Original Article

Immunohistochemical Expression of BubR1 and Telomerase in Minor Salivary Gland Tissue Adjacent to Oral Squamous Cell Carcinoma

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Abstract

Objectives: Evaluation of the expression of tissue markers in the parenchyma within the area adjacent to a primary tumor is critical in cancer progression, prevention, and identification of early changes in the field of cancerization. The present study aimed to evaluate BubR1 and telomerase activity in minor salivary gland tissue adjacent to oral squamous cell carcinoma (OSCC) with their clinicopathological features.

Methods: Four-micron tissue sections from 21 formalin-fixed paraffin blocks of OSCC surgical margins were prepared and stained for anti-BubR1 and anti-telomerase antigens.

Results: The studied sample included 71.4% males and 28.6% females, and the most prevalent site was buccal mucosa (42.9%). The total sample showed a high (71.4%) BubR1 expression, while telomerase was expressed in 42.9%. BubR1 was expressed significantly in the buccal mucosa ($p=0.000$) in the myoepithelial (cytoplasmic) and ductal cells (cytoplasmic and mixed). In comparison, telomerase showed significant myoepithelial mixed and cytoplasmic expression of buccal mucosa ($p=0.013$) and nuclear expression in ductal cells within the tongue ($p=0.05$). Both markers showed altered expression and predominated significantly in cases adjacent to poorly differentiated OSCC ($p<0.05$). Lastly, a moderate/strong correlation between the aberrant accumulation of BubR1 and telomerase was found ($r=0.5$-0.8, $p<0.05$).

Conclusions: Elevated expression of BubR1 and telomerase was found in the minor salivary gland adjacent to OSCC. Therefore, they represent a valuable tool to identify molecular changes in the field of cancerization. Cases of OSCC excised from buccal mucosa and tongue required a regular follow-up, especially poorly differentiated OSCCs. The elevated expression of both markers positively regulates each other.

Keywords: *BubR1, Telomerase, Minor salivary gland; OSCC.*

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Introduction

Cancer surgery aims to remove as much diseased tissue and retain as much healthy tissue as possible. One key predictor of locoregional relapse is the presence of carcinoma in or close to the primary tumor's surgical margins, as relapse can occur in cases with clear margins. Genotypic alterations can affect hundreds of genes, leading to phenotypic changes in critical cellular functions such as cell death resistance, high proliferation rate, angiogenesis, and ability to invasion and metastasis[1].

Spindle assembly checkpoint (SAC) is the mechanism by which the cells control chromatin segregation during mitosis. Bub1 and BubR1 are two key SAC protein factors, which have distinct functions during mitosis[2]. BubR1 acts as a guardian to monitor chromosome-spindle attachment to mitotic checkpoint signaling. Dysfunction of BubR1 results in chromosomal instability, uncontrolled checkpoint, premature anaphase, and early tumorigenesis[3]. BubR1 overexpression in OSCC is related to the normal gene's up-regulation, leading to loss of a normal checkpoint function. It is also possibly attributable to dysregulated expression of other mitotic SAC protein complex components, such as Bub1 and Bub3[4]. This Bub1-Bub3 complex normally binds to telomeres, promotes their replication during the S phase, and promotes telomere DNA replication. Bub1-Bub3 complex prevents telomere shortening and fragility. Besides, SAC proteins BubR1 and MAD2 also bind mitotic telomeres in a TRF1-dependent manner. The depletion of RTEL1 leads to telomere loss[5].

Telomeres are DNA-protein complexes (tandem repeats of TTAGGG) that cap the chromosome ends, synthesized by telomerase, a specialized reverse transcriptase. It protects chromosomes from incomplete replication, nuclease degradation, and end-to-end fusion during replication and is required for chromosome segregation during meiosis and mitosis. Telomerase is inactivated in most somatic cells, except in germ cells, hemopoietic stem cells, proliferating cells in self-renewal tissues, and many cancers but not in precancerous lesions[6]. Approximately 85% of human malignant tumors express increased levels of telomerase. Simultaneously, most of the remaining 15% find alternative lengthening of telomeres to maintain their telomere lengths in the absence of telomerase. Telomerase activity is higher in malignant than in non-neoplastic or benign salivary gland tumors[7]. A TRAP-assay-based study showed no telomerase activity in hyperkeratotic epithelia, normal epithelium, and stroma adjacent to head and neck SCC[8]. On the contrary, another study demonstrated telomerase activity in normal oral mucosa adjacent to or distal to head and neck SCC[9].

Thus, this study aims to characterize BubR1 and telomerase's subcellular expression within normal-looking minor salivary gland tissues neighboring OSCC invasive islands.

Materials and methods

The study was conducted at the University of Sulaimani /College of Dentistry /Department of oral and maxillofacial pathology in January 2020. The Ethical Committee of the College of Dentistry and Medical science approved this study (no. 158, 1 September 2020). The study sample comprises 21 retrospective formalin-fixed paraffin-embedded blocks of primary OSCC, containing minor salivary glands in the perilesional tissue. Clinicopathological information such as age, sex, location of the tumor, and Bryne's grades of OSCC, was obtained from the patients' histopathological reports.

The BubR1 protein and telomerase were detected immunohistochemically. Four µm tissue sections from each block were deparaffinized and rehydrated. Antigen retrieving was performed through boiling in citrate buffer (pH-6.0, 30 min). Slides were allowed to cool for 10 minutes and washed twice with phosphate-buffered saline (PBS) (3 min for each) at room temperature. The slides were incubated first with 3% hydrogen peroxidase (10 min) to block endogenous peroxidase, then with primary antibodies (diluted rabbit polyclonal anti-BubR1, 1:200, Abcam) (diluted rabbit polyclonal anti-telomerase, 1:200, Abcam) for one hour, followed by four times washing by PBS. Then they were incubated with complement for (15 min) and washed by PBS (5min). Goat anti-rabbit HRP conjugate was applied for 15 min and then washed by PBS. Sections were stained by diaminobenzidine (DAB) as chromogen for 5 min and four times washed by PBS, then immersed in hematoxylin (20 sec). A tissue section from the normal tonsil (for BubR1) and spleen (for telomerase) was used as a positive control (according to manufactural protocols). A negative control sample was performed by replacing the specific primary antibody with PBS.

The immuno-stained sections were compared with the corresponding Hematoxylin and Eosin-stained slides to

establish a topographic relationship between immuno-stained areas and Bryne's grades of adjacent OSCC. Under a light microscope, both markers' immunohistochemical evaluation was done at five high power (X400) hot spots. The immunohistochemical reaction was identified semiquantitatively in different cell types of salivary gland parenchyma (myoepithelial, ductal, and acinar) and localized with different subcellular compartments (cytoplasm alone (cyto), nuclear alone (n), or mixed)[10].

Statistical analysis

Sample normality was tested by Shapiro-Wilk's test histogram, Q-Q plots, and box plots. Since our data were not normally distributed ($P<0.05$), the interferential analyses for studied parameters were performed by Fisher's exact test and Kruskal-Wallis test. Spearman's rho test was used to correlate both markers' expression throughout different components of salivary gland tissue. SPSS version 24.0 software for Windows was used for data analysis. P-value ≤ 0.05 was considered significantly different.

Results

Demography

The studied sample was related to 15 males and six females; their ages were between 45 and 71 years, with 58 years. Primary OSCC samples were predominantly from the buccal mucosa (42.9%) (Table 1).

Semiquantitative evaluation for BubR1 protein

The immunohistochemical expression results of BubR1 are shown in Table 2. BubR1 staining was expressed in fifteen cases (71.4%). It was cytoplasmic in all positive myoepithelium (nine cases) (Fig 1.A). Ductal cells showed positivity in all fifteen cases; twelve of them were cytoplasmic (Fig 1.B), and three cases were mixed (cytoplasmic/nuclear) expression (Fig 1.C). Serous acinar cell expression was cytoplasmic in six cases (Fig 1.D) and mixed in three cases (Fig 1.A, C, E), whereas mucous acinar cell expression was cytoplasmic in six cases (Fig 1.B) and nuclear in three cases (Fig 1.A, C, E).

Regarding the lesion site, when all samples were analyzed together (Table 2), BubR1 expressed significantly in all minor salivary glands in the buccal mucosa ($p=0.000$). Contrary, all samples were taken from the lip and floor of the mouth were negative. Cytoplasmic expression of myoepithelial cells had a higher mean rank and was seen significantly in the buccal mucosa (17.0, 9 cases, $p=0.000$). Ductal cells showed a significantly higher number of cases expressing BubR1 (six with cytoplasmic, three with mixed expression, $p=0.000$), and the higher mean rank (15.0, $p=0.000$) was in the buccal mucosa. Acinar cells (serous and mucous) did not show a significant distribution and relation between BubR1 subcellular expression and different locations ($p=0.221$ for each). Nevertheless, the higher mean rank was in the buccal mucosa (14.0, $p=0.073$).

The histopathological grading of the sample showed an equal number of WDSCC and MDSCC (each 9 cases) and three PDSCC (Table1). Immuno-expression of BubR1 in the minor salivary gland related to the grade of adjacent OSCC was significantly not different ($p=.666$). However, there was a significant relation in its distribution within different cell types ($p\leq 0.05$) (Table 2). The minor salivary gland adjacent to PDSCCs had a higher mean rank in all cell types ($p<0.05$). In myoepithelial cells, cytoplasmic expression was seen with cases adjacent to WDSCCs (6) and PDSCCs (3) ($p=0.001$). Ductal cells showed cytoplasmic expression with cases of WDSCCs and MDSCCs (six cases each), while mixed expression was significantly found only in the adjacent area of PDSCCs ($p=0.005$). Adjacent minor salivary gland to PDSCCs had significant mixed expression in serous acini and nuclear expression in mucous acini (three cases each) ($p=0.005$).

Semiquantitative evaluation for telomerase protein

The immunohistochemical expression results of telomerase are summarized in table 3. Telomerase expression in the minor salivary gland was negative in 12 cases (57.1%), and it was expressed in nine cases (42.9%). Its expression was cytoplasmic (Fig 2.A) and mixed (Fig 2.B) in myoepithelial cells (three cases each). Ductal cells showed positivity in nine cases, which were cytoplasmic (Fig 2.A), mixed (Fig 2.C), and nuclear (Fig 2.B) (three cases each). Serous acinar cell expression was cytoplasmic in six cases (Fig 2.E) and mixed in three cases (Fig 2.D). In contrast, mucous acinar cells did not show positivity in 15 cases, and they were cytoplasmic (Fig 2.E) and nuclear (Fig 2.F) (three cases each).

Telomerase expression in the minor salivary gland was negative in the lip and floor of the mouth, and its expression in the total sample did not show a significant difference to different sites ($p=0.116$) (Table 3). However, telomerase expression in myoepithelial cells was positive in the buccal mucosal site of the salivary gland only. It was cytoplasmic and mixed expression (three cases each), with a significant high mean rank (15.0, $p=0.013$). Also, ductal cells showed telomerase

Table 1: Frequency and distribution of studied sample summary.

		No.	Percentage
Gender	Male	15	71.4
	Female	6	28.6
Age	Range	45-71 years	
	Median	58 years	
Location	Buccal mucosa	9	42.9
	Tongue	6	28.6
	Lip	3	14.3
	Floor of mouth	3	14.3
Adjacent OSCC Bryne's histopathological grades	WD-SCC	9	42.9
	MD-SCC	9	42.9
	PD-SCC	3	14.3

Table 2: BubR1 expression in the total 21 studied cases in different cell types of minor salivary gland adjacent to primary OSCC in relation to the location and Bryne's histopathological grades.

Variables		Total			Location				p-value	Bryne's grades			P-value
		Expression	no	%	Tongue	Buccal	Lip	Floor		WD	MD	PD	
Total sample positivity		negative	6	28.6	0	0	3	3	.000*	3	3	0	.666
		positive	15	71.4	6	9	0	0		6	6	3	
Myoepithelial cells		negative	12	57.1	6	0	3	3	.000*	3	9	0	.001*
		cytoplasmic	9	42.9	0	9	0	0		6	0	3	
		Kruskal-Wallis test			6.5	17.0	6.5	6.5	.000§	13.5	6.5	17.0	.001§
Ductal cells		negative	6	28.6	0	0	3	3	.000*	3	3	0	.005*
		cytoplasmic	12	57.1	6	6	0	0		6	6	0	
		mix	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			12.5	15.0	3.5	3.5	.000§	9.5	9.5	20.0	.003§
Acini	Serous	negative	12	57.1	3	3	3	3	.221	6	6	0	.005*
		cytoplasmic	6	28.6	3	3	0	0		3	3	0	
		mix	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			11.0	14.0	6.5	6.5	.073	9.5	9.5	20.0	.004§
	Mucous	negative	12	57.1	3	3	3	3	.221	6	6	0	.005*
		cytoplasmic	6	28.6	3	3	0	0		3	3	0	
		nuclear	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			11.0	14.0	6.5	6.5	.073	9.5	9.5	20.0	.004§

* P-value of Fisher's exact test significant difference p≤.05, § P-value of Kruskal-Wallis test significant difference p≤.05 no; number, WD; well differentiated, MD; moderate differentiated, PD; poorly differentiated, mix; cytoplasmic and nuclear

cytoplasmic and mixed expression (three cases each) in the buccal mucosa (p=0.05). But the higher mean rank was in the tongue (13.25) and expressed as a nuclear expression (p=0.134). Telomerase in serous acini was cytoplasmic in buccal mucosa and tongue (three cases each) and mixed in the other three buccal mucosa sites. The higher mean rank was in the buccal mucosa region (14.0, p=0.073). Mucous acini showed cytoplasmic expression in the tongue and nuclear expression in another three buccal mucosa cases. The mean rank was nearly the same in the tongue and buccal mucosa site (12.5, 12.0). Total sample positivity did not show a significant difference in minor salivary glands telomerase expression adjacent to different grades (p=0.144). Nevertheless, telomerase expression of each salivary gland cell type was significantly different in relation to the grade of the adjacent OSCC. The minor salivary gland adjacent to PDSCC had a higher mean rank in all cell types (p<0.05), except for ductal cells (p=0.091). Myoepithelial cell expression was cytoplasmic with WDSCCs and mixed with PDSCCs (three cases each, p=0.000). Ductal cells showed significantly different expressions in different grades. It was cytoplasmic with WDSCCs, nuclear with MDSCCs, and mixed with PDSCCs (three cases each, p=0.000). Serous acini adjacent to PDSCC (mixed, 3 cases) had significantly different expression than WDSCCs and MDSCCs (cytoplasmic, three cases each)

(p=0.005). Mucous acinar cells had a significantly different expression with MDSCCs (cytoplasmic) and PDSCCs (nuclear) (p=0.000).

Correlations between BubR1 and telomerase
Statistically, a significant positive moderate to high linear correlation was found between BubR1 and telomerase expression in total sample positivity and their expression between all cell types of minor salivary glands (r = 0.5-0.8, P<0.05) (Table 4).

Table 3: Telomerase expression in the total 21 studied cases in different cell types of minor salivary gland adjacent to primary OSCC in relation to the location and Bryne's histopathological grades.

Variables		Total			Location				p-value	Bryne's grades			p-value
		Expression	no	%	Tongue	Buccal	Lip	Floor		WD	MD	PD	
Total sample positivity		negative	12	57.1	3	3	3	3	.116	6	6	0	.144
		positive	9	42.9	3	6	0	0		3	3	3	
Myoepithelial cells		negative	15	71.4	6	3	3	3	.098	6	9	0	.000*
		cytoplasmic	3	14.3	0	3	0	0		3	0	0	
		mix	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			8.0	15.0	8.0	8.0	.013§	11. 0	8.0	20.0	.000§
Ductal cells		negative	12	57.1	3	3	3	3	.050*	6	6	0	.000*
		cytoplasmic	3	14.3	0	3	0	0		3	0	0	
		mix	3	14.3	0	3	0	0		0	0	3	
		nuclear	3	14.3	3	0	0	0		0	3	0	
		Kruskal-Wallis test			13.25	12.5	6.5	6.5	.134	9.0	11.0	17.0	.091
Acini	Serous	negative	12	57.1	3	3	3	3	.221	6	6	0	.005*
		cytoplasmic	6	28.6	3	3	0	0		3	3	0	
		mix	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			11.0	14.0	6.5	6.5	.073	9.5	9.5	20.0	.004§
	Mucous	negative	15	71.4	3	6	3	3	.098	9	6	0	.000*
		cytoplasmic	3	14.3	3	0	0	0		0	3	0	
		nuclear	3	14.3	0	3	0	0		0	0	3	
		Kruskal-Wallis test			12.5	12.0	8.0	8.0	.420	8.0	11.0	20.0	.000§

* P-value of Fisher's exact test significant difference p≤.05, § P-value of Kruskal-Wallis test significant difference p≤.05 no; number, WD; well differentiated, MD; moderate differentiated, PD; poorly differentiated, mix; cytoplasmic and nuclear

Table 4: Correlation between BubR1 and telomerase expression in different cell types in minor salivary gland adjacent to primary OSCC.

		r	p- value
Total sample positivity		0.5	.010*
Myoepithelial cells		0.7	.000**
Ductal cells		0.6	.003*
Acini	serous	0.6	.004*
	mucous	0.8	.000**

r; Spearman's rho coefficient of correlation, * p-value significant difference p≤.05, ** p-value highly significant difference p≤.001

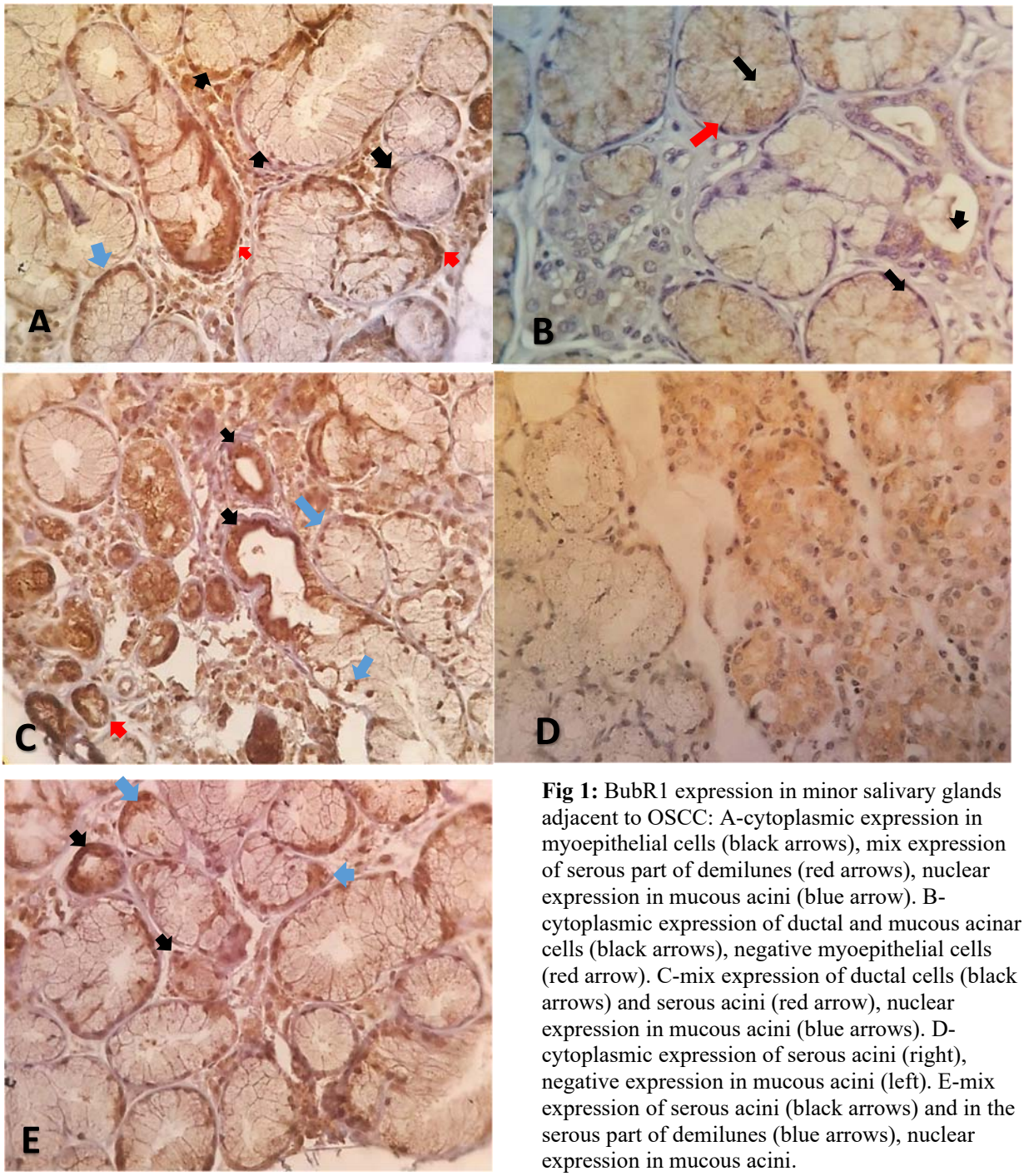

Fig 1: BubR1 expression in minor salivary glands adjacent to OSCC: A-cytoplasmic expression in myoepithelial cells (black arrows), mix expression of serous part of demilunes (red arrows), nuclear expression in mucous acini (blue arrow). B-cytoplasmic expression of ductal and mucous acinar cells (black arrows), negative myoepithelial cells (red arrow). C-mix expression of ductal cells (black arrows) and serous acini (red arrow), nuclear expression in mucous acini (blue arrows). D-cytoplasmic expression of serous acini (right), negative expression in mucous acini (left). E-mix expression of serous acini (black arrows) and in the serous part of demilunes (blue arrows), nuclear expression in mucous acini.

Figure 1: BubR1 expression in minor salivary glands adjacent to OSCC: A-cytoplasmic expression in myoepithelial cells (black arrows), mix expression of serous part of demilunes (red arrows), nuclear expression in mucous acini (blue arrow). B-cytoplasmic expression of ductal and mucous acinar cells (black arrows), negative myoepithelial cells (red arrow). C-mix expression of ductal cells (black arrows) and serous acini (red arrow), nuclear expression in mucous acini (blue arrows). D-cytoplasmic expression of serous acini (right), negative expression in mucous acini (left). E-mix expression of serous acini (black arrows) and in the serous part of demilunes (blue arrows), nuclear expression in mucous acini.

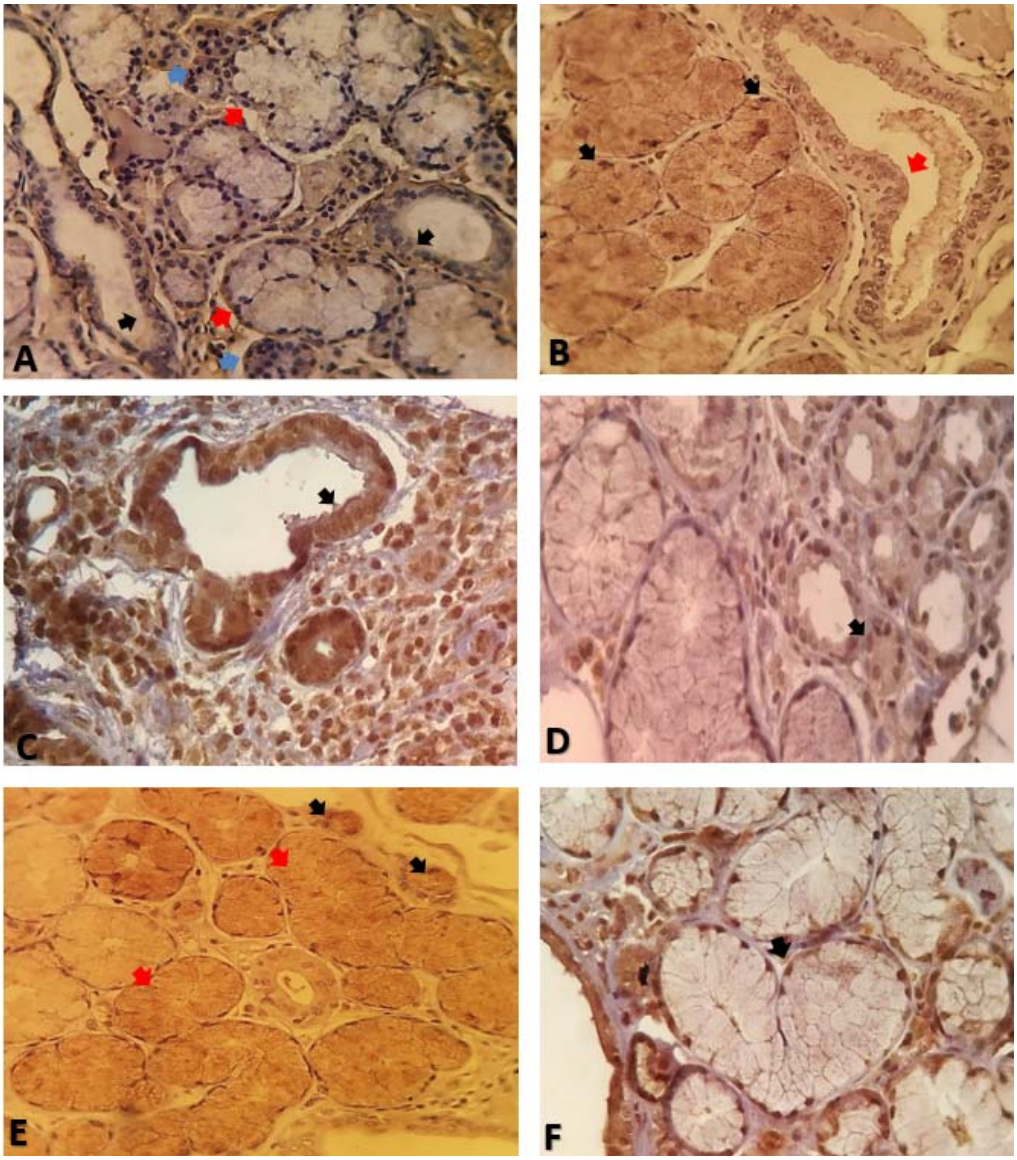

Figure 2: Telomerase expression in minor salivary glands adjacent to OSCC: A-cytoplasmic expression in ducal cells (black arrow), and myoepithelial cells (red arrows), negative expression of serous acini (blue arrow), and mucous acinar cells. B-mixed expression in myoepithelial cells (black arrows), nuclear expression in ductal cells (red arrow). C-mixed expression of ductal cells (black arrow). D-mixed expression of serous acini (black arrow). E-cytoplasmic expression of mucous acini (red arrows), and serous acini cells (black arrows). F-nuclear expression of mucous acini (black arrow).

Discussion

The present study is considered an original study in investigating mitotic checkpoint proteins BubR1 and telomerase in the minor salivary gland next to OSCC. Perilesional area of malignant growth cannot be considered a biologically normal area since molecular changes and early precancerous genetic aberrations were observed in the nearby blood vessels, fibroblast cells, and normal-looking surface epithelium[11-13]. It had shown that within 20 years of diagnosis of primary oral cancer, 30% of males and 20% of female patients might develop a second primary tumor. About 50% of oral cancers develop from precursor lesions, and despite treatment modalities, there is no reduction in the rate of malignant transformation[13].

Few studies documented molecular alteration in the oral epithelium[14-16] and minor salivary glands[10,17] neighboring to OSCC. The present study showed a high predominant cytoplasmic expression of BubR1 in normal-looking minor salivary gland (71.4 % of cases), being more in the ductal and myoepithelial cells than secretory acini. BubR1 is one of the most important

mitotic phosphoproteins, which serves as a sensor for proper attachment between microtubules and the kinetochore. As an executor to delay the onset of the anaphase[18]. It is expressed in various human tissues with a high mitotic index, such as fetal tissues, but not in differentiated tissues. Thus, the hBubR1 gene expression is undetectable in normal tissues[4]; besides, normal oral mucosa did not express BubR1 protein[19]. However, Rizzardi et al. and Burum-Auensen et al. studies showed cytoplasmic BubR1 expression of normal oral epithelial cells in the basal and parabasal layers. Simultaneously, dysplastic epithelium neighboring SCC had more BubR1-positive cells above the parabasal layer[16,20].

Taking into consideration that an elevated BubR1 cytoplasmic expression was previously ranged between 61% -100% of OSCC cases (61%[16], more than 70%[21], 96.7%[19], 100%[4]). As well as an elevated BubR1 was found in the oral potentially malignant epithelial disorders (97.4% at the basal and parabasal layers)[4]. Overexpression in premalignant lesions suggests an early event during step-wise malignant transformation and in head and neck cancers reflects the aggressiveness of these tumors[4]. On the other hand, increased BubR1 cytoplasmic expression was seen in 84% of pleomorphic adenoma and 75% of malignant salivary gland tumors[22]. At the same time, a small percentage (11%) was seen mixed in pleomorphic adenoma in comparison to malignant salivary gland tumors (25%)[22]. At the molecular level, it had been found that the expression levels of BubR1 and Bub3 mRNA were higher in malignant salivary gland tumors than benign salivary gland tumors and normal submandibular gland tissue[23].

Further analysis of BubR1 expression showed a significant relation with the anatomical SCC site. Thus, its expression in minor salivary glands' myoepithelial and ductal cells was high in the buccal mucosa, and it was absent in the lip and floor of the mouth. In the same context, Lira et al. showed high BubR1 expression within OSCC arises in the tongue compared to that in the gums[21]. Also, minor salivary gland tissue adjacent to OSCC showed a significant association with tumor grades; thus, parenchymal tissue adjacent to PDSCCs had a high mean rank and altered expression (mix in ductal and serous acini, nuclear in mucous acini). This indicated that high graded tumors greatly affected the surrounded tissue to show abnormal expression. Since BubR1 expression within OSCC islands was correlated with its higher histological grade[19].

In contrast, another study showed no difference in BubR1 expression in different OSCC grades[4]. Thus, BubR1 overexpression is likely the consequence of an interaction of many regulatory signaling complexes, considered BubR1 as a biomarker for carcinogenesis. The loss of its function may be associated with altered expression in different cell types, which might cause cell cycle progression and high proliferative activity.

Telomere shortening at every replication cycle is postulated to limit the life span of human somatic cells. In contrast, telomerase activation is proposed to be an essential step for cancer cell immortalization[24]. A steady increase in the level of nuclear expression of hTERT was reported in oral carcinogenesis[25]. Telomerase was positive in 30%[26], 35%[27], 74%[28], and 100%[8] of epithelial tissue adjacent to OSCC (normal looking, hyperplastic, and dysplastic). On the other hand, 54.5%[29], 75%[9], 100%[28] of leukoplakia samples, and 100%[28] of submucous fibrosis had telomerase expression. At the same time, 30-33% of oral mucosa from a distant site of OSCC showed telomerase expression[26,30].

Negative expression of telomerase was detected in normal salivary gland tissue[7,29,31,32]. However, 7% of normal-looking salivary glands lobes adjacent to different salivary gland tumors showed positive reactivity for telomerase[32]. Telomerase positive expression in normal looking minor salivary glands nearby OSCC growth presented in 42.9%, mainly in ductal and serous acinus, whereas myoepithelial cells and mucous acini had 71.4 % negative expression. Cytoplasmic positive expression was found in all cells; also, mixed expression was detected in all cells except mucous acini, while nuclear expression was found in ductal cells and mucous acini. Similarly, in salivary gland tumors, Abdulrahman *et al.* reported[31] an abnormal telomerase cytoplasmic localization, while a nuclear localization was illustrated in OSCC[33]. This cytoplasmic expression may indicate non-functional protein production that later on would be translocated to the nucleus.

Minor salivary glands in the lip and floor of the mouth were predominantly telomerase negative. In contrast, all cell types in those within the buccal site, and only acinar and ductal cells in the tongue showed positivity. This was in agreement with Kannan *et al.*, who reported that 71% of buccal mucosal SCC with inadequate treatment response exhibited a high telomerase activity[9]. On the other hand, Patel *et al.* study showed no significant

association between telomerase expression and head and neck cancer location[28].

Telomerase activity could be used as a specific marker for distinguishing malignancy in the salivary gland tumors[31]. The telomerase activity would have been greatly reduced if salivary gland tumor cells had a non-proliferative condition[7]. This study indicates that increasing telomerase expression in certain compartments of minor salivary glands suggests its association with structural changes within different adjacent tissues.

In a similar manner, telomerase expression in the present study had no significant association with the grade of histodifferentiation of adjacent OSCC. However, different salivary gland cells adjacent to OSCC showed a significantly different expression in different OSCC grades. In accordance with previous studies, which showed no such correlation neither between OSCC histopathological grading and telomerase expression[33] nor between its expression in normal adjacent tissue (from buccal mucosa) to that in OSCC islands[24]. But, telomerase in OSCC was related to the histological differentiation, and a lower histological grade of differentiation is associated with higher telomerase expression[9] since grades II and III contained more immortal cells than the more differentiated histological type (Grade I)[34, 35].

It had been suggested that chronic inflammation, long-term exposure to inflammatory cytokines, and progressive epithelial dysplasia lead to telomerase expression; even the degree of telomerase activity in oral cancer cells differed considerably according to tissue origin[36]. Chang *et al.* suggest that weak telomerase activity of oral submucous fibrosis might be due to the contamination of inflammatory cells[37]. Others suggest that the decrease in the thickness of epithelium is associated with reducing the stem cell or transition cell populations of the basal layer, leading to low nuclear expression among the oral submucous fibrosis sample[38]. Taken together, the telomerase activity is linked to cellular proliferation, immortal cell clones involved in field cancerization, which might be migrating from neighboring malignant areas, interfering with cell-mediated immunoreaction.

Finally, there was a direct correlation between the expressions of these two markers. Therefore, they had a dependent role in cellular changes of adjacent minor salivary gland tissue to OSCC. Epithelial malignant cells had more influence on the ductal lining epithelia of minor salivary glands than secretory ones (acini). Since the dysfunction of BubR1 results in chromosomal instability, uncontrolled checkpoint, premature anaphase, and early tumorigenesis[3], and telomerase showed to be activated in proliferating cells[6].

Conclusions

Aberrant expression of BubR1 and telomerase was found in minor salivary glands close to OSCC that cannot ensure proper function. Those glands in the buccal mucosa had a significant BubR1 elevated expression, while telomerase expression increased within myoepithelial cells at the buccal mucosa and in the tongue's ductal cells. Both markers' expression in different compartments of the adjacent minor salivary glands was significantly associated with the dedifferentiation of nearby OSCC. Both markers dependently could predict genotypic alterations in the field of cancerization.

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