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ORIGINAL RESEARCH

Comparison of COX2 Expression between Oral Squamous Cell Carcinoma, Leukoplakia and Normal Mucosa

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ABSTRACT

Aim: To compare cyclooxygenase 2 expression (COX2-E) between normal, oral leukoplakia lesions and different grades of oral squamous cell carcinoma (SCC).

Materials and methods: Around 90 paraffin embedded blocks consisting of 45 SCC, 15 leukoplakia and 17 controls were selected for immunohistochemistry (IHC) for detection of COX2-E. COX2-E was divided in four grades, as A (0-10%), B (11-40%), C (41-70%) and D (> 70%) cellularity.

Results: Mean age of the patients was 55.17 ± 18.41 (M:57.92 ± 16.87, F:52.19 ± 19.74). A significant difference was found in COX2 expression between SCC total and, basal and spinous layers of leukoplakia ($p < 0.05$). COX2-E in spinous layer of normal tissue was significantly lower than SCC ($p = 0.000$). COX2-E was significantly different in SCC grade 3 and leukoplakia ($p = 0.001$) and normal tissue ($p = 0.000$). COX2-E was significantly higher in SCC grade 3 compared to leukoplakia (basal layer) ($p = 0.000$).

Conclusion: We showed a significant higher COX2-E in SCC lesions compared to leukoplakias and normal controls. In our study COX2-E was not significantly different in SCC grades 1, 2 and 3 ($p > 0.05$).

Keywords: Oral cancer, Cyclooxygenase 2, Leukoplakia, Head and neck cancer, Expression.

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INTRODUCTION

Despite advances in diagnostic techniques, oral squamous cell carcinoma (SCC) has a high incidence in many parts of the world including Asia. Recent studies show an increase in early onset oral SCC among young patients in Iran.[1-4] Iran is a large country located in the Middle East and Khorasan (including North, Razavi and South Khorasan) is the largest province in Iran covering 7.8% of the total area of this country with a population about 7 millions. Oral SCC is among the 10 most common cancers in our province.[5]

It seems that before appearance of clinical lesions of SCC of oral cavity and esophagus, some molecular changes occur in malignant tissues, such as increase in expression of cyclooxygenase 2 (COX2) enzyme.[6,7] COX2 can increase prostaglandin synthesis in many malignant cells including esophagus, colon, lung and prostate.[7-9] This increase can drive tumor extention by angiogenesis and cell proliferation, modulating inflammatory and immune responses and inhibiting apoptosis.[7,9-12]

Lio et al showed that aspirin reduced the *in vitro* and *in vivo* proliferation of esophageal cancer cells and induced apoptosis , in a dose dependent manner.[6]

Goulart Filho et al showed that COX2 expression (COX2-E) was significantly higher in oral SCC than normal epithelium but there were no significant differences in COX2-E between high and low grade tumors.[10]

Although many studies have shown overexpression of COX2 in oral malignant lesions, only few researches have compared COX2-E between different grades of SCC and also between normal and premalignant oral lesions. This study was conducted to compare COX2-E in different grades of SCC and leukoplakia with normal epithelium.

MATERIALS AND METHODS

A total 90 blocks of paraffin embedded archival tissue were obtained from the Department of Oral and Maxillofacial Pathology of Mashhad Dental School. All hematoxylin-eosin stained sections were reviewed for the quality of the material, stage of SCC and dysplasia for leukoplakia lesions and the best section from each specimen was selected.

A total of 77 blocks were selected: 45 blocks concerning oral SCC specimens (15 grade 1, 15 grade 2 and 15 grade 3), 15 blocks of oral leukoplakia as a premalignant lesion (5 with dysplasia and 10 without dysplasia) and 17 paraffin embedded blocks of normal tissue (from patients undergoing oral surgery for various benign disorders).

Histologic grading[1-3] was performed based on WHO classification[13] and three grades were stablished. The study protocol was approved by the Committee on Ethics of Mashhad University Of Medical Sciences (MUMS). Immunohistochemical expression of COX2 (COX2-E) was blindly examined independently by two pathologists,who used light microscopes. The mean number COX2 positive cells was graded semiquantitatively and each sample was assigned to one of the following categories: Grade A (0-10%), grade B (11-40%), grade C (41-70%) and grade D (> 70%) cellularity.

Immunohistochemistry Staining

Four µm sections from each formalin-fixed, paraffin embedded blocks were cut. IHC was then performed on sections fixed on poly-L-lysine-coated glass slides. Deparaffinized and rehydrated slides were incubated for 30 minutes in 3% H_2O_2/methanol to stop endogenous peroxidase activity, and then irrigated with phosphate-buffered saline (PBS) for 20 minutes. Specimens were incubated with the primary monoclonal antibody COX2, human source, isotype IgG1 kappa, clone CX-294, code M3617, Dako, Denmark (UK). The sections were rinsed three times with PBS at room temperature. After washing with PBS, the immunoreactivity was visualized by diaminobenzidine and hydrogen peroxide. The procedure was followed by counterstaining with hematoxylin and then slides examined by light microscopy. Epithelial cells were counted with light microscope at hot spot in five areas at 100× and 400× magnification. The areas were marked by highlighter pens to prevent counting one area twice.

The results were analyzed using SPSS version 16.0 (SPSS, Chicago, III) COX2 expression (COX2-E) and the intensity of their staining on epithelial cells were compared by nonparametric Mann-Whitney and Kruskal-Wallis as well as Fisher's exact tests. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 77 blocks were evaluated in this study. Mean age of the patients was 55.17 ± 18.41 (M:57.92 ± 16.87 , F:52.19 ± 19.74). Different grades of SCC were enrolled (15 grade 1,15 grade 2, and 15 grade 3). Most of SCC samples were in grade D COX2- (75.6%) whereas none of leukoplakia or normal mucosa samples were in grade D.

In spinous layer of leukoplakia 53.3% of samples were in grade B and in basal layer of leukoplakia 53.3% of samples were in grade C. In basal layer of normal mucosa 52.9% of samples were in grade D and in spinous layer 47.1% of samples were in grade A (Table 1) (Figs 1A and B).

A significant difference was found in COX2-E between SCC total and basal layer of leukoplakia (p = 0.001) and spinous layer of leukoplakia (p = 0.000).

COX2-E in spinous layer of normal tissue was significantly lower than SCC total (p = 0.000). Although COX2-E in basal layer was not different with SCC (p = 0.03).

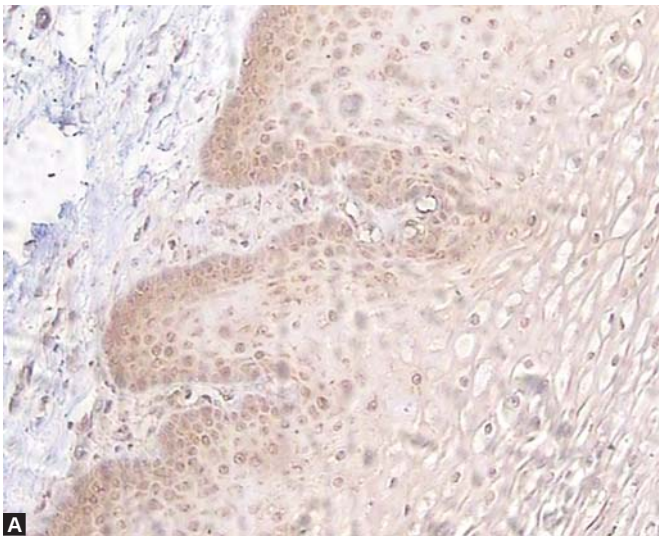
No differences was found in COX2-E between normal tissue and leukoplakia lesions and also between dysplastic and nondysplastic leukoplakia (p = 0.371 spinous layer, p = 0.513 basal layer) (Figs 2A and B).

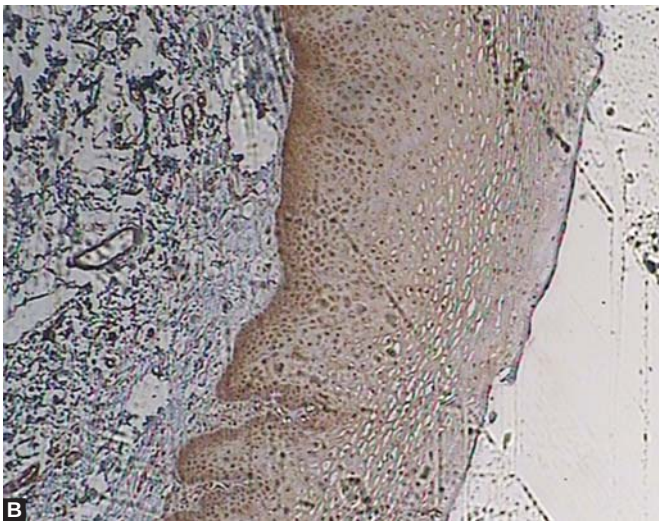
COX2-E was significantly different in SCC grade 3 and leukoplakia (p = 0.001) and normal tissue (p = 0.000). COX2-E was significantly higher in SCC grade 3 compared to leukoplakia (basal layer) (p = 0.000) (Figs 3A and B).

No significant differences was found in COX2-E between different grades of SCC in this study.

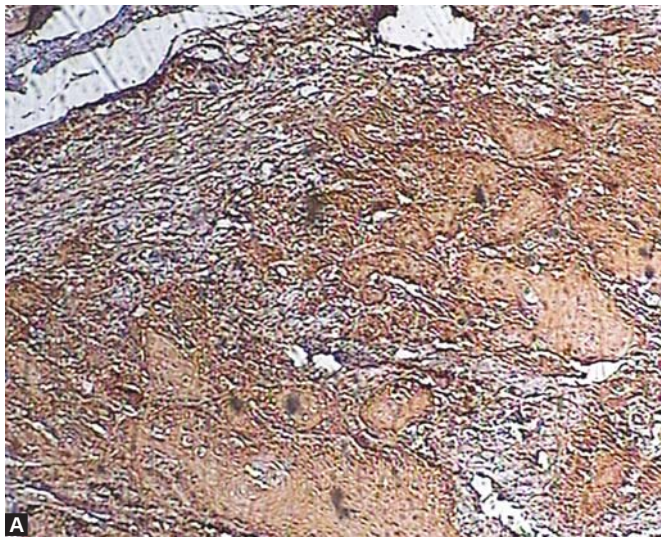
Table 1: Expression of COX2 in different grades of SCC and leukoplakia and normal mucosa

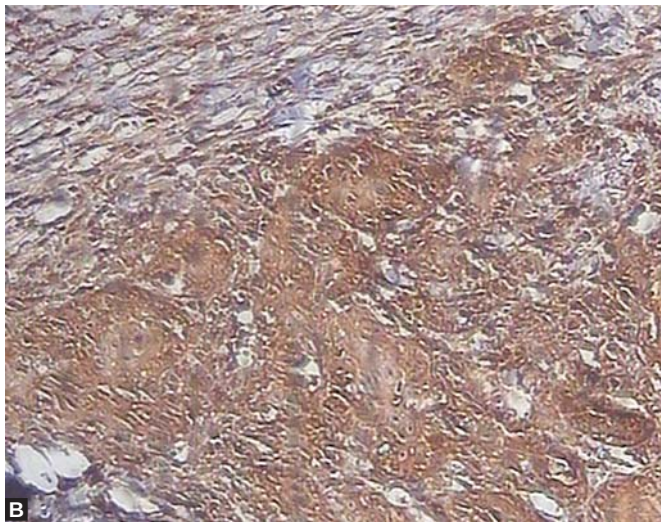
Groups	*Expression of COX2*				*Total*
	Grade A	*Grade B*	*Grade C*	*Grade D*	
SCC total	0 (0%)	6 (13.3%)	5 (11.1%)	34 (75.6%)	45
Grade 1	0 (0%)	3 (20%)	3 (20%)	9 (60%)	15
Grade 2	0 (0%)	3 (20%)	1 (6.7%)	11 (73.3%)	15
Grade 3	0 (0%)	0 (0%)	1 (6.7%)	14 (93.3%)	15
Leukoplakia					
Basal layer	0 (0%)	4 (26.7%)	8 (53.3%)	3 (20%)	15
Spinous layer	5 (33.3%)	8 (53.3%)	2 (13.3%)	0 (0%)	
Normal mucosa					
Basal layer	1 (5.9%)	4 (23.5%)	3 (17.6%)	9 (52.9%)	17
Spinous layer	8 (47.1%)	6 (35.3%)	3 (17.6%)	0 (0%)	





Figs 1A and B: Cytoplasmic COX2 expression in normal buccal epithelium of basal and parabasal layers (×40 and ×100 magnification)





Figs 2A and B: Severe COX2 expression in SCC grade 3 (×40 and ×100 magnification)

DISCUSSION

COX2-E in squamous cell carcinoma and chemoprotective effects of nonsteroidal anti-inflammatory drugs and selective COX2 inhibitors in early stages of carcinogenesis has been shown in many studies.[7,14-17]

Allici et al found overexpression of COX2 is related to tumor stage and specific tumor markers in SCC of esophagus.[14]

Pendi et al showed a significant higher exposure of COX2 gene in oral cancer patients compared to both normal controls and premalignant lesions.[9] They also found a higher exposure in premalignant patients compared to normal controls.

We showed a significant higher expression of COX2 in SCC lesions compared to leukoplakias and normal controls. This result was in agreement with result of Kawata and Pandy.[8,9]

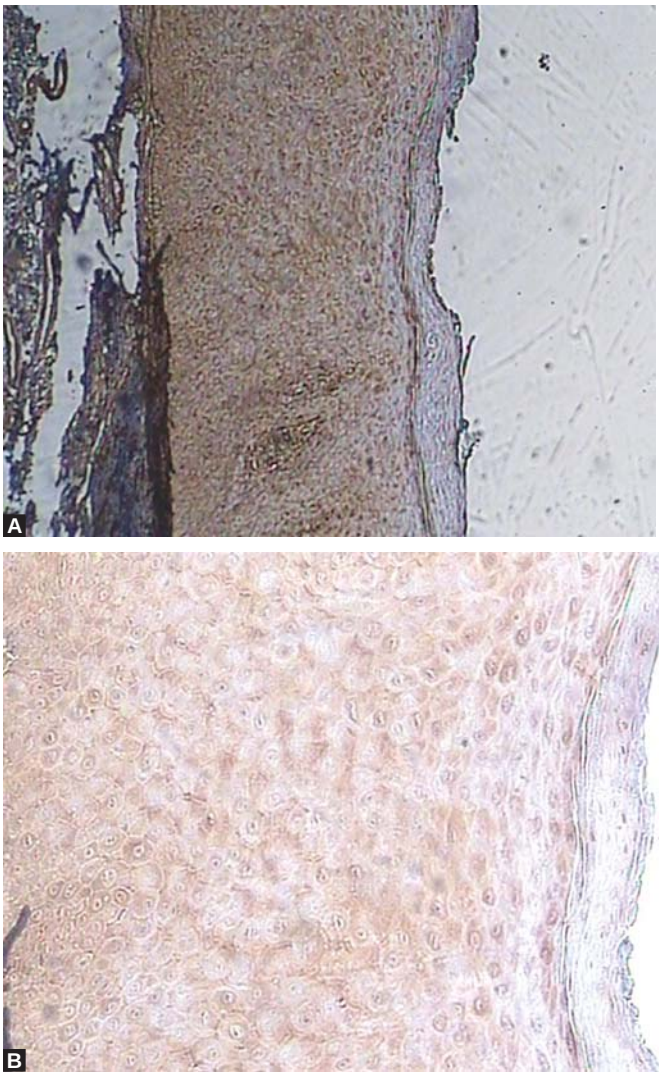
Kawata showed that expression of COX2 in well-differentiated head and neck SCC was stronger than poorly differentiated SCC.[8]

Kyzas showed a relation between COX2 expression and lymph node metastases at the time of diagnosis. He also showed a correlation between COX2-E and clinical stage, but no correlation was observed between COX2-E and histologic grade.[18] Although Goulart Filho showed no difference in all grades of SCC,[10] however several studies demonstrated that COX2-E was higher in grade 1 SCC.[19-22]

In our study, COX2-E was not significantly difference in SCC grade 1, 2, 3 ($p > 0.05$), so previous data was not confirmed by our results.

CONCLUSION

It was shown that COX2-E was high in SCC compared to leukoplakia and normal controls. This correlation can be



Figs 3A and B: Cytoplasmic COX2 expression in entire dysplastic epithelium of oral mucosa (×40 and ×100 magnification)

employed for therapeutic and diagnostic means. Although some studies evaluated relation between survival and clinical stage with COX2 expression, but the results were not reliable and further studies to reveal relation between COX2 expression and clinical stage is recommended.

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