Journal of Kerman University of Medical Sciences

JKMU

Journal of Kerman University of Medical Sciences, 2020; 27 (2): 183-189

Immunohistochemical Eexpression of Endothelin A Receptor in Dysplastic Oral Mucosa

Taymaz Shirali, D.D.S.[1], Hamid Abbaszadeh, D.D.S.[2], Forough Foroughi, M.D.[3], hemmat Gholinia, M.Sc.[4]

1- Babol University of Medical Sciences, Babol, Iran

2- Associate Professor, Oral and Maxillofacial Pathology Department, Faculty of Dentistry, Birjand University of Medical Sciences, Birjand, Iran (Corresponding author; E-mail: hamidabbaszade@yahoo.com)

3- Assistant Professor, Pathology Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

4- Institute of Health, Babol University of Medical Sciences, Babol, Iran

Received: 20 June, 2019 Accepted: 14 September, 2019

ARTICLE INFO

Article type:
Short Communication

Keywords:
Dysplasia
Normal mucosa
Endothelin A receptor

Abstract

Background: Recent researches have provided evidences of the importance of endothelin axis in carcinogenesis. According to our knowledge, no data exists about endothelin A receptor (ET_A) expression in dysplastic oral mucosa (DOM). Therefore, the aim of the present study was to evaluate immunohistochemical expression of ET_A in DOM.

Methods: In this cross-sectional study, paraffin-embedded tissue blocks of 20 cases of DOM and 20 cases of normal oral mucosa (NOM) were studied. Three-micron sections were prepared from tissue blocks and stained with ET_A antibody using immunohistochemistry. Percentage of stained cells and staining intensity were compared between DOM and NOM groups and also between different grades of DOM using Mann-Whitney, Chi-Square and Kruskal-Wallis statistical tests.

Results: In DOM group, 11 cases were stained positive for ET_A and in NOM group 17 cases were not stained. Comparison of percentage of stained cells and staining intensity for ET_A revealed significant difference between DOM and NOM groups (P=0.01 and 0.02, respectively). There were significant differences among different grades of DOM with respect to the percentage of stained cells (P=0.001) and staining intensity (P=0.02), so that higher grades showed greater expression for ET_A.

Conclusion: Our results supported ET_A receptor role in the initiation of carcinogenesis process in oral cavity.

Citation: Shirali T, Abbaszadeh H, Foroughi F, Gholinia H. Immunohistochemical Eexpression of Endothelin A Receptor in Dysplastic Oral Mucosa. *Journal of Kerman University of Medical Sciences*, 2020; 27 (2): 183-189.

Introduction

Oral dysplasia is a relatively common premalignant condition that affects 2.5-5 persons per 1000 persons. The importance of oral dysplasia is embedded in that a percentage of such lesions progress to oral cancer (1). Gene alterations have been implicated in the development of oral cancer by affecting protein expression (2).

Endothelin (ET) axis (including ET-1, ET-2, ET-3 and their Endothelin A [ET_A] and Endothelin B [ET_B] receptors) plays an important physiologic role in vascular tone, tissue differentiation and cellular proliferation (3- 6).

Recent researches have provided evidences of importance of ET axis in cancers (4). Components of ET axis may help the growth and progression of tumors via direct and indirect

mechanisms (5). Emergence of ET_A receptor antagonists have provided a new opportunity for targeted-therapy in cancers (4).

According to our knowledge, no data exists about endothelin A receptor (ET_A) expression in dysplastic oral mucosa (DOM). Therefore, the aim of present study was to evaluate immunohistochemical expression of ET_A in DOM.

Materials and Method

In this cross-sectional retrospective research, the studied group included paraffin-embedded tissue blocks of 20 DOMs (8 cases with mild dysplasia, 7 cases with moderate dysplasia and 5 cases with severe dysplasia). DOMs were comprised of leukoplakia lesions associated with dysplasia. Tissue blocks of 20 normal oral mucosa or NOMs (gingival tissue without clinically and histologically inflammation or with minimal inflammation achieved from crown lengthening surgery) were used as control group.

In DOM group, criteria for the diagnosis and determination of dysplasia grade were based on Neville *et al*. (7). Based on these criteria, DOMs were divided into three histopathologic grades: mild, moderate and severe. Specimens were selected from those patients who received no treatment for their lesions prior to the biopsy. Recurrent lesions were excluded from the study. Tissue blocks without enough tissue for evaluation or with improper quality and fixation were also excluded from the study (2).

In the present study, 3µ sections were prepared from tissue blocks and stained with ET_A antibody [Novocastra™ Liquid Mouse Monoclonal Antibody Endothelin-1 Receptor (ET_A); Leica Biosystems, Newcastle, United Kingdom, Product Code: NCL-L-ETA, Clone: RJT24, Ig Class: IgG2b] using immunohistochemistry. Sections of ductal carcinoma of breast were used for positive control and omission of primary antibody was used for negative control (2). Stained histopathologic slides were assessed under Olympus CX21 light microscope (Olympus Corporation, Tokyo, Japan) at ×100 and ×400 magnifications by a pathologist. For this assessment, percentage of stained cells (8) and staining intensity (9) for ET_A were taken into account. Cytoplasmic staining for the immunomarker was considered positive (9).

Five microscopic fields were selected as hot spots (fields in which epithelial cells had the greatest staining) at ×100 magnification under light microscope; in these fields percentage of stained cells were counted at ×400 magnification. The average of these five selected fields was recorded as the final percentage of the stained cells for each case. Percentage of the stained cells was semi-quantitatively categorized into four groups as follows:

Negative (percentage of stained cells ≤ 25%), weak positive (26% ≤ percentage of stained cells ≤ 50%), positive (51% ≤ percentage of stained cells ≤ 75%) and strongly positive (percentage of stained cells > 75%) (8).

Staining intensity of epithelial cells was also semi-quantitatively categorized into four groups as follows:

Negative (score 0): absence of staining; weak positive (score 1+): weak/hardly appreciable cytoplasmic staining in most of cells or light brown staining; moderately positive (score 2+): moderate cytoplasmic staining in most of cells or oak brown staining and strongly positive (score 3+): strong cytoplasmic staining in most of cells or dark brown staining (9).

Finally, data were entered into SPSS20 statistical software and analyzed using Kruskal-Wallis, Man-Whitney and Chi-square statistical tests. The significance level was P-value < 0.05.

Ethical Approval

The study was independently reviewed and approved by ethical board of our university (Code: MUBABOL.REC.1392.157).

Results

Average age of patients affected by dysplasia was 65 years. Of those, 7 ones (35%) were female and 13 ones (65%) were male. Dysplastic lesions were located in tongue (11cases), floor of the mouth (3 cases), lip (3 cases), buccal mucosa (2 cases) and gingiva (1 case) in decreasing order of frequency.

ET_A expression in DOM and NOM has been presented in figure 1. Seventeen cases of NOMs and 9 cases of DOMs were not stained for ET_A immunomarker. Means ± standard deviations (SD) of percentage of stained cells for ET_A in DOM and NOM groups were 0.43 ± 0.40 and 0.08 ± 0.14 respectively (mean ranks were 25.50 and 15.50 respectively). There was significant difference between these groups (P-value = 0.006). Means ± SD of percentage of stained cells in mild, moderate and severe dysplasia were 0.05 ± 0.13, 0.37 ± 0.26 and 0.83 ± 0.15 respectively (mean ranks were 5.25, 11.29 and 17.80 respectively).

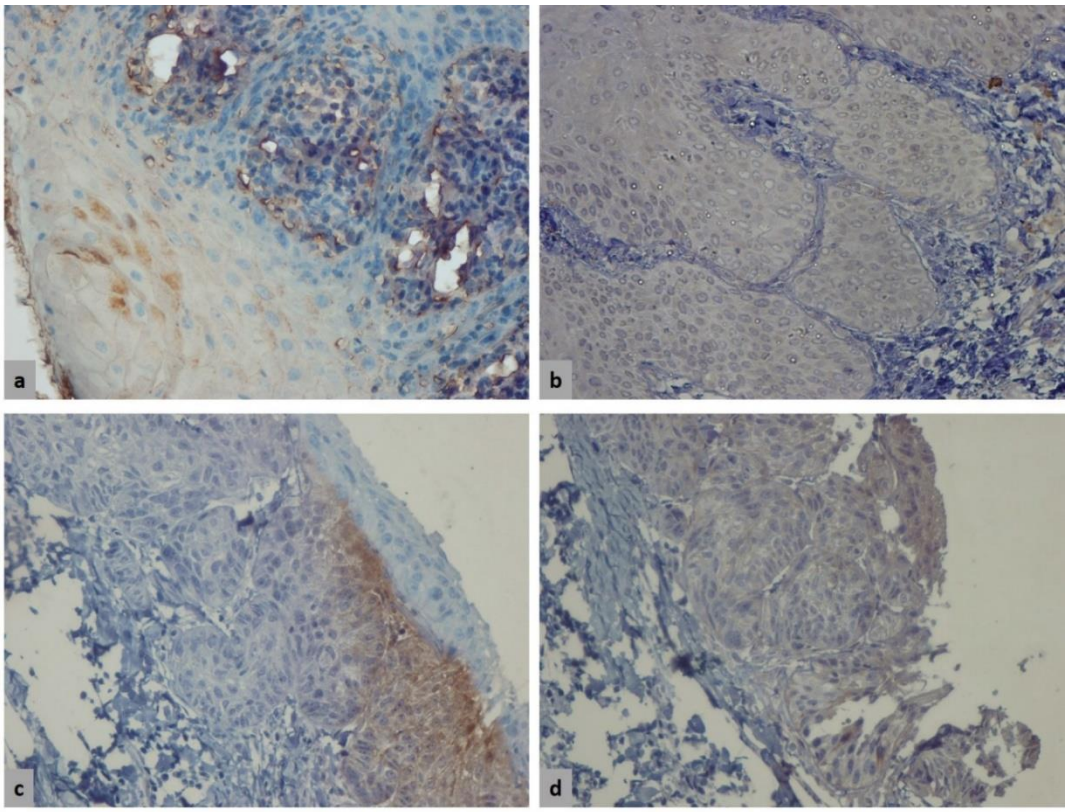

Figure 1. a) weak ET_A staining in normal mucosa (×400 magnification); b) weak staining intensity for ET_A in mild dysplasia (×100 magnification); c) moderate staining intensity for ET_A in moderate dysplasia (×100 magnification); d) strong staining intensity for ET_A in severe dysplasia (×100 magnification)

Categories of percentage of stained cells for ET_A in NOM and DOM groups have been presented in table 1. There was significant difference between NOM and DOM groups in the light of percentage of stained cells (P value<0.05); percentages of stained cells were significantly higher in DOM group than in NOM group.

Table 1. Percentage of stained cells for ET_A in normal oral mucosa (NOM) group and dysplastic oral mucosa (DOM) group

Category / group	percentage of stained cells				Pvalue
	negative	weak positive	positive	Strongly positive	
NOM	17	3	0	0	0.01
	85%	15%	0%	0%	
DOM	9	3	4	4	
	45%	15%	20%	20%	

Categories of percentage of stained cells for ET_A in different grades of DOMs have been presented in table 2. There was significant difference among different grades of DOMs in the light of percentage of stained cells (P value<0.05); higher grades showed significantly higher expression of ET_A (severe>moderate>mild).

Table 2. Percentage of stained cells for ET_A in different grades of dysplastic oral mucosa (DOM)

Category / DOM	Percentage of stained cells				Pvalue
	negative	weak positive	positive	Strongly positive	
Mild	7	1	0	0	0.001
	87.5%	12.5%	0%	0%	
Moderate	2	2	3	0	
	28.6%	28.6%	42.8%	0%	
Severe	0	0	1	4	
	0%	0%	20%	80%	

Categories of staining intensity for ET_A in NOM and DOM groups have been presented in table 3. There was significant difference between NOM and DOM groups in the light of staining intensity (P value<0.05).

Table 3. Staining intensity for ET_A in normal oral mucosa (NOM) group and dysplastic oral mucosa (DOM) group

Category / group	staining intensity				Pvalue
	negative	weak positive	Moderately positive	Strongly positive	
NOM	17	2	1	0	0.02
	85%	10%	5%	0%	
DOM	9	7	3	1	
	45%	35%	15%	5%	

Categories of staining intensity for ET_A in different grades of DOM group have been presented in table 4. There was significant difference between different grades of DOMs in the light of staining intensity (P value<0.05).

Table 4. Staining intensity for ET_A in different grades of dysplastic oral mucosa (DOM)

Category / Grade	staining intensity				Pvalue
	negative	weak positive	Moderately positive	Strongly positive	
Mild	7 87.5%	1 12.5%	0 0%	0 0%	0.02
Moderate	2 28.6%	4 57.1%	1 14.3%	0 0%	
Sever	0 0%	2 40%	2 40%	1 20%	

Discussion

Recent researches have provided evidences of the importance of endothelin axis in carcinogenesis. According to our knowledge, no data exists about endothelin A receptor (ET_A) expression in dysplastic oral mucosa. Therefore, the present study was done to evaluate immunohistochemical expression of ET_A in DOM.

In this study, there were significant differences between NOM and DOM groups in the light of percentage of stained cells for ET_A and staining intensity, so that percentage of stained cells and staining intensity was significantly higher in DOM group than in NOM group. The overexpression of ET_A in DOMs in our study suggested the role of ET_A in initiation of carcinogenesis process in oral cavity.

In our study, there were significant differences among different grades of dysplasia in the light of percentage of stained cells for ET_A and staining intensity; that is, higher grades of dysplasia showed greater expression for ET_A than lower grades. This finding implies the relationship of ET_A expression with dysplasia grade. It can be suggested that increase in ET_A expression might help the progression of dysplasia to higher grades and consequently to get closer to the squamous cell carcinoma and involvement of the surrounding stroma. In Ishibashi *et al.* study on endothelin protein expression in esophageal squamous cell carcinoma (SCC) and proximal dysplastic and normal mucosa, high endothelin protein expression reduced the recurrence-free survival in patients affected by esophageal SCC; they concluded that measurement of endothelin expression by a simple immunohistochemistry analysis may help to predict the prognosis of patients with esophageal SCC (10).

In Awano study, expressions of all components of endothelin axis were observed in human OSCC cells but only ET-1, ET_B and endothelin converting enzyme-1 (ECE-1) increased in comparison with normal epidermal keratinocytes (11). In our study, overexpression of ET_A was observed in dysplasia compared to normal mucosa.

Pickering *et al.* observed overexpression of endothelin-1 protein and mRNA in OSCCs in comparison to the normal control group (12).

In Hinsley *et al.* study, ET-1 increased migration of head and neck SCC cells via releasing EFGR ligands from fibroblasts. They concluded that endothelin axis activation in head and neck SCC might help SCC progression by stimulating cancer cell motility via increasing epithelial-stromal interactions (13).

According to Ishimoto *et al.*, both ET_A and ET_B endothelin receptors overexpressed in tumor cells of tongue SCC. They

concluded that endothelin signaling pathway may play somewhat an import role in cell growth in SCC (3).

Alaizari *et al*. observed endothelin-1 protein immunoreactivity in all OSCC samples and in their study, poorly-differentiated OSCCs had significantly more immunoexpression than moderately-differentiated OSCCs, since moderately differentiated OSCCs showed significantly more immunoexpression than well-differentiated OSCCs. They concluded that overexpression of endothelin-1 can increase invasive behavior of poorly-differentiated OSCCs and therefore endothelin-1 can be a therapeutic target in OSCC (5). More ET_A immunoexpression in dysplasia with higher grade in our study is somewhat in accordance with more endothelin-1 immunoexpression in poorly-differentiated SCC in the mentioned study.

In Salem *et al*. study, endothelin-1 and ET_A were expressed significantly more in CSCCs and psoriasis than in control and BCC groups; they concluded that overexpression of ET-1 and ET_A implies to their involvements in keratinocyte proliferation in CSCC and psoriasis and that ET_A is the predominant expressed receptor in psoriasis and SCC (9).

In Cong *et al.* study, ET_A was overexpressed in hepatocellular carcinoma tissues and cells and ET_A and ET-1 overexpression were associated with vascular invasion and tumor stage in hepatocellular carcinoma; they concluded that ET_A may play an important role in hepatocellular carcinoma progression (14).

Conclusion

Our results suggest an important role for ETA in initiation of carcinogenesis process and development of dysplasia.

Acknowledgement

We thank Research Vice Chancellor of Babol University of Medical Sciences for supporting this study.

Conflict of interest statement

The authors declare no conflict of interest.

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