Original Article

Expression of β-catenin in Minor Salivary Glands Adjacent to Oral Squamous Cell Carcinoma

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Abstract

Objective: This study aims to evaluate the expression pattern, localization, and stain intensity of β-catenin in minor salivary glands adjacent to surgically excised oral squamous cell carcinoma (OSCC).

Methods: A retrospective study was held with 16 samples of formalin-fixed paraffin-embedded blocks with minor salivary glands adjacent to OSCC. Sections were stained and evaluated immunohistochemically with β-catenin. The staining expression was assessed according to cellular localization, stain intensity, and, lastly, the pattern of stain distribution throughout acini. Statistical analysis was performed using SPSS version 24.0 software for Windows, and data analyzed by Fisher's exact test. P-value <0.05 was considered as statistically significant.

Results: All minor salivary glands in the studied sample showed β-catenin staining with different expression in their functional units, as all had ductal and myoepithelial cells staining with a predominant cytoplasmic localization. While the mucous acini showed β-catenin expression in 10 cases (62.5%), this marker was significantly less frequently detected in serous acini of two cases of poorly differentiated OSCC (p= .008). A highly significant relation was found between the β-catenin cellular localization and stain distribution pattern in mucous and serous acini.

Conclusions: β-catenin had altered cytoplasmic expression in all of the minor salivary glands adjacent to OSCC. Furthermore, the diffuse pattern of distribution throughout the acini could identify the multi-patches pathological alteration of this area. The current study clarifies that the adjacent clinically normal-appearing salivary glands could harbor genetic aberrations of their subsequent malignant transformation.

Keywords: *Adjacent, β-catenin, Minor salivary gland, OSCC.*

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Introduction

Salivary glands involved paired major organs, and about 600– 1000 minor salivary glands spread throughout the oral cavity[1]. These glands have two morphologically well-defined functional segments: the acinar cells and a complex ductal system associated with contractile myoepithelial cells, the contractile ability responsible for the salivary secretion, and subsequently plays a significant role in acinar salivary flow[2,3]. Immunohistochemical findings showed dual epithelial and contractile properties of these cells with the expression of keratin filaments and contractile proteins such as actin, caldesmon, calponin, and smooth muscle actin[4,5].

Wnt/β-catenin signaling pathway is one of the essential intercellular signaling pathways responsible for the proliferation, differentiation, and functioning of many cell and tissue types[6,7]. β-catenin has a double function; it acts as a significant structural component of cell-cell adherence junctions with E-cadherin and a signaling molecule in the Wnt pathway[8]. The intracellular β-catenin protein is responsible for the regulation of the Wnt/ β-catenin pathway, as it consists of highly conserved genes for proteins with various biological functions[9]. During salivary gland development, the Wnt/β-catenin pathway is activated initially in the mesenchyme and then in the ductal epithelial cells[1,10].

As Wnt signaling is under tight control in normal salivary glands, its dysregulation would develop many head and neck lesions. The over-expression of β-catenin is associated with tumorigenesis, promotes cell migration and invasion[11].

On the other hand, tissues adjacent to malignancy are not considered to be biologically normal since perilesional molecular changes seemed to affect the close structures, including the blood vessels, the fibroblast, and even normal-looking surface epithelium[12]. Thus, even clinically normal-appearing mucosa harbors early precancerous genetic aberrations[13,14]. Also, minor salivary glands adjacent to salivary gland cancers might show abnormal expression of β-catenin[15].

Thus this study aims to evaluate the expression pattern, localization, and stain intensity of β-catenin in minor salivary gland tissues adjacent to surgically excised OSCC, which may strictly relevant to the therapeutic plan and prognosis.

Materials and methods

The present study was held at the University of Sulaimani /College of Dentistry / Department of Oral Diagnosis between December 2019 and February 2020. It was accepted by the Local Ethical and Scientific Committee in the College of Medical Sciences/University of Sulaimani(4-February-2020). The sample was composed of 16 retrospective formalin-fixed paraffin-embedded blocks of minor salivary glands adjacent to primary OSCC. Two serial 5µm sections were cut; one was stained with hematoxylin and eosin to observe histopathological grading of OSCC by using Bryne's grading system[16] and indicate the presence of adjacent salivary glands in perilesional areas. The other section was mounted on a positively charged slide and stained immunohistochemically with β-catenin. Sections were deparaffinized, rehydrated, and then retrieved by boiling tissue sections in citrate buffer pH6 (15 min at 95°C). Sections were incubated with H_2O_2 for 10 min to block endogenous peroxidase activity, then blocking serum was used for another 10 min to avoid non-specific binding. Then sections were incubated with the primary rabbit polyclonal antibody of β-catenin (abcam®; dilution 1:100) for 45 min at 37°C in a humid chamber. Complement was added and incubated for 10 min. Then goat anti-rabbit HRP conjugate was applied and incubated for 15 min. Washing with phosphate buffer saline four times for 5 min each was done after each step. The reaction was visualized by incubation with DAB for 5 min, and then sections were counterstained with hematoxylin for 20 seconds. Then sections were dehydrated, cleared, and mounted with DPX and evaluated under a light microscope. Negative control was done in each run by omitting the primary antibody. The normal oral epithelium was used as a positive control. Immuno-evaluation was done for different functional structures of the minor salivary glands, including the acini (both serous and mucous), ducts in addition to myoepithelial cells. Three observers evaluated the immunoreactivity separately and semi-quantitatively. The expression of staining was assessed in response to the following parameters: cellular localization with membranous (M), cytoplasmic (Cyto) or mixed (both cytoplasm and membranous), stain intensity as (faint, moderate, strong)[17], and lastly the pattern of stain distribution throughout acini as (focal, diffuse) homogenous distribution.

Statistical analysis

Data were assessed using SPSS version 24.0 software for Windows and analyzed by Fisher's exact test. p-value <0.05 was considered statistically significant.

Results

The study included 16 patients, twelve males and four females with an age range of 29-71 years and a median of 50 years old. Samples were taken from 8 buccal mucosa, two lower lip, and six tongue cases. Normal oral epithelium showed strong membranous β-catenin expression (Figure 1A). All minor salivary glands samples adjacent to OSCC revealed β-catenin staining (100%) with different functional units positivity. All the cases had ductal and myoepithelial cells expression with high cytoplasmic localization (Figure1B C). The mucous acini showed β-catenin expression in 10 cases (62.5%), with 25% for each cytoplasmic and mixed localization (Figure1D). At the same time, β-catenin expression was less frequently detected with mixed localization in serous acini (12.5%) (Figure 1E) (Table1).

OSCC samples were previously sorted according to Bryne's grading system into well-differentiated (8 cases), moderately differentiated (6 cases), and poorly differentiated (2 cases). The localization of β-catenin expression among different functional units of salivary glands was related to the adjacent OSCC. Serous acini showed significant mixed expression in poorly differentiated OSCC (p =.008). Regarding the staining intensity, moderate-intensity was found in 8 cases (50%), while 2 cases (12.5%) had faint intensity (Table1). There was no significant difference between the staining intensity and adjacent OSCC different grades (p=.32). According to the pattern of distribution of staining throughout acinar cells, 50% of cases showed diffuse homogeneous distribution (Figure1F), 12.5% had focal distribution (Figure 1G). Still, no significant difference was found between the distribution of staining and OSCC in different grades (p=.32). Finally, the localization of staining was related to the pattern of distribution throughout the acini; highly significant relations were found in mucous and serous acini, which were (p = .000) and (P=.008**)** respectively, as shown in (Table2).

Table 1: β-catenin sub cellular localization in different functional units, stain intensity, and pattern of distribution throughout acini in relation to adjacent OSCC Bryne's grades.

		Localization	No. (%)	Bryne's grade			p-value
				Well (8) (50%)	Moderate (6) (37.5%)	Poor (2) (12.5%)	
Cell type	Ductal	Cyto	12 (75%)	6	4	2	1.0
		Mixed	4 (25%)	2	2	0	
	Myoepithelial	Cyto	16 (100%)	8	6	2	
	Mucous acini	Negative	6 (37.5%)	2	4	0	0.13
		Cyto	4 (25%)	2	2	0	
		M	2 (12.5%)	2	0	0	
		Mixed	4 (25%)	2	0	2	
	Serous acini	Negative	14 (87.5%)	8	6	0	0.008*
		Mixed	2 (12.5%)	0	0	2	
Stain intensity	Faint		2 (12.5%)	2	0	0	0.32
	Moderate		8 (50%)	4	4	0	
	Strong		6 (37.5%)	2	2	2	
Pattern of distribution	Negative		6 (37.5%)	2	4	0	0.32
	Focal		2 (12.5%)	2	0	0	
	Diffuse		8 (50%)	4	2	2	

*p-value < 0.05; significant difference, Cyto; cytoplasmic, M; membranous.

Table 2: β-catenin cellular localization in relation to the pattern of distribution throughout acini.

			Pattern of distribution			p-value
			Negative	Focal	Diffuse	
Acini type	Mucous acini	Negative	6	0	0	0.000*
		Cyto	0	0	4	
		M	0	2	0	
		Mixed	0	0	4	
		Total	6	2	8	
	Serous acini	Negative	14	0	0	0.008*
		Mixed	0	0	2	
		Total	14	0	2	

* P-value < 0.001; highly significant difference; Cyto; cytoplasmic, M; membranous.

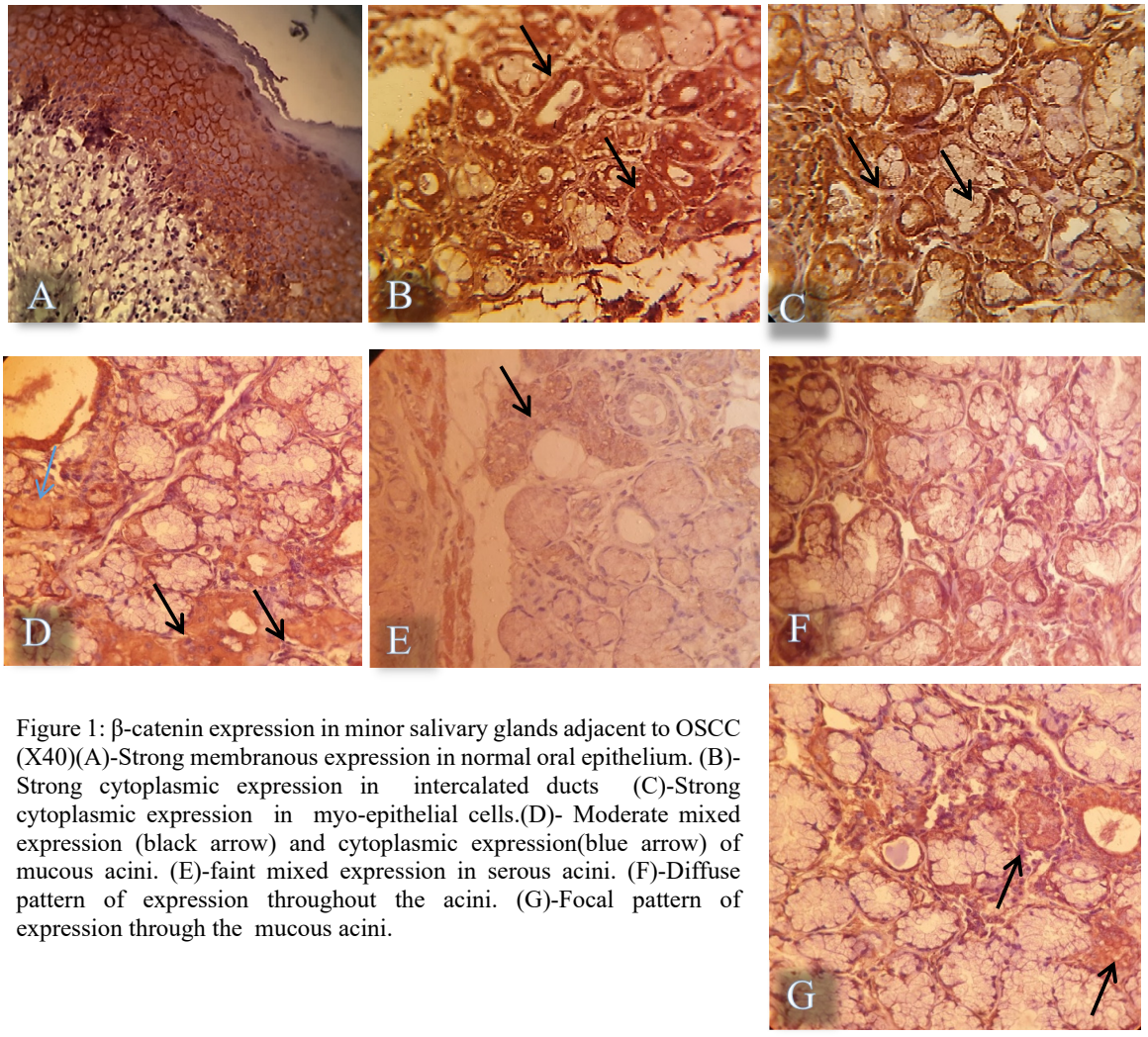

Figure 1: β-catenin expression in minor salivary glands adjacent to OSCC (X40)(A)-Strong membranous expression in normal oral epithelium. (B)-Strong cytoplasmic expression in intercalated ducts (C)-Strong cytoplasmic expression in myo-epithelial cells.(D)- Moderate mixed expression (black arrow) and cytoplasmic expression(blue arrow) of mucous acini. (E)-faint mixed expression in serous acini. (F)-Diffuse pattern of expression throughout the acini. (G)-Focal pattern of expression through the mucous acini.

Discussion

Structures adjacent to malignancy are considered histologically abnormal and subsequently related to local tumor recurrence and metastasis[18]; therefore, margin adequacy and perilesional molecular nature are an essential prognostic factor that would help to reach more advanced therapeutic approaches[19].

β-Catenin has a vital role in tissue development and function. It acts as a structural protein at cell-cell junctions by forming complexes with E-cadherin; any abnormality of cadherin-catenin compounds may dissociate cells and subsequent invasion and metastasis[20]. Also, β-catenin functions as a transcriptional activator mediating Wnt signal transduction, regulating cell proliferation, differentiation, and oncogenesis[20-23]. It is highly suggested that the functions of β-catenin depend on its subcellular localization since it is synthesized in the cytoplasm and transported to the nucleus to perform its transcriptional function; thus, membranous β-catenin mediates cell-cell adhesion, whereas the accumulation of cytoplasmic and nuclear pool of this protein is associated with oncogenic functions[24]. The exact role of β-catenin in the pathophysiology of salivary gland neoplasms is still not understood[25], even its expression in normal salivary gland had some variation, as Yamada et al. found negative expression of β-catenin in ductal and acinar compartments except for the striated duct of major salivary gland[26]. While others demonstrated positive membranous expression in the acinar and ductal compartments[27, 28].

The oral carcinoma field of this sample previously showed high altered cytoplasmic β-catenin expression[29]. This study revealed β-catenin expression in all studied samples (ductal and myoepithelial cells), with predominant cytoplasmic expression (100% myoepithelial cells, 75% ductal cells), and to a lower extent acinar cells (25% of the mucous acini). This cytoplasmic subcellular localization and stabilization demarcate this marker's role in the activation of other nuclear transcription factors associated with proliferation and differentiation[30,31]. Unfortunately, no previous studies have examined the expression of β-catenin in salivary glands adjacent to SCC; most of the research involved β-catenin in salivary gland tumors[8,10,11,15,30]. Thus it wasn't easy to find studies to compare our results with. However, a previous study of the perilesional normal-looking epithelium of the same sample of OSCC indicated altered cytoplasmic β-catenin expression in 16% of the sample[12]. This altered cytoplasmic expression of β-catenin in both perilesional tissues adjacent to OSCC might explain the occurrence of second primary tumors or other unrelated tumors in the same field of cancerization.

Comparing our results to normal salivary glands adjacent to salivary tumors showed some similarity, as ductal and myoepithelial cells of normal salivary glands adjacent to pleomorphic adenoma showed positive expression of 100% and 1.2%, respectively[32]. While inconsistency was seen as dominating membranous expression was illustrated in ductal and acinar cells, according to Furuse et al. 2006[30].

Variations in subcellular localization of β-catenin in salivary tumors reported in different studies, as it was found that nuclear expression is highly specific for basal cell adenoma and basal adenocarcinomas[24,33]. Other studies indicated that predominant cytoplasmic localization was prominent among the malignant salivary tumors, reflecting their aggressiveness and biological behavior[21,25].

The present study revealed significant remarkable aberrant β-catenin expression in adjacent serous acini of two poorly differentiated OSCC (P-value =.008). Thus altered localization of staining of this marker might indicate loss of differentiation of the adjacent cancerous area and predict subsequent invasion and recurrence ability of this cancer. Lastly, the diffuse cytoplasmic pattern of distribution of β-catenin throughout the acini (50%) reflects the same cancerization field of clonal unit patches that acquire more mutations with growth advantage and spread laterally, replacing the normal compartment[14,34].

However, the preliminary finding of this study needs more confirmation by further studies, with larger sample sizes, further genetic analysis of β-catenin, and studying Wnt expression to establish better the signaling pathway and biological significance and the clinical importance of β- catenin in salivary glands adjacent to malignancy.

Conclusions

Aberrant diffuse β-catenin expression in all salivary glands samples adjacent to OSCC might demarcate the valuable role of this marker in the initiation of other adjacent tumors and/ or second primary tumors in the field of cancerization. This study can be considered as base evidence for future studies to be held concerning other marker's expression in salivary glands adjacent malignancies.

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