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The expression of Cox-2 and Cyclin D1 with clinicopathological characteristics in oral epithelial dysplasia: A retrospective study

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Abstract

Background: Oral mucosal cancers are often preceded by potentially malignant diseases that may exhibit diverse morphological and histological alterations. The expression of COX 2 has been related to tumor vascularization, dissemination, and survival in oral cancer studies. High levels of cyclin D1 expression to precancerous lesions and oral malignancies. This study was designed to evaluate and compare the immunohistochemical expression of the above-mentioned markers.

Materials and Methods: 50 retrospective formalin fixed-paraffin embedded histopathologically confirmed blocks of oral epithelial dysplasia. Immunohistochemical staining was performed using monoclonal antibodies against COX-2 and Cyclin D1. Using a standard camera, photomicrographs of the slides were taken.

Results: No significant difference in the age distribution of the patients studied, as well as age pivoting at the fifth and sixth decades with mean and standard deviation (53.36 2.63). There was no significant difference in gender among the studied patients. Furthermore, there was a highly significant difference in grade distribution.

Conclusions: The COX-2 and Cyclin D1 markers may assist in diagnosing oral epithelial dysplasia based on severity. It's easy to gather image-analytic data on the stained tissue specimens.

Keywords: Epithelial Dysplasia; Malignancy; Cox-2; Cyclin D1; Immunohistochemistry

1. Introduction

Oral epithelial dysplasia (OED) can be defined as a histopathological term that shows a spectrum of different cellular and architectural changes that are limited to the oral epithelium [1]. It is a potentially cancerous lesion of the oral mucosa that may or may not turn into squamous cell carcinomas [2]. Although oral squamous cell carcinomas may arise from dysplastic lesions, they can develop from the lesions without any dysplastic changes [3]. The presence of epithelial dysplasia of oral mucosal lesions is generally considered a good predictor of the rate of progression to malignancy [4].

Oral Potential Malignant Disorders (OPMDs) are clinical terms used to describe different disorders and diseases such as leukoplakia (LP), erythroplakia (EP), erythroleukoplakia (ELP), oral submucous fibrosis (OSMF), nicotinic stomatitis, actinic cheilitis, oral lichen planus (OLP), and others. Histologically, these disorders and diseases may harbor oral epithelial dysplasia**. It** is represented a risk factor for malignant transformation of OPMDs [5]**.** It can be seen in many types of OPMDs with different grades, so the lesion with a high risk of malignant transformation usually has a high grade of epithelial dysplasia in its diagnosis [6]**.** The presence of any dysplastic changes in the oral epithelial tissue of a potentially disordered mouth can be detected early to prevent the development of oral squamous cell carcinoma [7]**.**

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Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme that controls the production of prostaglandins and thromboxanes. This controls inflammatory processes, growth, vasculature, tumor progression, and conversion [8]. Cyclooxygenases (COX-1 and COX-2) synthesize prostaglandins from arachidonic acid. The COX-2 enzyme is the most significant in cancer development [9].

Cyclin D1 is a protein of the cyclin family which is related to cell cycle progression by controlling the phosphorylation and inactivation of the retinoblastoma protein (RB)—a tumor suppressor protein—which allows cells to move from G1 to S phase [10]. Many researchers have noticed a gradual rise in cyclinD1 expression from normal oral tissues to dysplastic lesions and OSCCs [11]. Cyclins are considered as diagnostic and prognostic biomarkers in a number of cancers [12].

The aims of the present study are to evaluate and correlate the immunohistochemical (IHC) expression of COX-2 and CyclinD1in oral epithelial dysplasia samples.

2. Materials and Methods

2.1. Study design

Retrospective study includes cases of oral epithelial dysplasia were retrieved from the archives. Formalin-fixed paraffin-embedded histopathologically diagnosed tissue specimens of the cases were collected along with their relevant patient's clinical data. Hematoxylin and eosin (H&E) stained tissue sections of all the cases were evaluated by two experienced pathologists.

2.1.1. Ethical approval

The Ethics Board, College of Dentistry, University of Baghdad, granted ethical permission for this study. (Ref. number 294, date: 01/04/2021).

2.2. Study samples

The samples were obtained from the Oral Pathology Laboratory at the University of Baghdad's College of Dentistry. Age, gender, and the location of the biopsy from the mouth were extracted from the appropriate records for each case.

2.2.1. Samples processing

From each block, three slices with a thickness of 4 mm were cut out and put onto microscopic charge slides. Hematoxylin and eosin (H&E) staining was used to check the tissue quality and review the histopathological image of each case's OED. IHC analysis was performed on the final two slides.

2.3. Immunohistochemistry

The IHC process involves dealing with the slides through several sequential steps, which include deparaffinization, rehydration, antigen retrieval, blocking the endogenous peroxidase, and then the step of adding primary and secondary antibodies. The primary antibodies used in this study were anti-COX-2 and anti-CyclinD1- (1: 50 dilution),all purchased from PathnSitu Biotechnologies, Secunderabad, India. In the current study, negative and positive controls were used. The positive control for anti-COX-2 was colon cancer and human tonsil for Cyclin D1was taken

2.3.1. Scoring system

The cytoplasmic and membranous positivity of the COX-2 was evaluated by immunohistochemical score (HIS). This number was found by multiplying the estimated number of positive cells by the staining intensity score. ,"No staining" was scored as 0, 1-10% of positive cells received a score of 1, 11-50% of positive cells are classified as 2 , 51-80% of positive cells are 3, 81-100% of positive cells. The staining intensity was graded from 0 to 3, with 0 being negative, 1 being mild, 2 being moderate, and 3 being strong. The score could range from 0 to 12. A HIS score of 9–12 indicated high immunoreactivity, 5-8 indicated moderate immunoreactivity, 1-4 indicated mild immunoreactivity, and 0 indicated no immunoreactivity. If the HIS score was moderate to strong, COX-2 was considered overexpressed [13]. The semi-quantification of the nuclear expression of cyclin D1 was evaluated as follows: 0 (No positively stained cells), 1+ Mild (less than 5% positive cells), 2+ Moderate (5% to 30% positive cells), and 3+ Severe (more than 30% positive cells) [14].

2.4. Statistical Analysis

A statistician's opinion was requested. For statistical analysis, the Statistical Package for Social Sciences (SPSS) version (20) was used. Statistical significance was defined as a P value of less than 0.05.

3. Results

The two socio-demographical characteristic variables regarding the studied patients are randomly distributed among their different classes or not (Table 1). Age group's distribution of the studied patients has no significant difference at P>0.05, and accordance with this result, it could be indicating that the probability of recorded studied disorder does not differ according to the distribution of age groups, as well as age pivoted at the fifth and sixth decades with mean value and standard deviation (53.36 ± 12.63). With respect of gender, studied patients has no significant difference at P>0.05, and accordance with this result, it could be conclude that probability of recorded studied disorder does not differ according to the gender's patients (Table 1).

Table 1 Distribution Socio-Demographic Significant comparisons of the studied subjects' characteristics variables

SDC v.	**Classes**	**No.**	**%**	**(*) C.S. P-value**
Age Groups Yrs.	< 40	7	14	χ^2= 5.600 P=0.231 NS
	40 _ 49	12	24	
	50 _ 59	13	26	
	60 _ 69	13	26	
	≥ 70	5	10	
	Mean ± SD	53.36 ± 12.63		
Gender	Male	24	48	P=0.888 NS
	Female	26	52	
(*) C.S. (Between Age & Gender)		CC = 0.334 P = 0.178 NS		
(*) NS: Non Sig. at P>0.05; Testing based on One-Sample Chi-Square test, Binomial test, and Contingency coefficient test.				

Table 2 Distribution of Site and Grade variables for studied patients with comparison's significant

Site and Grade	**Classes**	**No.**	**%**	**C.S. (*)**
Site	Tongue	12	24	χ^2= 8.720 P=0.033 S
	Buccal Mucosa	21	42	
	Lips	10	20	
	Others	7	14	
Grade	Mild	27	54	χ^2= 13.240 P=0.001 HS
	Moderate	17	34	
	Sever	6	12	
(*) C.S. (Site & Grade)		C.C. = 0.318 P = 0.469 NS		

The distribution of the "Site and Grade" variables concerning of the diagnosed patients with oral epithelial dysplasia. Sits group's distribution of the patients were significant different at P<0.05, it could be indicating that the probability of recorded patients have meaningful differences that was demonstrated by the high number of patients with "Buccal Mucosa" site, since they are accounted 21(42%), then followed with "Tongue" site, accounted 12(24%), then followed

with "Lips" site, accounted 12(24%), and finally followed with others sites, accounted 7(14%). With respect of grade's diagnoses, patients have highly significant difference at P<0.01, it could be conclude that probability of recorded patients have meaningful differences that were demonstrated by the high number of patients with "Mild" grade, since they are accounted 27(54%), then followed with "Moderate" grade, accounted 17(34%), and finally followed with "Sever" grade, accounted 6(12%), (Table 2).

Brown cytoplasmic and/or membrane positivity of the COX-2 presented in different grades of oral epithelial dysplasia cases. The vast majority mean of score was accounted in the sever grade (2.17), while followed descending ordered by the mild grade (1.37), and finally moderate grade has accounted the low mean of score (1.29) concerning studied marker. With reference to positive nuclear expression of the "Cyclin D1" marker, results showed that high mean of score was accounted in the moderate grade (1.76), while followed descending ordered by the mild grade (1.67), and finally sever grade (1.17) has accounted the low mean of score concerning studied marker (Fig. 1), (Fig.2).

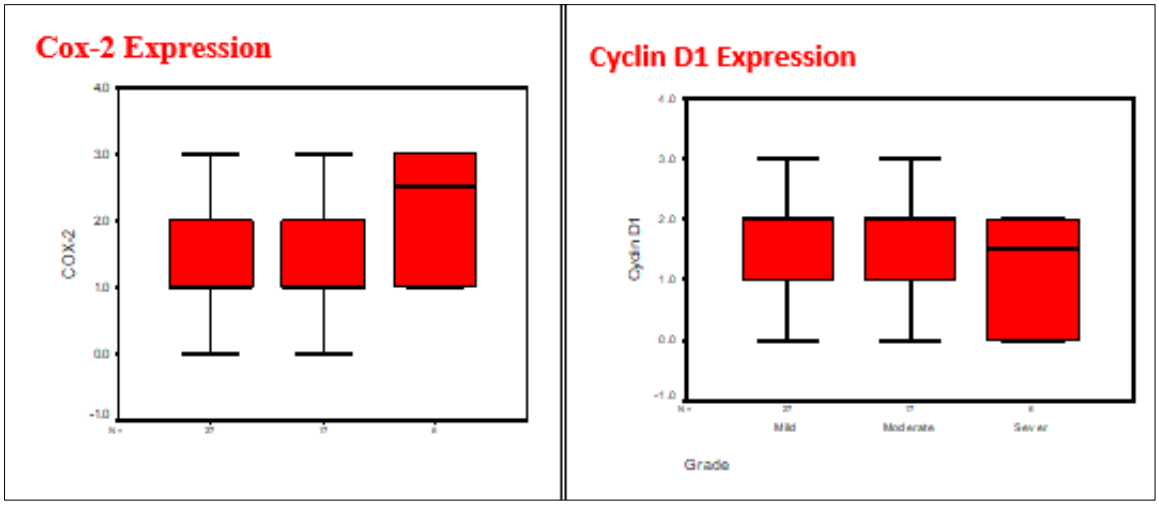
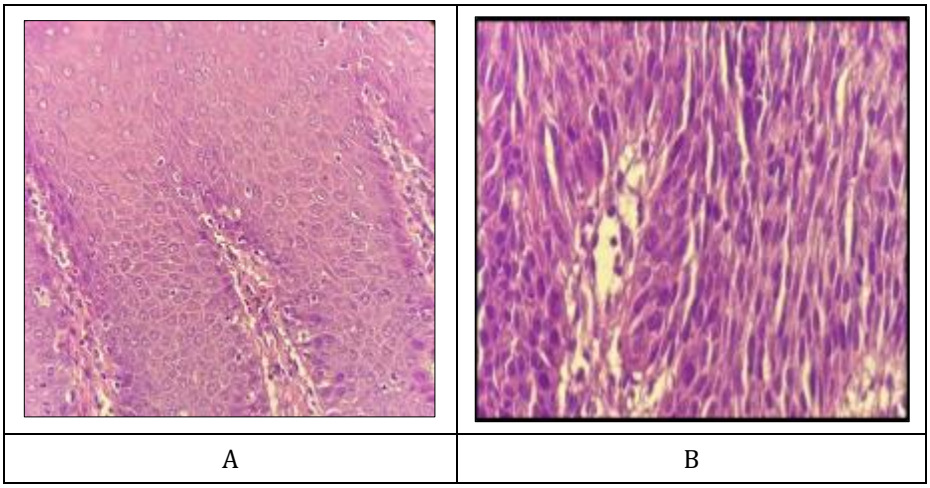


Figure 1 Stem-Leaf Plots for the distribution of the studied markers according to grade's scoring scales



A	B

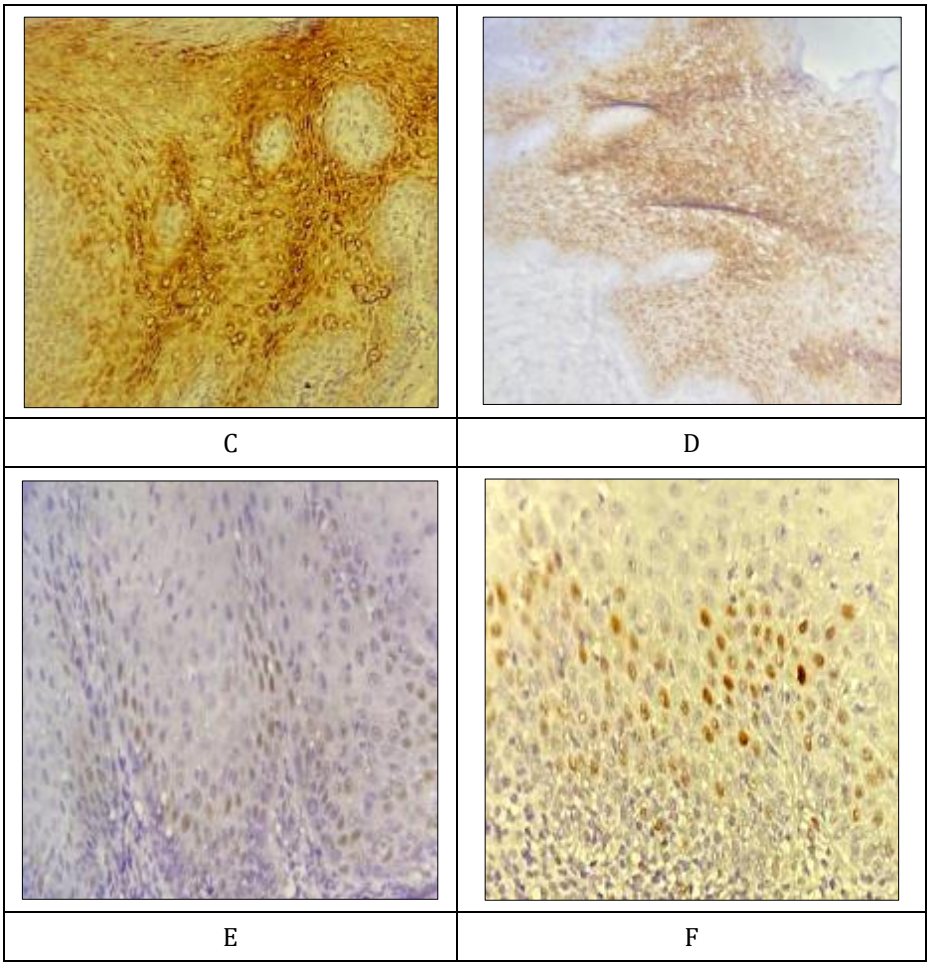


Figure 2 High power photomicrograph of oral epithelial dysplasia A-Mild grade, B-Sever grade (magnification 40X, H&E stain); Positive cytoplasmic and/or membrane expression of Cox-2; C-Mild grade, D-Sever grade (magnification 40X); Positive nuclear expression of Cyclin D1 E-Mild grade, F-Severe grade (magnification 20X)

4. Discussion

Although oral epithelial dysplasia (OED) is considered to be an intermediate step for transformation of varied precancerous lesions in oral cancer development, interestingly OSCC can develop from non-dysplastic lesions too [15].

In our study most of the oral dysplastic cases were distributed in the fifth and sixth decades of life which come in accordance with the results of other study [16]. This similarity might be due to similar sample size with similar gender distribution in their study. The association of oral epithelial dysplasia development with aging could be explained by the prolong accumulation of genetic changes which are caused by genetic and/or environmental factors such as tobacco and alcohol drinking. Concerning gender distribution, the results showed that females affected with oral epithelial dysplasia (52%) more than males (48%). Similar finding reported in other parts of the world [17] that revealed there is no gender predilection was found, but, in contrast to our study [18] showed that (80%) of oral dysplastic samples belonged to males with M:F 4:1**.** There is controversy as to which gender is most affected. This could be explained by specific habits in each region, such as betel nut, tobacco chewing or other factors. According to site, the current study revealed that the "Buccal mucosa" was the commonest site affected with oral epithelial dysplasia. This finding was in agreement with previous study, [19] that showed higher prevalence in buccal mucosa site as a result of the widespread habit of betel/ areca nut chewing in some regions and increased malignant transformation in oral leukoplakia lesions of the buccal mucosa over other sites.

There are increased expression of COX-2 in the sever grade of oral epithelial dysplasia cases which come in accordance with the results of other study [20] which revealed that increased expression of COX-2 in high grade of dysplastic epithelia reflects the role of this enzyme in the early stages of oral carcinogenesis. Some studies showed that the expression of COX-2 progressively increased from low grade to sever grade of epithelial dysplasia and the highest levels of COX-2 expression were found in severe grade of dysplasia and also showed that COX-2 may be involved in regulation

of cell proliferation [21]**.** The increased expression of COX-2 during oral carcinogenesis may depend on the developmental stage of the tumor, as well as etiologic factors such as the types of mutations and distinct types of injuries affecting different regions [22]. Few researchers reported prevalence rates of the cyclin D1 overexpression range from low-grade oral epithelial dysplasia to high-grade in all degrees of oral epithelial dysplasia [23] Similar results were observed in other studies with significant increase in the expression from mild to moderate to severe dysplasia [24,25]. The results of present study showed that high expression of Cyclin D1 in moderate grade of oral epithelial dysplasia. This findings accordance with many researchers [26]. Which stated that the overexpression of Cyclin D1 was in grades of the epithelial dysplasia cases especially low grade when compared to high grade.

The present study followed a retrospective design depending on archived samples from patients with OED, the absence of severity measurements of the tissue grading and followup are the main limitations of the study. Large study samples are recommended for future studies.

5. Conclusion

The COX-2 and Cyclin D1 markers may be useful diagnostic markers for diagnosing the examined oral epithelial dysplasia. The ordinary photography can be used to analyze the stained sections.

Compliance with ethical standards

Disclosure of conflict of interest

I declare that there is no possible conflict of interest in the publication of this work. Furthermore, I have personally observed ethical difficulties like plagiarism, consent forms, misconduct, data fabrication and/or deception, multiple publishing and/or submission, and duplication.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Author contributions

I not only collected, analyzed, and interpreted the data, but also wrote, analyzed, and put together the paper.

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