Original Article

Immunohistochemical Expression of BubR1 and Telomerase in Oral Squamous Cell Carcinoma

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Abstract

Objective: Aberrant BubR1 and Telomerase expressions are considered as important markers in tumor progression and clinical outcome. This study purposed to describe, compare, and correlate the immunohistochemical expression of BubR1 and Telomerase in oral squamous cell carcinoma (OSCC).

Methods: Immunohistochemical staining was performed for 28 OSCC samples. The samples graded according to Bryne's grading system. The reaction positivity, intracellular localization, and intensity were recorded. The expression distribution and mean-ranks of these markers were related to OSCC grades by Fisher's exact and Kruskal Wallis tests. Finally, the correlation between them was achieved by Spearman's rho test.

Results: BubR1 was detected in 89.3% of OSCCs, equally within scores 2 and 3 (35.7%), presented with 50% faint intensity, and 64.3% cytoplasmic localization. Grade III had a significantly higher mean-rank of BubR1 scoring. Whereas Telomerase observed in 75% of OSCCs, mainly found at scores 2 and 3 (60.7%), and 42.9% was within the cytoplasm. Half of OSCCs had faint intensity. No significant differences reported in the mean-rank and stain intensity among the grades. There was no significant correlation between BubR1 and Telomerase expression.

Conclusions: OSCCs had high cytoplasmic BubR1 and telomerase expression that cannot ensure proper function. Mean-rank of BubR1 expression related to OSCC grading and being more in high grade. It determines the loss of differentiation and aggressiveness of OSCC. While the mean-rank of Telomerase did not relate to histopathological grading and considered an early event in carcinogenesis, it can use for the diagnostic approach of oral carcinogenesis. These markers have an independent role in cancer progression.

Keywords: *BubR1, Telomerase, OSCC, Immunohistochemical.*

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Introduction

Despite improvements in diagnosis and treatment of oral squamous cell carcinoma (OSCC) in the past two decades, the overall survival of these patients remains poor [1]. Inventing new molecular studies, at protein or gene level, can help early detection, identify response to treatment, estimate the rate of regional and distant metastases, and clinical outcomes[1].

BubR1 mitotic-checkpoint protein directs proper attachment of microtubules to kinetochores (a complex of proteins, that during cell division is associated with the centromere of a chromosome, to which the microtubules of the spindles attach) and links regulation of chromosome-spindle attachment to mitotic checkpoint signaling. Thus, disruption of BubR1 activity results in loss of checkpoint control, chromosomal instability caused by premature anaphase, or early onset of tumorigenesis[2]. Lira et al. analyzed a series of OSCCs; and found overexpression of BubR1 associated with shorter survival rate and metastasis[3].

Telomerase is a ribonucleoprotein enzyme complex that maintains the length of telomeres. It is not found in normal somatic cells but is found in 'immortal' cells or cells which divide rapidly, including germ cells, inflammatory cells, and tumor cells[4]. Telomerase contains both RNA and protein components. The protein component, hTRT (human Telomerase Reverse Transcriptase), catalyzes phosphodiester bond synthesis during telomere DNA elongation. Through the extension of the 3' template strand ends, telomere loss on the daughter strand's 5' end no longer results in a net loss[5]. It had been reported that telomerase activity is higher in poorly differentiated SCC, increases with tumor stage, and is associated with lymph node involvement or extracapsular extension of lymph node metastases, reduced response to treatment, ultimately resulting in poor clinical outcomes[4,6]. The aim of the present study was to describe, compare, and correlate the immunohistochemical expressions of BubR1 and Telomerase in OSCC samples.

Patients and methods

A randomly The present study approved by the Committee of Ethics of the Sulaimani Medical College at the University of Sulaimani, Sulaimani, Iraq (3698/29/7, research code 361). It was a cross-sectional retrospective study, involved 28 neutral-buffered formalin-fixed paraffin-embedded blocks previously diagnosed as OSCC, which were collected from histopathological centers in Sulaimani. Informed consent was obtained from histopathological centers before sample collection. The study was conducted in the Histopathological department of College of Dentistry between July and December 2019. Three serial 5µm tissue sections were cut from each block and mounted on positively charged slides. One stained with Hematoxylin and Eosin to perform histopathological grade according to Bryne's invasive front grading system[7] and to demarcate the representative cancerous area for immunohistochemical (IHC) staining.

The other two sections were used for BubR1and Telomerase immunostaining by a biotin free immunoenzymatic antigen detection system (expose mouse/rabbit specific HRP/DAB micro polymer detection IHC kit-ab80436 (ready to use) from **abcam®,** UK). Sections were deparaffinized in xylene (Himedia, India) and rehydrated through a series of ethanol (Scharlau, Spain). Antigens were retrieved by boiling in citrate buffer (ab64236) (pH-6, 15min), and allowed to cool at room temperature, then washed twice with PBS (3 min each). Endogenous peroxidase activity was blocked by hydrogen peroxidase (10 min), and then protein block applied (10 min). Sections were incubated with primary antibodies (Mouse monoclonal to BubR1-ab54894, and rabbit polyclonal anti-Telomerase-ab216625, dilution 1:100, Abcam; UK) for 45 min and then washed four times with PBS. After that, they were incubated with complement (10 min) and washed by PBS (3min). The secondary antibody conjugate was applied for 15 min and washed. Sections were stained by DAB (5 min in the dark) and counterstained with Hematoxylin (20 sec). Then they were dehydrated, cleared, and mounted with DPX to be ready for microscopical examination. The tissues recommended by the manufacturer protocol served as positive controls. In contrast, the negative control for both antibodies included a non-immune serum by omitting the primary antibody and applying antibody diluents alone. The negative and positive control tissue specimens run with each batch of stain.

Two oral pathologists examined the slides. Images of five selected high-spot fields of each case were captured and analyzed by ImageJ software (National Institutes of Health). The count of positive immune-stained cells was performed after applying a grid on the images. The positive reaction was further studied for intracellular localization (nuclear, cytoplasmic, and mixed). BubR1 expression evaluated as follow: score 0 (negative), score 1 (<10%), score 2 (10-50%), score 3 (51-80%), and score 4 (>80%)[8]. The percentage of telomerase expression graded into: score 0: (0-10%), score 1: focal positivity 11-25%, score 2: regional positivity 26-75%, and score 3: diffuse positivity 75-100% of immunoreactive cells[9]. The intensity of the stain of both markers was assessed subjectively into mild,

moderate, and strong when compared with positive control slides[8,9].

Statistical analysis

SPSS 24.0 software for Windows was used to analyze the data. The descriptive analysis, Fisher's exact test, Kruskal Wallis-test, and Spearman's rho test used to analyze, compare, and correlate the data, respectively. For all tests, P≤0.05 was deemed to be significant.

Results

The paraffin blocks related to 16 males and 12 females; their ages ranged from 25 to 85 years with a median of 56.5 years. OSCCs predominantly were in the tongue (39.3%), clinically presented as ulcerated lesions (64.3%), and well-differentiated cases (46.4%) details were presented in Table 1.

BubR1 positive labeling in the total OSCC samples was detected in 25 cases (89.3%), had equal frequency in score 2 and 3 (10 samples each out of 28, 35.7%) with 50% faint intensity (Table 2), and predominant cytoplasmic localization (64.3 %) (Figure 1, 2-A). Nuclear expression was found in 21.4%, while mixed expression (cytoplasmic and nuclear) was only in 3.6% (Figure 1, 2-B, C).

Meanwhile, grade III had a significantly higher mean rank of BubR1 scoring (23.75, p=0.014, Table 2). There Grade II OSCCs dominated by Score 2 (7 cases out of 11, 63.63%), while score 3 was most frequently seen in grade I (6 samples out 13, 46.2%) (Table 2). were no significant differences in stain intensity among the histopathological grades (p=0.56, Table 2). Telomerase showed positive staining in 21 cases (75%). The majority of the sample was scored in scores 2 and 3 (60.7%). Cytoplasmic expression was mainly detected in 42.9% (Figure 1, Figure 2-D). While mixed expression (cytoplasmic and nuclear) was only illustrated in 10.7% (Figure 2-E, F). Half of the positive cases had faint staining intensity (50%). The mean rank of grade I (14.77) was slightly more than grade II (11.18) and near equal to that of grade III (14.5) with no significant relation (p=0.98, Table 2). Again, no significant difference was noticed among telomerase staining intensity and histopathological categories (p=0.97, Table 2).

Spearman's rho statistical test revealed a non-significant low direct correlation between the BubR1 and Telomerase scoring (r=+0.3, P=0.08). Besides, a non-significant, very low direct correlation of the two markers stain intensity was detected (r=+0.2, p=0.16).

Table 1: Study sample clinical data summary.

Variables		No.	Percentage
Gender	Male	16	57.1
	Female	12	42.9
Age	Range	25-85 years	
	Median	56.5 years	
Location	Tongue	11	39.3
	Buccal mucosa	4	14.3
	Lip	4	14.3
	Gum	4	14.3
	Palate	3	10.7
	Floor of mouth	2	7.1
Clinical presentation	Mass	4	14.3
	Ulcer	18	64.3
	White lesion	4	14.3
	Ulcerated mass	2	7.1
Bryne's grade	I	13	46.4
	II	11	39.3
	III	4	14.3

Table 2: BubR1 and Telomerase expression in OSCC different histological grades.

Parameter	Subgroups	Total		Bryne's grade			p value
		No.	%	I	II	III	
BubR1 score (25 case positive)	Score 0	3	10.7	2	1	0	0.09*
	Score 1	2	7.1	1	1	0	
	Score 2	10	35.7	3	7	0	
	Score 3	10	35.7	6	2	2	
	Score 4	3	10.7	1	0	2	
	§ Mean ranks			14.62	11	23.75	0.014*
BubR1 intensity	Negative	3	10.7	13.46	14.36	18.25	0.56§
	Faint	14	50				
	Moderate	6	21.4				
	Strong	5	17.9				
Telomerase score (21 case positive)	Score 0	7	25	3	3	1	0.93*
	Score 1	4	14.3	2	1	1	
	Score 2	13	46.4	6	6	1	
	Score 3	4	14.3	2	1	1	
	§ Mean ranks			14.77	11.18	14.5	0.98§
Telomerase intensity	Negative	7	25	14.38	14.68	14.38	0.97§
	Faint	14	50				
	Moderate	6	21.4				
	Strong	1	3.6				

* p-value significant difference; $p < 0.05$, § Kruskal Wallis test, * Fisher's exact test.

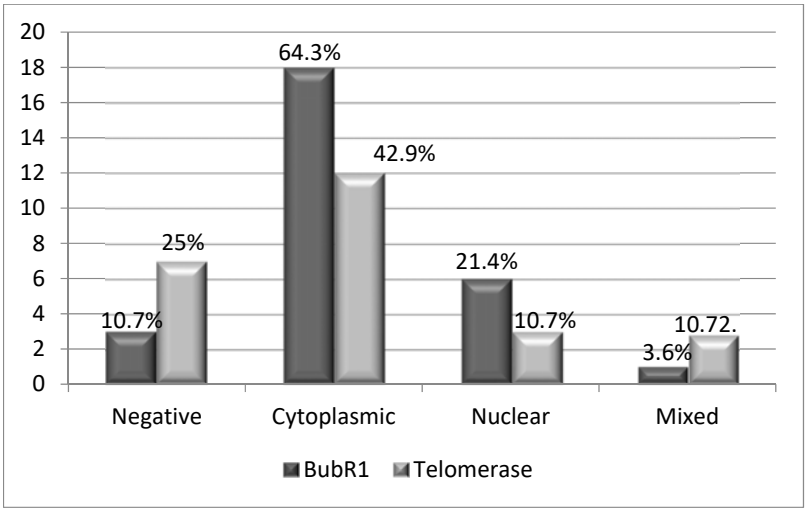

Figure 1: Frequency and percentage distribution of intracellular localization of BubR1 and Telomerase expression in OSCC.

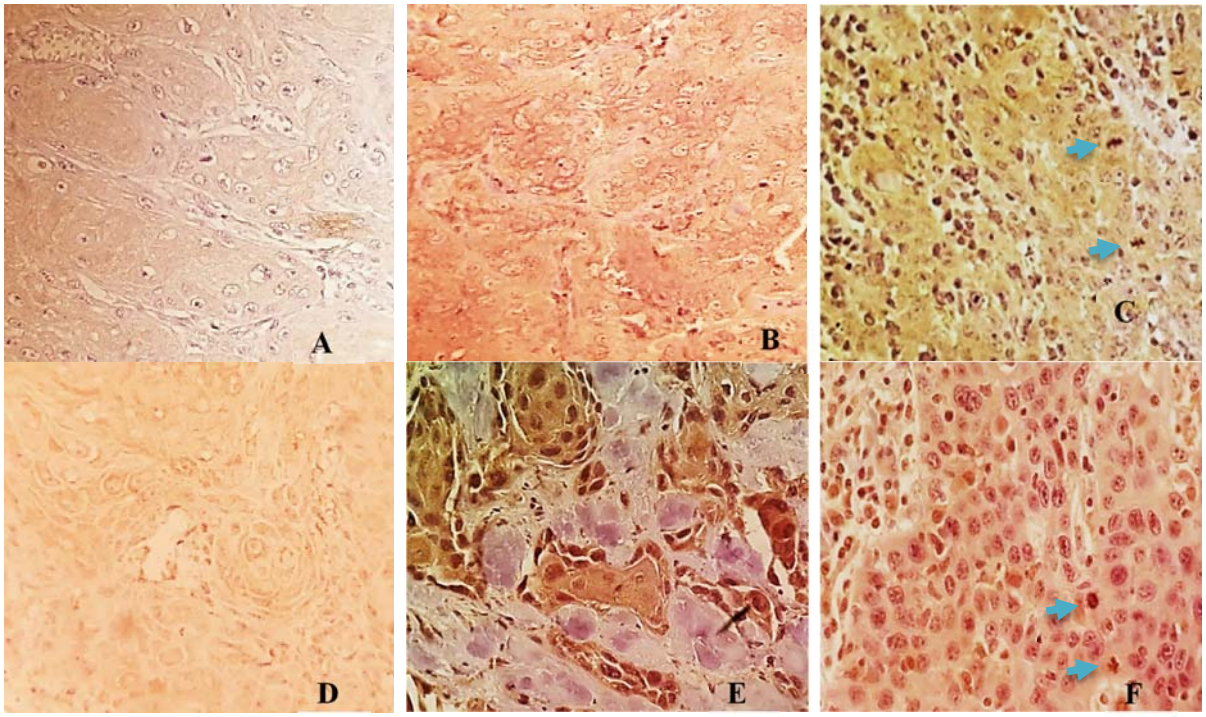

Figure 2: BubR1 and Telomerase immunohistochemical expression in OSCC (X 400): (A): BubR1 faint cytoplasmic expression, score 2. (B): BubR1 moderate cytoplasmic and nuclear expression, score 3. (C): BubR1 positive, strong expression in the mitotic cell (arrows). (D): Telomerase faint cytoplasmic expression, score 3. (E): Telomerase strong cytoplasmic and nuclear expression, score 2. (F): Telomerase positive, strong expression in abnormal mitotic cells (arrows).

Discussion

The clinical features registered in this sample are similar to Iraqi studies[10,11] and others[12] as they revealed male predominance and ulceration as the most common clinical presentation in their study. However, Jainkittivong *et al.,* in their research, found an equal male to female ratio, the alveolar ridge and gingiva being the frequently involved site, and the majority of the OSCC cases existed as ulcers or masses[13].

The spindle assembly checkpoint (SAC) is a highly conserved system and plays an essential role during mitosis to ensure accurate chromosome distribution between the two dividing cells[14]. Proper chromosome segregation during mitosis is achieved by this signaling pathway that inhibits anaphase onset until all chromosomes are aligned at the metaphase equator[15]. The high BubR1 immune labeling detected in this sample is similar to two studies in a deal with invasive OSCC[3,16], while Rizzardi et al., in their research, reported low BubR1 expression (22.4%)[17]. Further identification for the expression within cellular compartments, we found BubR1 mainly expressed in the cytoplasm, besides, a possibility to see a low percentage of the antigens in the nucleus. Lira *et al*. showed only cytoplasmic BubR1 expression in their OSCC samples[3]. It seems that BubR1 overexpression related to OSCC grading and being more in high grade. Thus, BubR1 activity results in a loss of checkpoint control with chromosomal instability and premature anaphase that remarkably observed with loss of differentiation and could predict the aggressiveness and recurrence of OSCC. A similar finding with a statistically significant difference was reported by Sravya et al.[18]. On the contrary, Rizzardi et al. stated that BubR1 overexpression is associated with less advanced pathologic stage and had more extended survival periods[17].

The acquisition of unlimited proliferative potential and immortalization of cells is essential for the development of cancer. Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA fragments de novo, using its RNA template, and compensates for the loss of telomere during the cell division[19]. This study showed Telomerase positive expression in 75% of the sample. A similar finding was found by Carkic *et al.* study as they detected Telomerase expression in 74% of their OSCC sample[20], while Raghunandan *et al.*

demonstrated 100% positive Telomerase expression[21]. Lower expression was observed by Miyazaki *et al*. studied sample (9/15, 60.0%)[22]. This study revealed abnormal cytoplasmic localization of Telomerase in 42.9% of the sample, similarly significant cytoplasmic expression (19 out of 25, 76%) reported by Carkic *et al.*[20]. While high nuclear localization was illustrated by Raghunandan *et al*., which was 65%[21], the cytoplasmic expression of Telomerase in this study may indicate non-functional protein production that later on would be translocated to the nucleus. The mean of Telomerase scoring did not relate to the increase in the histopathological grade of OSCC. This result supports the finding of other studies that indicated that high Telomerase expression is an early event in oral carcinogenesis[23-25]. While Raghunandan et al., in their research demarcated, a gradual increase in mean of expressions through the grades of differentiation of OSCC from 74.01 for low-grade OSCC to 86.18 in high-grade OSCC[21]. This difference could be attributed to variant evaluation manner and different sample sizes.

Finally, there is no correlation between the expressions of these two markers. Therefore, each of them has its independent role in cancer progression, as telomerase overexpression started in the early stage of cancer, which can affect a diagnostic approach of oral carcinogenesis. While BubR1 was highly expressed in high-grade OSCC, so it could determine the loss of differentiation and aggressiveness and prognosis of OSCC.

Conclusions

BubR1 and Telomerase work independently in the progression process of oral carcinogenesis, as the Telomerase mean rank increased early in low-grade OSCC, which can facilitate early identification. On the other hand, BubR1 showed high expression in high-grade OSCC, which might be associated with aggressiveness and invasion ability of OSCC.

References

1. Mao L, El-Naggar AK, Fan YH, Lee JS, Lippman SM, Kayser S. Telomerase activity in head and neck squamous cell carcinoma and adjacent tissues. Cancer Res. 1996;56(24):5600-4.
2. Park HY, Jeon YK, Shin HJ, Kim IJ, Kang HC, Jeong SJ, et al. Differential promoter methylation may be a key molecular mechanism in regulating BubR1 expression in cancer cells. Exp Mol Med. 2007;39(2):195-204.
3. Lira RC, Miranda FA, Guimarães MC, Simões RT, Donadi EA, Soares CP et al. BUBR1 expression in benign oral lesions and squamous cell carcinomas: correlation with human papillomavirus. Oncol Rep. 2010;23(4):1027-36.
4. Boscolo-Rizzo P, Da Mosto MC, Rampazzo E, Giunco S, Del Mistro A, Menegaldo A et al. Telomeres and telomerase in head and neck squamous cell carcinoma: from pathogenesis to clinical implication. Cancer Metastasis Rev. 2016;35(3):457-74.
5. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of Telomerase. Science. 1997;276(5312):561-7.
6. Raju KL, Haragannavar VC, Patil S, Rao RS, Nagaraj T, Augustine D et al. Expression of hTERT in oral submucous fibrosis and oral squamous cell carcinoma- an immunohistochemical analysis. Pathol Oncol Res. 2019:1-10.
7. Rastogi V, Puri N, Mishra S, Sharma R, Yadav L, Sabharwal R. Dilemmas in grading epidermoid carcinoma. Int J Head Neck Sur. 2014;5(1):9-14.
8. Maciejczyk A, Szelachowska J, Czapiga B, Matkowski R, Hałoń A, Györffy B, et al. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem Cytochem. 2013;61(5):330-9.
9. Maraei AA, Hatta AZ, Shiran MS, Tan GC. Human telomerase reverse transcriptase expression in ovarian tumors. Indian J Pathol Microbiol. 2012;55(2):187-91.
10. Al Talabani NG, Gaphor SM, Abdul Wahab RH. Serum and salivary levels of proinflammatory cytokines as potential biomarkers in the diagnosis of oral squamous cell carcinoma. J Baghdad Coll Dent. 2013;21(1):60-5.
11. Sarkis SA, Abdullah BH, Abdul Majeed BA, Talabani NG. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in oral squamous cell carcinoma in relation to proliferation, apoptosis, angiogenesis and lymphangiogenesis. Head Neck Oncol. 2010;2:13-21.
12. Alves AM, Correa MB, Silva KDD, Araújo LMA, Vasconcelos ACU, Gomes APN et al. Demographic and clinical profile of oral squamous cell carcinoma from a service-based population. Braz Dent J. 2017;28(3):301-6.
13. Jainkittivong A, Swasdison S, Thangpisityotin M, Langlais RP. Oral squamous cell carcinoma: a clinicopathological study of 342 Thai cases. J Contemp Dent Pract. 2009;10(5):E033-40.

14. Silva P, Barbosa J, Nascimento AV, Faria J, Reis R, Bousbaa H. Monitoring the fidelity of mitotic chromosome segregation by the spindle assembly checkpoint. Cell Prolif. 2011;44(5):391-400.
15. Holland AJ, Cleveland DW. Boveri revisited: chromosomal instability, aneuploidy, and tumorigenesis. Nat Rev Mol Cell Biol. 2009;10(7):478-87.
16. Hsieh PC, Chen YK, Tsai KB, Shieh TY, Chang YY, Chang JG et al. Expression of BUBR1 in human oral potentially malignant disorders and squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(2):257-67.
17. Rizzardi C, Torelli L, Barresi E, Schneider M, Canzonieri V, Biasotto M et al. BUBR1 expression in oral squamous cell carcinoma and its relationship to tumor stage and survival. Head Neck. 2011;33(5):727-33.
18. Sravya T, Sivaranjani Y, Rao GV. Immunohistochemical expression of budding uninhibited by benzimidazole related 1 in leukoplakia and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2016;20(1):71-7.
19. Rai A, Naikmasur VG, Sattur A. Quantification of telomerase activity in normal oral mucosal tissue and oral squamous cell carcinoma. Indian J Med Paediatr Oncol. 2016;37(3):183-8.
20. Carkic J, Nikolic N, Radojevic-Skodric S, Kuzmanovic-Pficer J, Brajovic G, Antunovic M et al. The role of tertclptm1l SNPs, hTERT expression and telomere length in the pathogenesis of oral squamous cell carcinoma. J Oral Sci. 2016;58(4):449-58.
21. Raghunandan BN, Sanjai K, Kumaraswamy J, Papaiah L, Pandey B, Jyothi BM (2016) Expression of human telomerase reverse transcriptase protein in oral epithelial dysplasia and oral squamous cell carcinoma: An immunohistochemical study. J Oral Maxillofac Pathol. 2016;20(1):96-101.
22. Miyazaki Y, Yoshida N, Nozaki T, Inoue H, Kikuchi K, Kusama K. Telomerase activity in the occurrence and progression of oral squamous cell carcinoma. J Oral Sci. 2015;57(4):295-303.
23. Kannan S, Tahara H, Yokozaki H, Mat hew B, Nalinakumari KR, Nair MK et al. Telomerase activity in premalignant and malignant lesions of human oral mucosa. Cancer epidemiol biomarkers prev. 1997;6(6):413-20.
24. Sumida T, Sogawa K, Hamakawa H, Sugita A, Tanioka H, Ueda N. Detection of telomerase activity in oral lesions. J Oral Pathol Med. 1998;27(3):111-5.
25. Palani J, Lakshminarayanan V, Kannan R. Immunohistochemical detection of human telomerase reverse transcriptase in oral cancer and pre-cancer. Indian J Dent Res. 2011;22(2):362-3.