucosa has the potential to transform into OSCC.Propagation of lesions with dysplasiais supported by a gradual increase in mutations and chromosomal derangements in dysplastic lesions.PD-L1 is an anti-apoptotic marker through the axis PD1/ PDL1. In the tumor microenvironment, expression of PD-L1 on tumor cells leads to its engagement with PD-1 of T cells creating T cell dysfunction, exhaustion, and neutralization.
Objective: The main purpose of this study is to study the varied expression of PDL-1 in squamous epithelial malignancy in oral epithelial dysplasia and to investigate the different clinic-pathological data of OED.
Material and methods:Routine hematoxylin and eosin staining on 10 archival blocks of OED to verify the diagnosis.Immunohistochemical staining was also used to analyze the expression of PD-L1 in these cases.
Results: The immunopositivity of PD-L1 was in 70% of the OED and negative observation in only three cases (30%) of this group. Weak positive reaction of PD-L1 in the 70% of with no statistically significant in this group.
Conclusion: The biological behavior and the prognostic significance of OEDcannot be predicted from the expression of PD-L1.Therefore, further investigations should be made.

I. INTRODUCTION

The term epithelial dysplasia is applied to a lesion in which part or full thickness of the epithelium is replaced by cells showing varying degrees of cellular atypia(Tilakaratne et al. 2019).**Epithelial dysplasia describes histological** changes only and has no clinical morphological equivalent(Odell et al. 2021).Recognition of dysplasia initially depended on identifying individual features of cytological atypia. Increasingly, architectural changes have been accepted as features of dysplasia, reflecting that malignant transformation may occur that shows minimal cytological abnormality. Nowadays,the most widely used grading system is the World Health Organization (WHO) classification system that subdivided OED into three categories: mild, moderate, and severe dysplasia(Khoury, Sultan, and Sultan 2022) .

The successful survival and proliferation of dysplastic cells involve the escape of cancer cells from immune surveillance(Alemohammad et al. 2022).Immune tolerance is mediated by inhibitory signaling pathways called immune checkpoints. One of the most important immunologic checkpoints is the axis PD1/ PDL1 representing the binding of PD-1 (programmed death 1) expressed by T cells to its ligand PD-L1 highly expressed by dysplastic cells (Zandberg and Strome 2014).In the tumor microenvironment, PD-1 and its ligand PD-L1 perform a vital role in tumor progression and survival by escaping tumor-neutralizing immune surveillance. The engagement of PD-L1 with PD-1 of T cells creates T cell dysfunction, exhaustion, neutralization, and interleukin-10 (IL10) production in a tumor mass (Dong et al. 2018).

Objectives

The present work aimed at studying the varied expression of PDL-1 in squamous epithelial malignancy inoral epithelial dysplasia and to investigate the different clinic-pathological data of OED.

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II. MATERIAL AND METHODS:

Tissues:

The present work is 10formalin-fixed paraffin sections of grades of OED collected from the archival files of the Oral Pathology Department, Faculty of Dentistry and Oncology Center Unit, Faculty of Medicine, Mansoura University.

Immunohistochemical marker:

Programmed cell death ligand - 1 (PD-L1),Provided as a vial containing 7 ml of ready-to-use, Rabbit Monoclonal Antibody. Germany cert. by TÜV Rheinland, Group EN ISO 13485:2016 & ISO 9001:201

Methods:

-Demographic as well as clinical data of all studied cases were collected from the recorded patients' reports regarding age, gender, site, and pain.
-Routine hematoxylin and eosin stainingto regard the grade of dysplasia.
-Immunohistochemical staining with the PD-L1 antibodywas also used to analyze the expression of iton these cases.

Evaluation and scoring of immunohistochemical reaction:

Through microscopic examination, a brown positive cytoplasmic reaction was seen in normal tonsil, which was used as a positive control of PD-L1. A positive reaction was detected among current study subjects in the cell membrane and/ or cytoplasmic staining.Immunohistochemically stained sections were interpreted independently by two of the authors by scanning the slides under an Olympus light microscope (CX31) at 200x magnification. For each IH section, three fields rich in lesional cells were selected to perform a semiquantitative evaluation for both variable staining intensity as well as proportion of immunoreactive cells.

Then the sum of the percentage of stained cells and intensity scores were calculated and categorized as the final score for each case as follows:
Absence of staining: 0.
Weak staining: 1-3.
Strong staining: 4-7.

Statistical analysis and data interpretation:

Qualitative data were described using numbers and percentages. Quantitative data were described using mean± Standard deviation for normally distributed data after testing normality using Shapiro Wilk test. The significance of the obtained results was judged at the ≤0.05 level (p-value).
Monte Carlo tests were used to compare qualitative data in the group as appropriate.

III. RESULTS:

1-Clinical results and Histopathological results:

The mean age among this group is 46.40 while a male predilection was more encountered among the OED group.The cheek and tongue were the most affected sites in the current studied cases. About pain, almost of the cases were reported as painless lesions.

According to the grading system of dysplasia (Muller and Tilakaratne 2022), three grades were encounteredin the OED group; mild (50%), moderate (30%), and severe (20%) (Table 1.figure 1,2,3).

Table (1): Clinical and histopathological data of the studied groups:

	Dysplasia n=10 (%)
Age/years mean±SD	46.40±15.03
Sex	
Male	6(60)
Female	4(40)
Site	
Cheek	3(30)
Mandible	1(10)
Palate	2(20)
Tongue	4(40)
Pain	
absent	6(60)
present	4(40)
Grade of Dysplasia	

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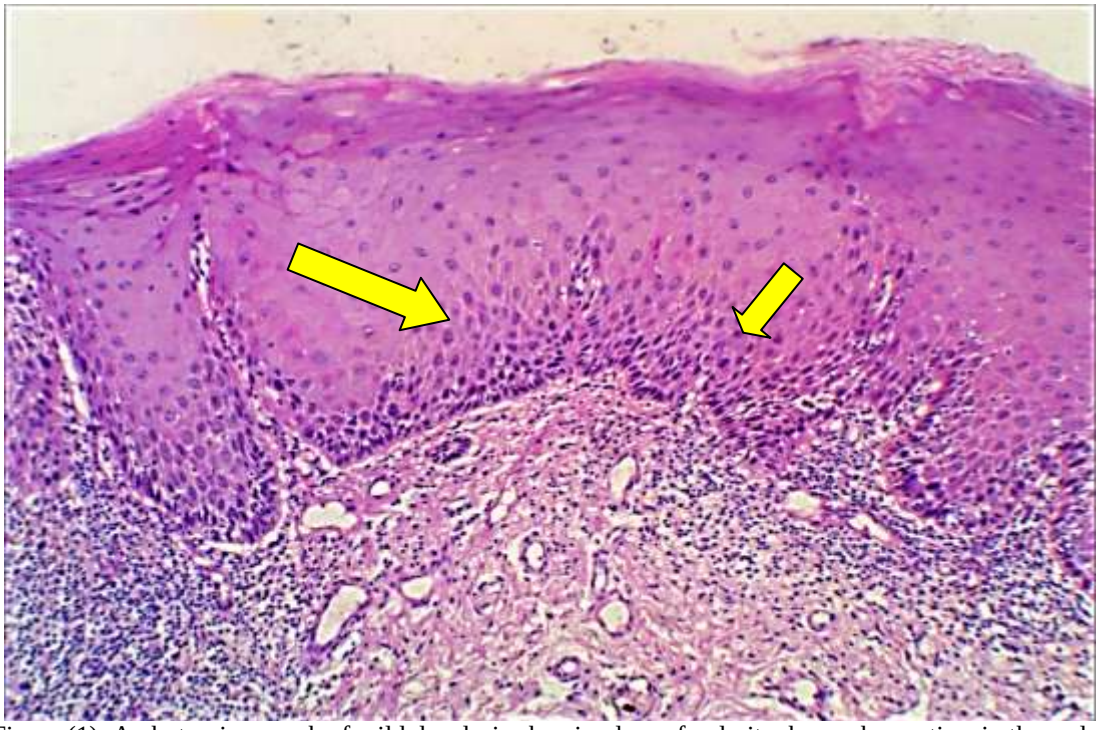
Mild	5(50)
Moderate	3(30)
Severe	2(20)



. Figure (1): A photomicrograph of mild dysplasia showing loss of polarity, hyperchromatism in thenuclei of prickle cells of the suprabasilar epithelium (yellow arrow). H&E stain100x.

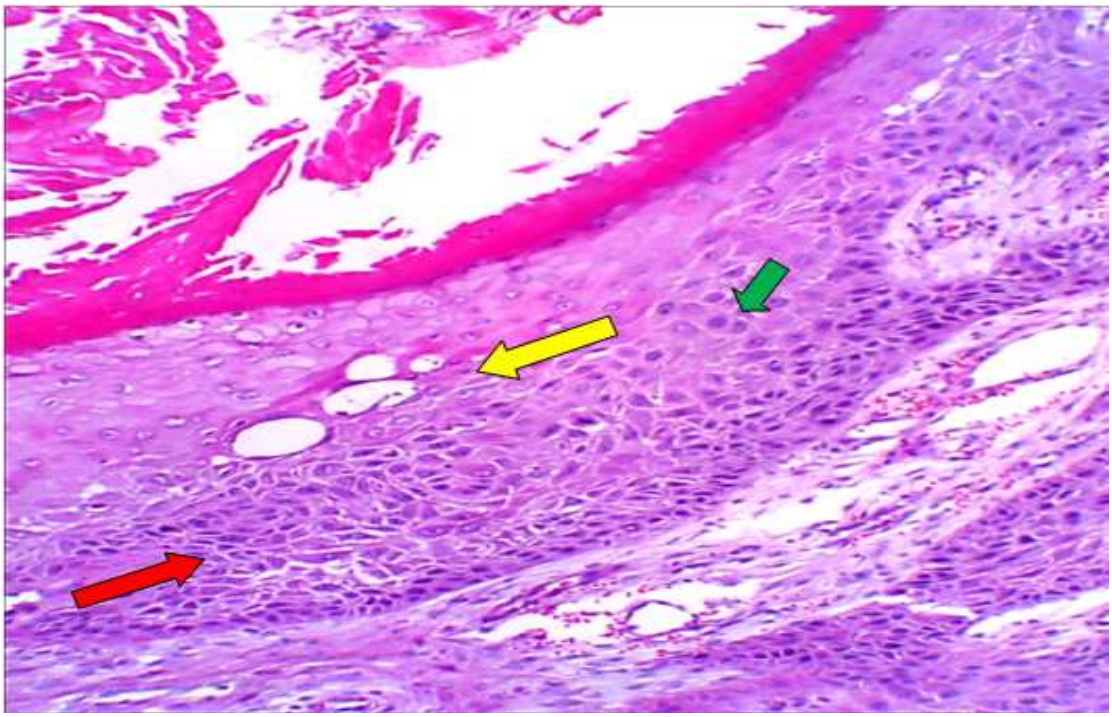


Figure (2): A photomicrograph of moderate dysplasia showing abnormal variation in cell and nuclear size and shape (red arrow) and loss of epithelial cell cohesion (yellow arrow) and increased nuclear-cytoplasmic ratio (green arrow) H&E 200x.

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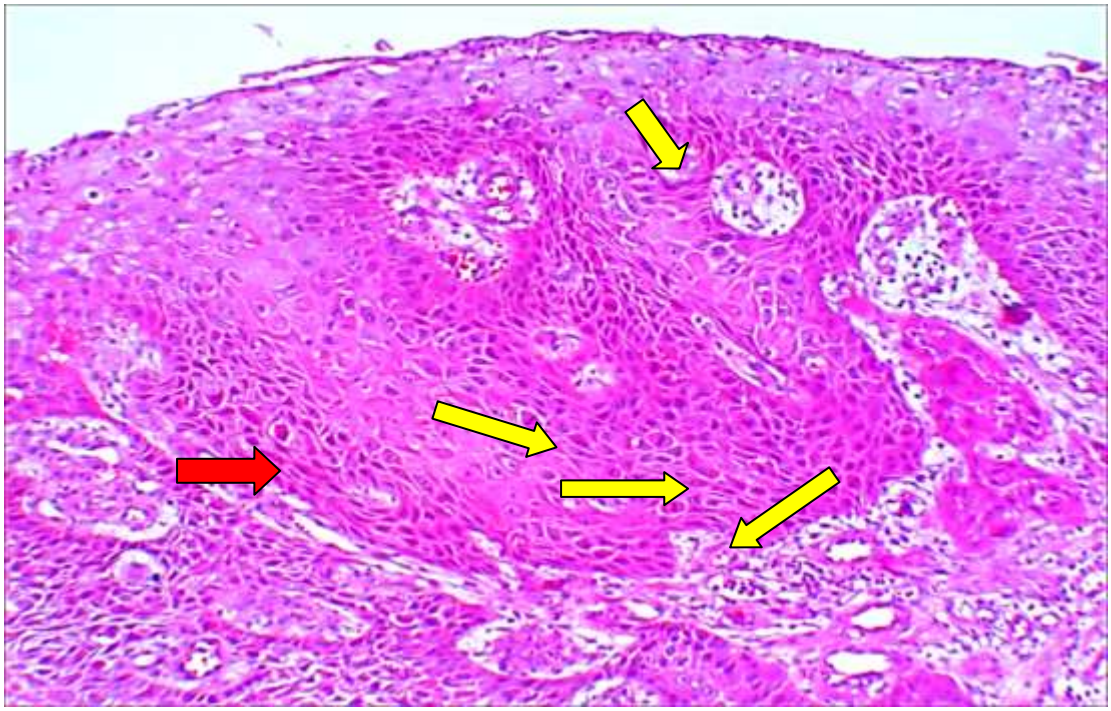

Figure (3): A photomicrograph of severe dysplasia showing pleomorphism (red arrow), increased number of mitotic figures (yellow arrow). H&E 200x.

2- Immunohistochemical Results

PD-L1 expression in grades of epithelial dysplasia:

Positive immunoreactivity was encountered in seven out of the 10 cases forming the dysplasia group. The reaction score was generally weak, distributed as two in the mild (50%)two in the moderate (66.7%), and three in the severe (100%) grades. The difference between the grades was insignificant.PD-L1 expression was represented as a cytoplasmic reaction located in the basal and suprabasal layer in the mild dysplasia grade, figures 4&5. In moderate dysplasia cases, PD-L1 expression was detected in the basal layer until half the thickness of the epithelium, figure 6. In severe dysplasia, PD-L1 expression was shown in the whole epithelium passing the midpoint of the thickness, Figure 7.

Table 2: Comparison of IH expression score among different grades of dysplasia

IH expression code	**Grade of Dysplasia**			**Test of significance**
	Mild N=4(%)	Moderate N=3(%)	Severe N=3(%)	
absent	2(50)	1(33.3)	0	p=0.356
weak	2(50)	2(66.7)	3(100)	

Used test: Monte Carlo test, significant where P≤ 0.05

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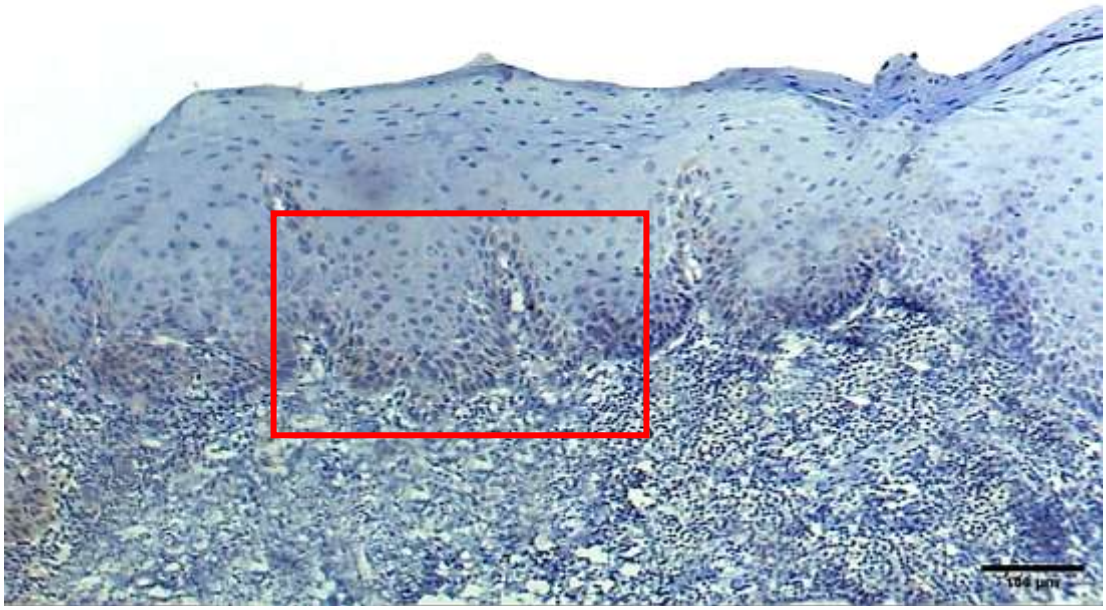

Figure 4:Photomicrograph of mild dysplasia showing weak reaction for PDL-1 in the basal and suprabasal cells (DABx100).

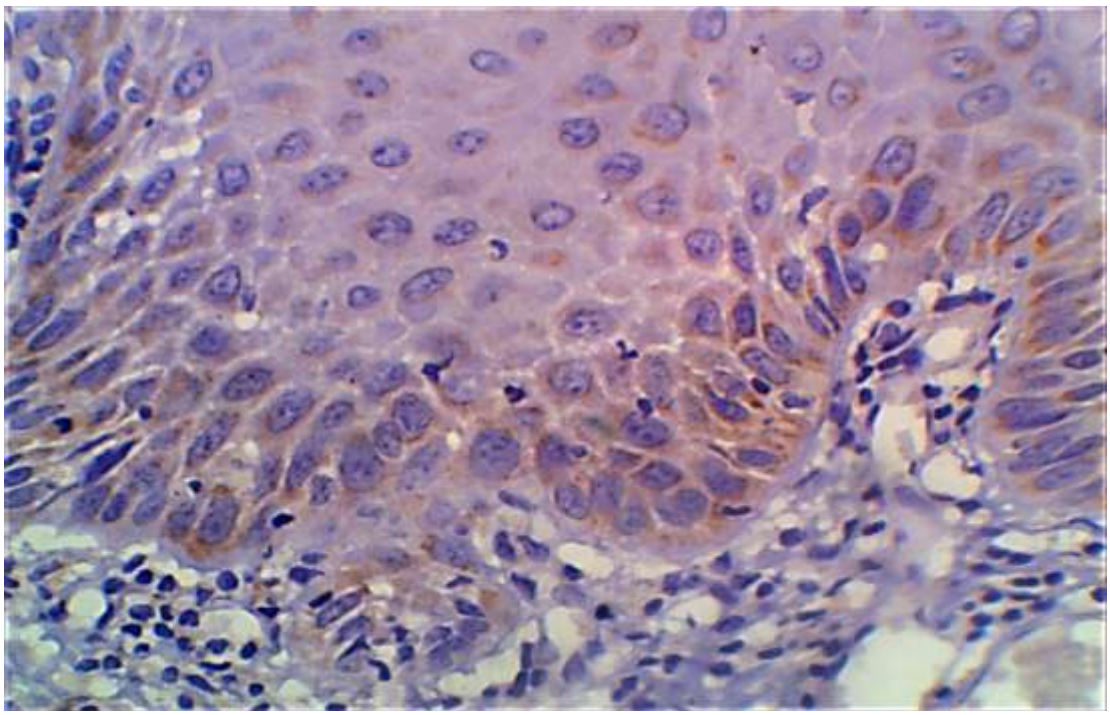

Figure 5: Higher magnification of basal cells of the previous case showing weak PDL-1 expression in the basal and suprabasal cells (DABx400).

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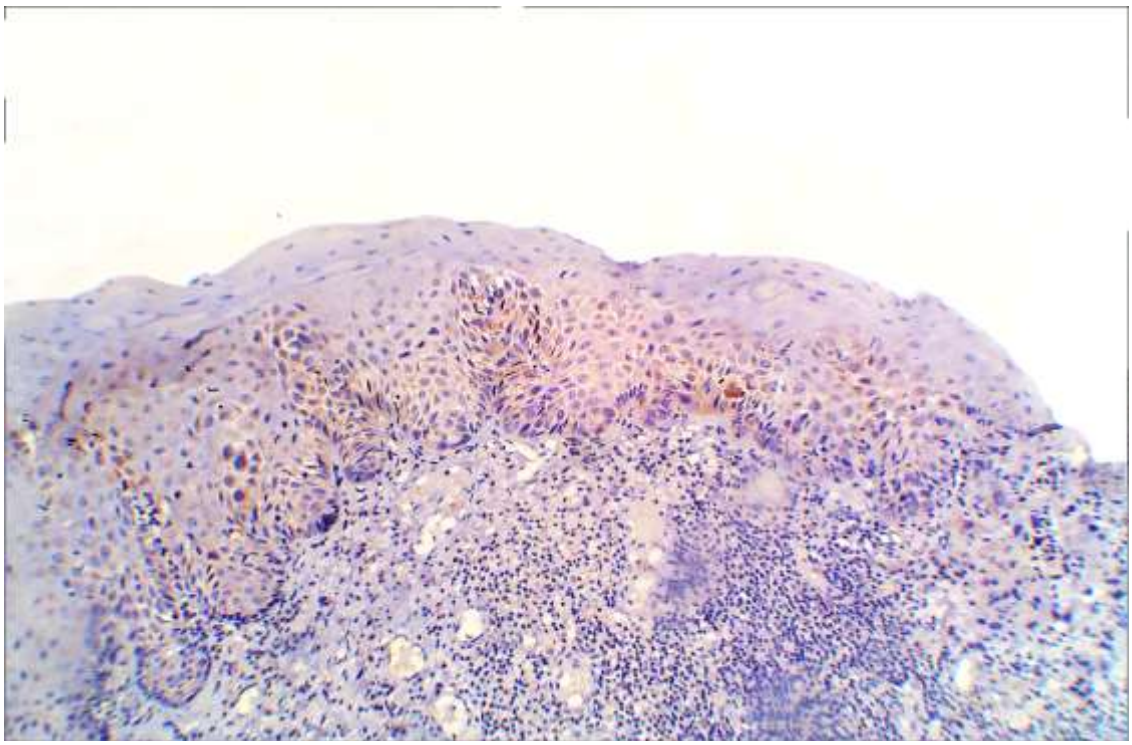

Figure 6: Photomicrograph of moderate dysplasia showing weak reaction in the epithelial cells (DABx100).

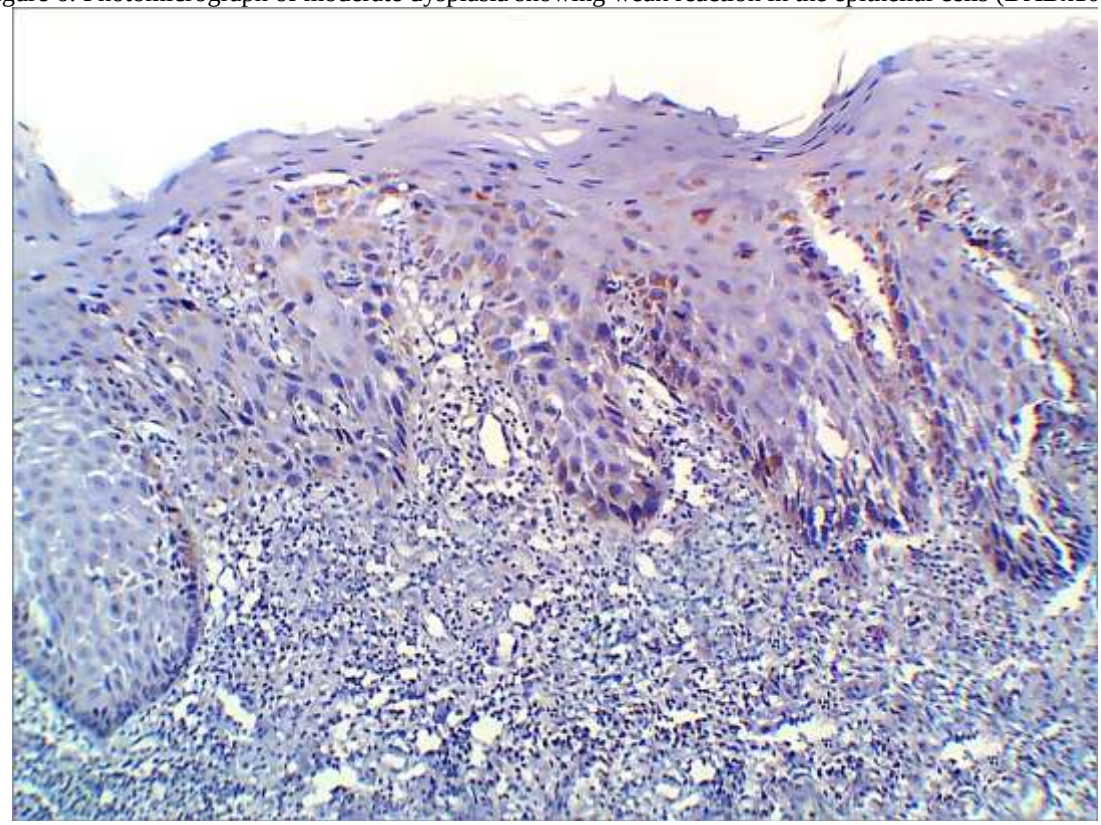

Figure 7: Photomicrograph of severe dysplasia showing weak reaction in the epithelial cells (DABx200).

IV. DISCUSSION:

Regarding age, OED showed a wide age ranging between 16 to 63 years, with a mean of 46.40, which was in harmony with previous studies (Singh et al. 2020). About gender,OED showed a higher incidence in males than females, and this

was in harmony with a previous study by (Singh et al. 2020), butwas contradictory to another investigator that showed OED was more common among women(Ellonen et al. 2023).This might be referred to the increased intensity of bad habits among them.

As regards the site, OEDwas located mainly in the tongue followed by the buccal mucosa and then the palate, this was in agreement with (Ellonen et al. 2023), while, the buccal mucosa was the predominant site in another study made by (Singh et al. 2020).

Regarding pain, almost half of the OED cases were painless,this was in agreement with(Viet et al. 2011).

According to the histological grade of OED, mild dysplasia was more frequently observed than moderate and severe dysplasia, which is in agreement with (Kierce et al. 2021).

Throughout the examination of cases of OED, PD-L1 positive immunoreactivity was in 70% of the OED and negative observation in only three cases (30%) of this group.

In most dysplasia cases, low immunoreactivity of PD-L1 was detected, which appeared in basal and parabasal, and spinous layers of the dysplastic epithelium whereas the other cases showed negative reactions. These findings were in concordance with a study performed by (Dave, Ali, and Magalhaes 2020) and (Pakkanen et al. 2022) who reported that PD-L1 is expressed in dysplastic lesions. Contradictory findings were reported by (Saeed et al. 2022) those who found that the majority of cases of dysplastic lesions were negative reactions. This might be referred to the small sample employed in the study.

V. CONCLUSION:

The current work was conducted to highlight the relation of PD-L1 protein with dysplastic epithelium. The study demonstrated that PD-L1 was as low reaction in all grades of dysplasia. So, PD-L1 staining is not efficient and not easy to perform for routine purposes and dose not help in determining the prognosis OED.

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