Predictivity ofHuman Telomerase Reverse Transcriptase(h-TERT) inOral Squamous Cell Carcinoma:Immunohistochemical Study.

ABSTRACT

Aims: Determine the potential role of h-TERT as a prognostic marker for oral squamous cell carcinoma by evaluating its immunohistochemical expression in different OSCC histologic grades in comparison with normal oral mucosa.

Study design: A retrospective study.

Place and duration: Oncology Center, Mansoura University, and Oral Pathology Department, Faculty of Dentistry, Mansoura University. The worked sample selected from the years (2015-2019).

Methodology: Our study was accomplished on 30 OSCC cases and a control group of normal oral mucosa. Histopathologic grading of OSCCs was made using the WHO grade and Anneroth`s grading systems. Immunohistochemical expression of h-TERT was assessed in relation to different clinicopathological parameters. The data was analyzed using Pearson`s chi-square test to compare the differences between groups. Spearman's correlation co-efficiency test was used to test the association between different variables. A P value ≤ 0.05 was considered to be statistically significant.

Results: A significant increased h-TERT expression was recorded from normal to OSCC groups (P=0.000). Additionally, elevated h-TERT expression was significantly correlated with the higher carcinoma histologic grade (P =0.025). There were high statistically significant differences in h-TERT expression concerning the parameters; Anneroth pattern of invasion (P= 0.049), depth of invasion (DOI, P=0.024), and lymphoplasmacytic infiltration (LPI, P=0.009).There were no statistically significant differences in h-TERT immunoexpression considering the clinical parameters; patient age, gender, anatomical sites, clinical shape of lesion, T, N, M, and TNM stages (Chi-square test, P values were > 0.05).

Conclusions: Increased h-TERT immunoexpression from normal to OSCC groups suggests its involvement in malignant transformation.Additionally, the significant differential expression of h-TERT among different OSCC histologic grades signaling its valuable use as a biomarker for assessing the cellular malignant progressionof oral carcinomas, but unreliable as a clinical tumor progression marker.Moreover,the significant correlation of h-TERT expression with LPI, DOI and pattern of invasion indicating its possible reliable role in tumor host relation during multistage carcinogenesis.

Keywords:*Oral squamous cell carcinoma, human Telomerase Reverse Transcriptase, immunohistochemistry, predictive marker.*

1. INTRODUCTION

More than 90% of Oral Cancer (OC) caseshave the diagnosis of OSCC [1]. The tongue and the mouth floor are the commonly involved sites than others, despite OC can happen anywhere in oral cavity[2, 3].In 2020, there were 377,713 new incident cases of OC worldwide, accounting for 2% of all cancers and resulting in 177,757 deaths, according to global cancer statistics compiled by GLOBOCAN [4]. Ages above 40 are more likely to experience OC. Males are more likely than females to have OC globally, with 5.8 versus 2.3 cases per 100,000 people [5].

At the ends of human chromosomes are hexameric DNA repeats called telomeres, which are linked to both genome replication and protection [6]. Additionally, telomeres function as a biological clock that guides a cell towards replicative senescence or apoptosis after DNA division is exceeded [7]. The components of human telomerase include the reverse transcriptase-active TERT (h-TERT) catalytic subunit, the telomerase RNA template (TERC) having a sequence complementary to the telomere sequence, and auxiliary proteins (localization factor) [8]. In order to prevent telomere erosion, this h-TERT can reduce telomerase activity [9]. Remarkably, telomerase's non-telomeric effects have also risen in popularity, with the primary focus being on its anti-apoptotic and anti-oxidant properties [9].While h-TERT expression in several cancer types is well-documented [10–13], its role in oral cancer is less well understood. In order to shed light on h-TERT's possible involvement in oral cancer, the current study compared the expression of h-TERT in various histologic grades of OSCC to that of normal oral mucosa.

2. MATERIAL AND METHODS

2.1. Patients selection and data retrieval

The present retrospective study was carried out on thirty paraffin-embedded blocks of formalin-fixed tissue of OSCC. The studied cases were collected from the archives of Oncology Center, Mansoura University at the duration (2015-2019). Five paraffin-embedded blocks of a formalin-fixed tissue of normal oral mucosa were obtained during the extraction of impacted third molar teeth and surgical crown lengthening from the outpatient clinic of the Faculty of Dentistry, Mansoura University. These cases were considered as a control group.All cases in our study were primary tumors that had undergone surgical removal. None of the worked cases had received chemotherapy or radiotherapy prior to their surgeries. Cases with tiny tissue biopsies were excluded from consideration.

The current study was approved by the research ethics committee of Faculty of Dentistry, Mansoura University. Informed written consent to participate in the study was obtained from each individual (Code Number: A10060421). The Sample size calculation was performed using G*Power version 3.1.9.2[14], University Kiel, Germany. Copyright (c) 1992-2014. The effect size f was 0.69 using alpha (α) level of 0.05 and Beta (β) level of 0.05, i.e., power = 80%; the estimated sample size (n) should be at least 30 samples for this study.

2.2. Immunostaining technique:

Four-microns thick sections were cut from each paraffin blocks for hematoxylin and eosin staining and h-TERT immunostaining according to the manufacturer instructions. The slides were immersed in xylene for 15 minutes, and then hydrated in graded alcohol series. The endogenous peroxidase activity was blocked by incubating the slides with 3% hydrogen peroxide in methanol for 30 minutes. Heat induced epitope retrieval was done by boiling the sections in citrate buffer solution for 10 minutes. Then the sections were cooled for 20 minutes. The primary antibody (h-TERT; ABclonal company, cat#. A16625, USA) was applied in the optimized dilution (1:100). The slides were incubated with the primary antibody for one hour at room temperature followed by incubation with secondary antibody for twenty minutes in room temperature, and then were washed in PSB three times for three minutes. The 3, 3 diaminobenzidine (DAB) was applied as a chromogen for antibody detection. The DAB chromogen yielded a brown reaction end product at the site of the target antigen. Sections were counterstained with Mayer's hematoxylin and covered with glass slip. For the negative control, the primary antibody was eliminated and replaced with PBS.

2.3. Evaluation and scoring of h-TERT immunohistochemical expression

Evaluation of the immunohistochemical (IHC) reaction was achieved by digital image analysis computer system to assess the percentage area of positivity and the intensity of the immune staining using the following steps: The slides were photographed using an Olympus® digital camera installed on an Olympus® microscope with a 1/2 X photo adaptor, using a 20X objective.In order to assess and measure the staining intensity as well as the percentage of positively reacting cells, the target stained area, which was designated as a ROI (region of interest), was represented by five high-power fields [15]. The final image was examined using Fiji ImageJ (version 1.51r; NIH, Maryland, USA) software on an Intel® core I7®-based computer in order to determine the staining intensity and percentage area. The modified Histoscore (H-score) was used to score the area percentage and immunostaining intensity data from the image analyzer.There are 300 points available on this scoring system (0-300). The number indicating the IHC stain intensity (rated as 0, non-staining; 1, weak; 2, medium; or 3, strong using nearby normal mucosa as the median) is multiplied by the total value of each percentage of positive cells (0-100)[16]. The tissue segment was rated as follows: 0–50 was deemed negative, 50–100 as weak, 101–200 as moderate, and 201-300 as strong after the final score.

2.4. Statistical analysis and data interpretation:

The data were displayed as the mean± standard deviation (SD) of every section that was studied, with results that were similar. The chi-square test by Pearson was employed to assess how the groups'

qualitative data varied from one another. To evaluate the relationship between the various variables, Spearman's correlation co-efficiency test was employed. A P-value of less than 0.05 was deemed statistically significant. The statistical package for social science (Statistical Package for Social Science, Armonk, NY: IBM Corp.) version 22 computer program was used for all statistical analysis.

3. RESULTS

3.1. Histological findings:

The current study included two groups; Cancerous tissue group and the normal oral mucosa group (control group).OSCC cases were microscopically graded according to the WHO(2017)classification, and Anneroth`s classification system[17].

According to the traditional histopathological grading system of OSCC (WHO, 2017), tumors were classified into well, moderately, and poorly differentiated carcinoma depending on the degree of epithelial cell differentiation. The well-differentiated tumors were the most encountered grade (43.3%), followed by moderately differentiated and then poorly differentiated carcinoma (30%) and (26.7%), respectively (Figs. 1,2,3).

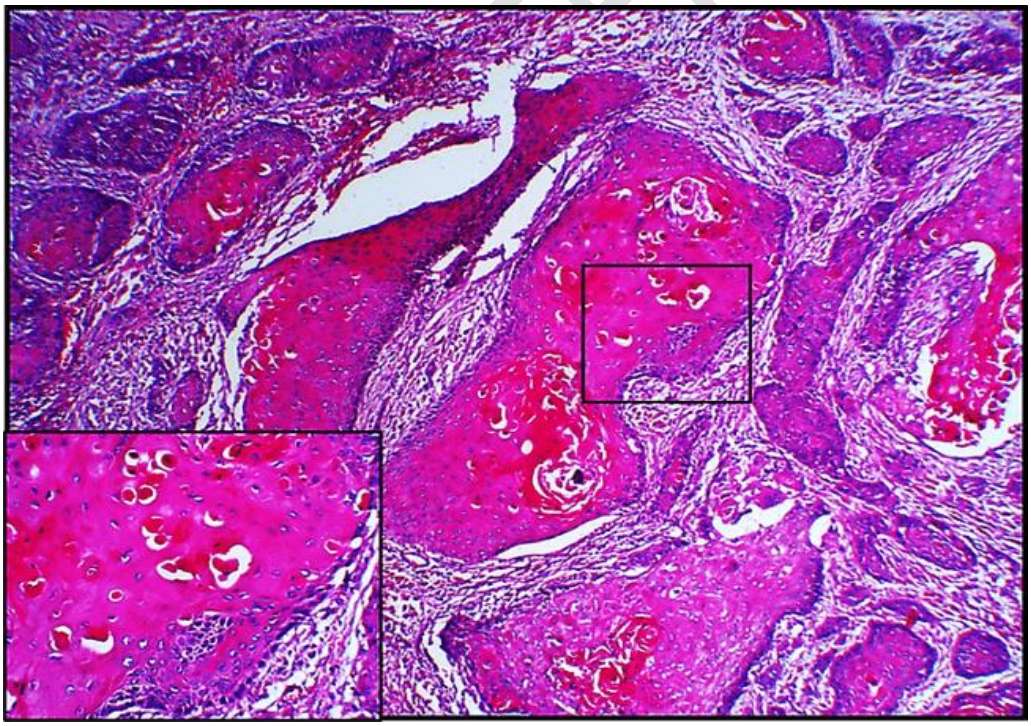

Figure 1: Photomicrograph of well-differentiated OSCC demonstrates cell nests with large keratin production.Scanty stroma with slight lymphoplasmacytic infiltration (LPI) and little small-sized blood vessels;Anneroth keratinization score 1, patterns of invasion score 2 of infiltrating solid bands, and score 1 pleomorphism (minimal pleomorphism) (H&E, x100).

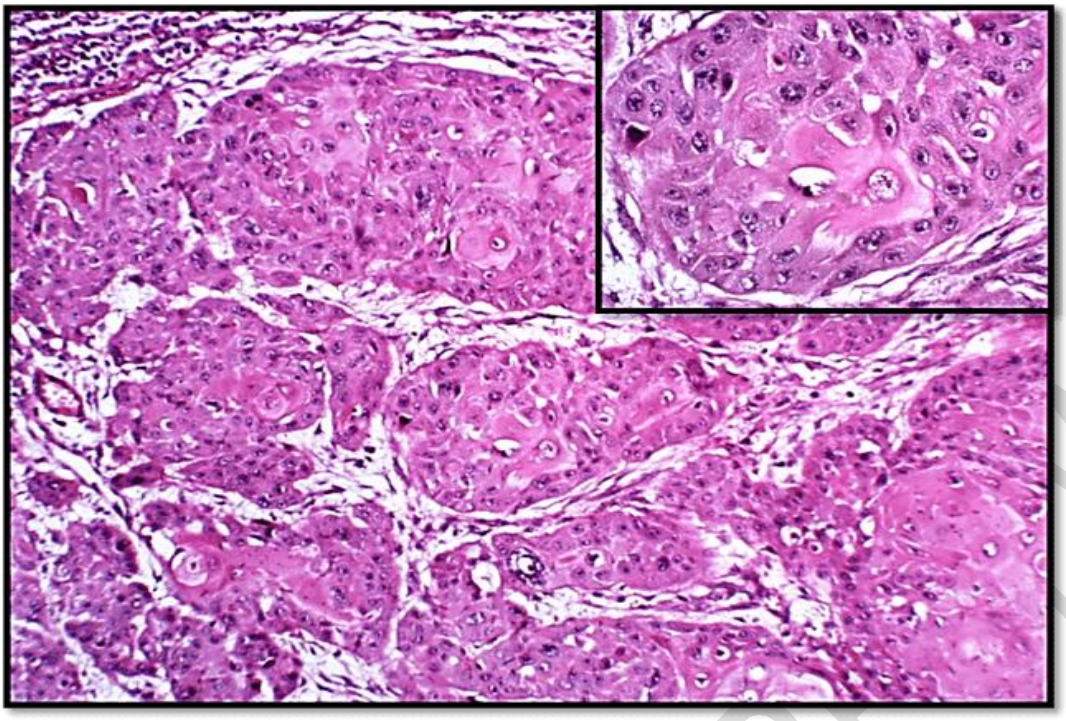

Figure 2: Photomicrograph of moderately differentiated OSCC demonstrates scanty stroma with decreased lymphoplasmacytic infiltration and blood vessels (H&E, X200). Inset showing tumor cells with nuclear and cellular pleomorphism, prominent nucleoli, and increased mitosis; Anneroth nuclear pleomorphism score 3, and mitosis score 2, (H&E, X400).

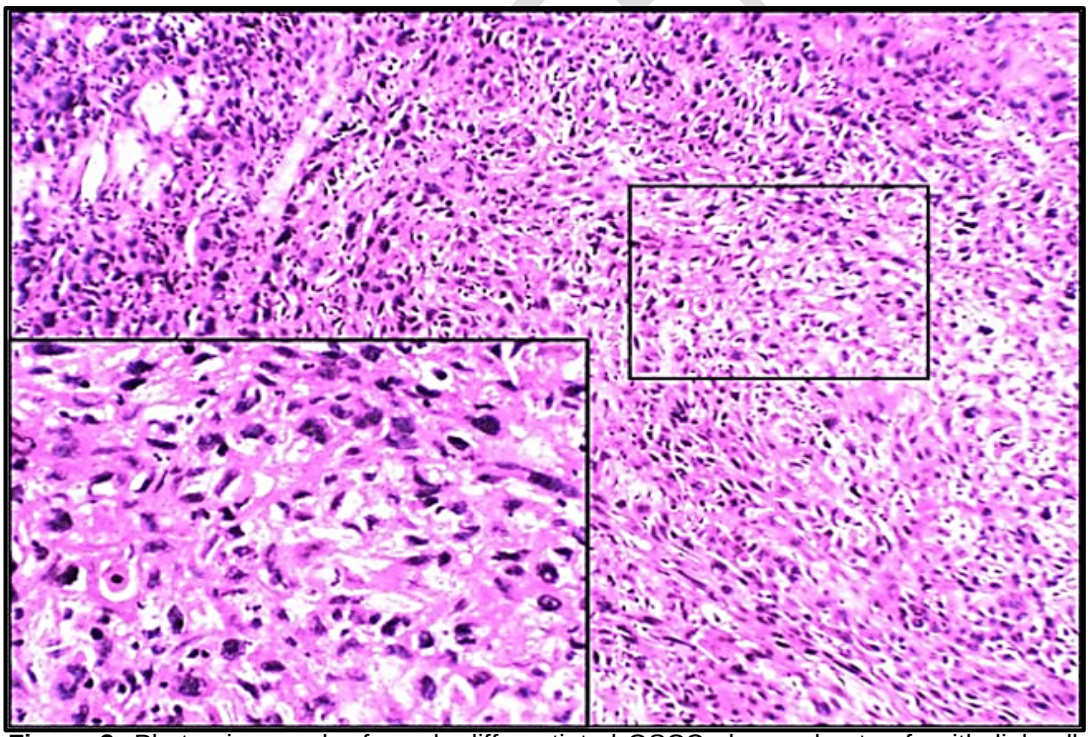

Figure 3: Photomicrograph of poorly differentiated OSCC shows sheets of epithelial cells with nuclear and cellular pleomorphism, and hyperchromatism. Stroma was mainly scanty with few blood vessels (H&E, X100). Higher magnification (inset) reveals, Anneroth`s nuclear pleomorphism score 4, mitosis (score 3), and none of LPI (score 4) (H&E, X400).

3.2. h-TERT immunoexpression concerning different clinical parameters

Through the microscopic examination, a positive reaction to h-TERT antibody was detected as brown staining in the nucleus and cytoplasm of epithelial cells. Meanwhile, the surrounding stromal tissue showed negative reaction except lymphocytes that mostly revealed an intense positivity.

There were no statistically significant differences in h-TERT immunoexpression concerning the different clinical parameters; patients age groups (P=0.28), gender (P=0.19), anatomical site (P=0.85), clinical shape of lesion (P=0.44), Tumor size, Nodal stage, Metastasis incidence, and TNM stage (Pearson`s Chi-square test, P values were > 0.05, **Table 1,2**).

Table 1: The expression of h-TERT in relation to different clinicopathological parameters.

Clinical parameters		**H- score for h-TERT**						**Total no.**	**P value**
		Weak		**Moderate**		**Strong**			
		no.	%	no.	%	no.	%		
Age	< 50years	0	0%	7	87.5%	1	12.5%	8	0.28
	50 -70 years	3	20%	8	53.3%	4	26.7%	15	
	≥ 70 years	2	28.6%	5	71.4%	0	0%	7	
Gender	Male	3	18.8%	12	75%	1	6.2%	16	0.19
	Female	2	14.3%	8	57.1%	4	28.6%	14	
Site	Tongue	3	20%	9	60%	3	20%	15	0.85
	Lip	1	20%	4	80%	0	0.0%	5	
	Cheek	1	16.7%	4	66.7%	1	16.7%	6	
	Alveolar mucosa	0	0.0%	2	100%	0	0.0%	2	
	Palate	0	0.0%	1	50%	1	50%	2	
Shape of lesion	Ulcerative	5	25%	12	60%	3	15%	20	0.44
	exophytic	0	0.0%	8	80%	2	20%	10	

*Chi-square test *Significant difference, (p values ≤ 0.05)*

Table 2: The expression of h-TERT concerning the clinical staging parameters.

Clinical parameters	**H- score for h-TERT**						**Total**	**P value**
	Weak		**Moderate**		**Strong**			
	no.	%	no.	%	no.	%		

Tumor size (T)	T1	1	16.7%	4	66.7%	1	16.7%	6	0.638
	T2	2	22.2%	6	66.7%	1	11.1%	9	
	T3	2	18.2%	8	72.7%	1	9.1%	11	
	T4a	0	0.0%	2	50%	2	50%	4	
Nodal stage (N)	N0	5	21.7%	14	60.9%	4	17.4%	23	0.481
	N1	0	0.0%	2	66.7%	1	33.3%	3	
	N2	0	0.0%	4	100%	0	0.0%	4	
Metastasis incidence (M)	M0	5	17.2%	19	65.5%	5	17.2%	29	0.772
	M1	0	0.0%	1	100%	0	0.0%	1	
TNM stage	I	1	25%	2	50%	1	25%	4	0.796
	II	2	25%	5	62.5%	1	12.5%	8	
	III	2	22.2%	6	66.7%	1	11.1%	9	
	IVa	0	0.0%	6	75%	2	25%	8	
	IVc	0	0.0%	1	100%	0	0.0%	1	

*Chi-square test *Significant difference, (p values ≤ 0.05)*

3.3. The expression of h-TERT concerning different pathological parameters

h-TERT revealed negative and weak expression in normal oral mucosa group. Regarding cancerous tissue, all the studied grades of OSCC showed positive staining for h-TERT protein with different expression scores. Moreover, the distribution of the h-TERT staining was typically heterogeneous among neoplastic cells with different expression intensities between tumor cells.

3.3.1.The expression of h-TERT in OSCCs concerning the WHO histologic grade

The greater percentage of the studied OSCCs revealed moderate h-TERT expression (66.6%), while strong h-TERT expression was observed only in 16.7% of all OSCC grades. Moreover, there was a statistically significant difference in h-TERT immunoexpression among the different OSCC grades (**Table.3, Figs. 4,5,6**). Weak h-TERT expression noted only among well-differentiated OSCCs, while moderately and poorly differentiated carcinomas reported increased levels of h-TERT expression (P =0.025, Table.3).

Table 3: The expression of h-TERT expression in OSCCs in relation to the WHO histologic grade

WHO grade of OSCC	**H-score for h-TERT**			Total	***P value***
	Weak	**Moderate**	**Strong**		

	No	%	No	%	No	%		
Well-differentiated	5	38.5%	8	61.5%	0	0.0%	13(100%)	0.025*
Moderately differentiated	0	0.0%	7	77.8%	2	22.2%	9(100%)	
Poorly differentiated	0	0.0%	5	62.5%	3	37.5%	8(100%)	
Total	5	16.7%	20	66.6%	5	16.7%	30(100%)	

*Chi-square test *Significant difference, (p values ≤ 0.05)*

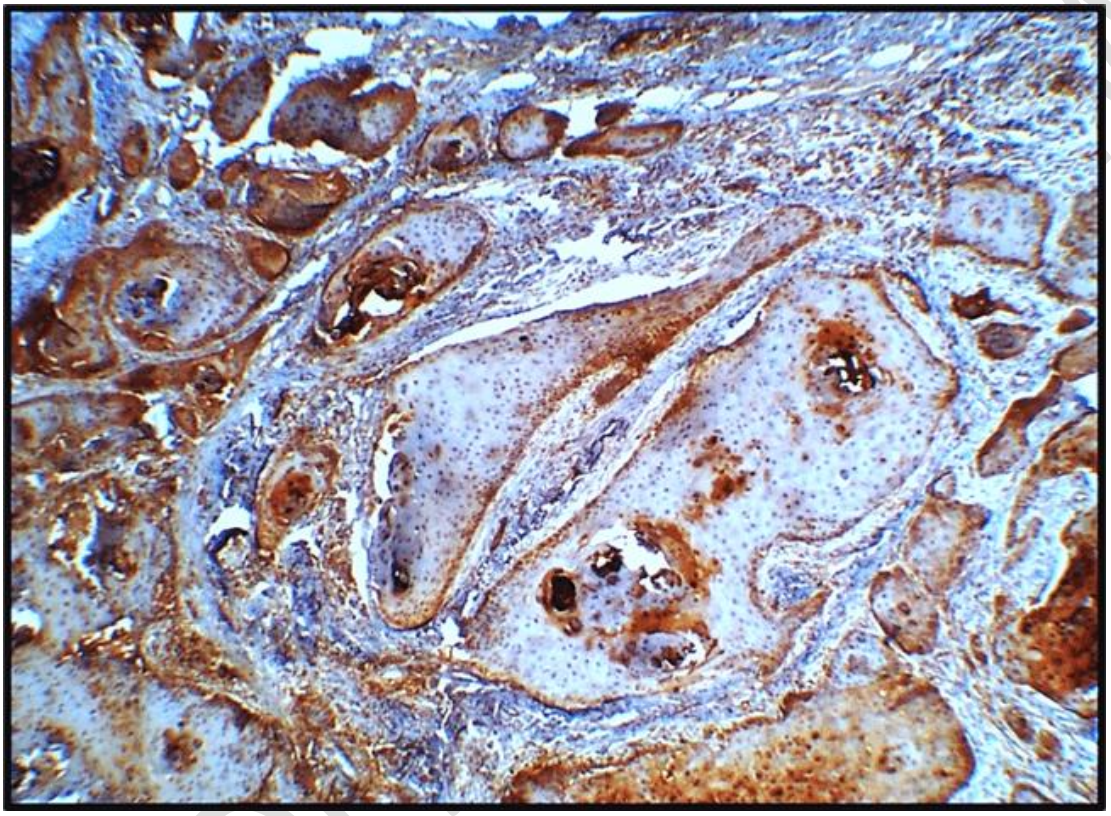

Figure 4: Photomicrograph of well-differentiated OSCC demonstrates strong h-TERT expression at the periphery of cell nests as well as central keratinized areas. Some central cells reveal moderate, while others reveal negative reactions. Stromal lymphocytes display a positive reaction. Anneroth score 1 degree of keratinization, and score 2 patterns of invasion (ABC/DAB.x100).

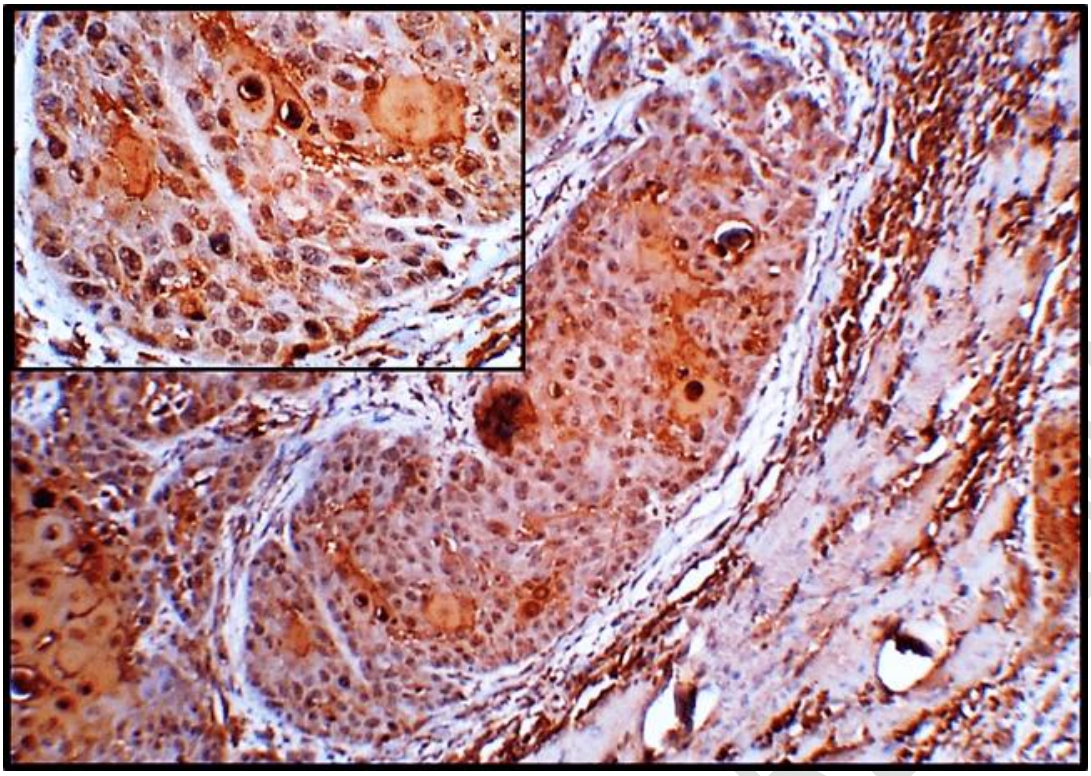

Figure 5: Photomicrograph of moderately differentiated OSCC demonstrates moderate h-TERT expression (ABC/DAB, x200). Lymphocytes display positive reaction peritumorally with Anneroth score 1 LPI. Higher magnification (inset) reveals nuclear and cytoplasmic h-TERT immunoexpression (ABC/ DAB,x400).

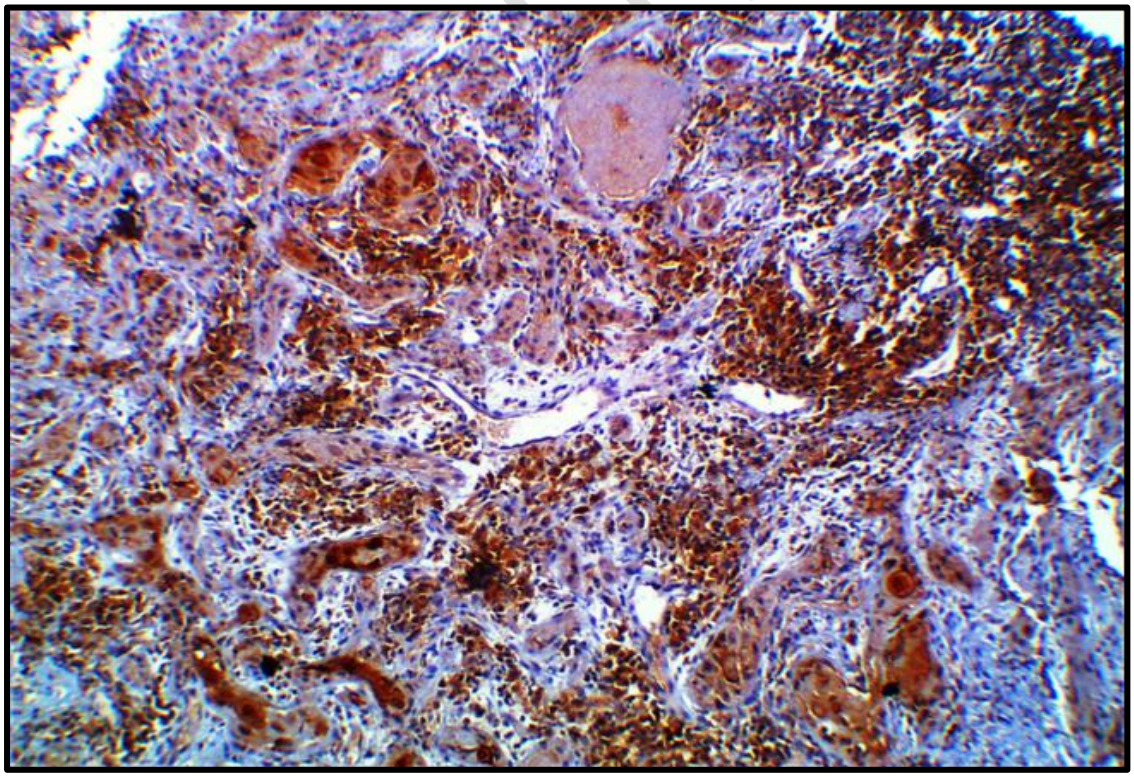

Figure 6: Photomicrograph of poorly differentiated OSCCs reveals multiple small sized cell nests with scattered tumor cells that reveal nuclear and cytoplasmic reaction with different staining intensities, Anneroth`s score 4 patterns of invasion, score 4 degrees of keratinization, and score 3 LPI (ABC/DAB, x200).

3.3.2. The expression of h-TERT in OSCCs concerning Anneroth`s histologic grade

Tumor cell population parameters; degree of keratinization, nuclear pleomorphism, mitotic figures count.

Regarding the degree of keratinization, it was noticed that the greater percentage of the studied OSCCs were presented with moderate H-score immunopositivity to h-TERT. The highest percentage of moderate H-score was presented with score 4 keratinization (no keratinization, 75%, **Fig.6**).

Regarding nuclear pleomorphism, all of score 4 cases (extreme nuclear pleomorphism),and the majority of score 1 (little nuclear pleomorphism, 66.7%), score 2 (moderate nuclear pleomorphism, 66.7%), and score 3cases (abundant nuclear pleomorphism, 62.5%) were presented with moderate H-score immunopositivity to h-TERT. Pearson`s chi-square test revealed no statistically significant differences among different degree of keratinization and nuclear pleomorphism scores concerning h-TERT expression (P= 0.085, and 0.219, respectively).

Regarding the number of mitotic figures, it was observed that score 3 (4-5 mitotic figures) was presented mainly (70.6%) with moderate h-TERT expression. Moreover, score 4 (> 5 mitotic figures) showed a predominantly moderate H-score for h-TERT immune expression (66.7%). However, Pearson`s chi-square test revealed no statistically significant differences was present amongthe different mitotic figures`scores concerning h-TERT immunoexpression (**Table 4**).

Table 4: h-TERT expression in relation to tumor cell population Anneroth`s grading parameters

Anneroth`s grading parameters	**Scores**	**H- score for h-TERT**			**Total**	***P value***
		Weak	**Moderate**	**Strong**		
		no. (%)	no. (%)	no. (%)		
Degree of keratinization	Score1	2 (28.6%)	5 (71.4%)	0 (0.0%)	7(23.3%)	0.085
	Score 2	3 (42.9%)	4 (57.1%)	0 (0.0%)	7(23.3%)	
	Score 3	0 (0.0%)	5 (62.5%)	3 (37.5%)	8(26.7%)	
	Score 4	0 (0.0%)	6 (75%)	2 (25%)	8(26.7%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	
	Score1	4 (33.3%)	8 (66.7%)	0 (0.0%)	12(40%)	

Nuclear pleomorphism	Score 2	1 (11.1%)	6 (66.7%)	2 (22.2%)	9(30%)	0.219
	Score3	0 (0.0%)	5 (62.5%)	3 (37.5%)	8(26.7%)	
	Score 4	0 (0.0%)	1 (100%)	0 (0.0%)	1(3.3%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	
Mitosis /HPF	Score 2	1 (25%)	2 (50%)	1 (25%)	4(13.3%)	0.890
	Score 3	2 (11.8%)	12 (70.6%)	3 (17.6%)	17(56.7%)	
	Score 4	2 (22.2%)	6 (66.7%)	1 (11.1%)	9(30%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	

*Chi-square test *Significant difference, (p values ≤ 0.05)*

Tumor to host relationship parameters; (pattern of invasion, depth of invasion, lymphoplasmacytic infiltration)

The highest percentage of strong h-TERT expression (37.5%) was observed among Anneroth`s score 4 patterns of invasion (marked cellular dissociation**)**. Meanwhile, moderate h-TERT expression was mainly seen among score 3 cases (small groups of infiltrating cells,87.5%). On the other hand, weak immune reaction was presented mainly in score 2 cases (Infiltrating solid cords or bands,35.8%). Pearson`s chi-square test revealed a statistically significant difference in h-TERT immunoexpression among the different pattern of invasion scores (P= 0.049).

As regard to the depth of invasion(DOI) parameter, the studied OSCCs were presented with only score 2 and score 3 invasion depth (46.7%, 53.4%). More than one half of the studied OSCCs (16 cases, 53.3%) presented score 3 DOI (Invasion below lamina propria adjacent to muscle). These cases demonstrated moderate and strong h-TERT expression (75%, 25%, respectively). On the other hand, DOI score 2 cases (distinctive invasion but involving lamina propria) presented mainly moderate (8 cases,57.1%) and weak (5 cases, 35.7%) h-TERT expression. Pearson`s chi-square test revealed a statistically significant difference in h-TERT expression considering the DOI score (P=0.024, Table 5). Additionally, there was a moderate strength positive correlation between h-TERT immunoexpression and the Anneroth 's DOI score*(r=0.463)*.

More than one-half of the studied OSCCs (56.7%) demonstrated score 1 lymphoplasmacytic infiltration (marked LPI) that revealed weak and moderate h-TERT expression (29.4% and 58.8% respectively, Table 5). All score 4 cases(none LPI) showed strong expression. It was observed that increased h-TERT expression was correlated with increasing LPI score*(r=0.440)*. Pearson`s chi-square test revealed a high statistically significant difference in h-TERT immunoexpression concerning different LPI scores (P=0.009).

Table 5: h-TERT expression in OSCCs concerningthe tumor to host Anneroth`s grading parameters.

Anneroth grading parameters	Scores	H- score for h-TERT			Total	*p*
		Weak	Moderate	Strong		
		no. (%)	no. (%)	no. (%)	no. (%)	
Pattern of invasion	Score2	5(35.8%)	8 (57.1%)	1 (7.1%)	14(46.6%)	0.049*
	Score 3	0 (0.0%)	7 (87.5%)	1 (12.5%)	8(26.7%)	
	Score 4	0 (0.0%)	5 (62.5%)	3 (37.5%)	8(26.7%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	
DOI	Score 2	5(35.7%)	8 (57.1%)	1 (7.1%)	14(46.7%)	0.024*
	Score 3	0 (0.0%)	12 (75%)	4 (25%)	16(53.3%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	
LPI	Score 1	5(29.4%)	10(58.8%)	2(11.8%)	17(56.7%)	0.009*
	Score 2	0 (0.0%)	8 (100%)	0 (0.0%)	8(26.6%)	
	Score 3	0 (0.0%)	2 (66.7%)	1 (33.3%)	3(10%)	
	Score 4	0 (0.0%)	0 (0.0%)	2 (100%)	2(6.7%)	
	Total	5(16.7%)	20(66.6%)	5(16.7%)	30(100%)	

*Chi-square test *Significant difference, (p values ≤ 0.05)*

3.4. The expression of h-TERT in carcinoma and control groups

Upon comparing h-TERT immunoexpression in OSCC group with the control group; the majority of OSCC demonstrated moderate h-TERT immune expression (66.7%). Meanwhile, the control group revealed negative (mainly) and weak h-TERT expression. Statistically,there was a significant difference in h-TERT immune expression between the carcinoma and the control groups (P=0.000, Fig. 7, **Table 6**).

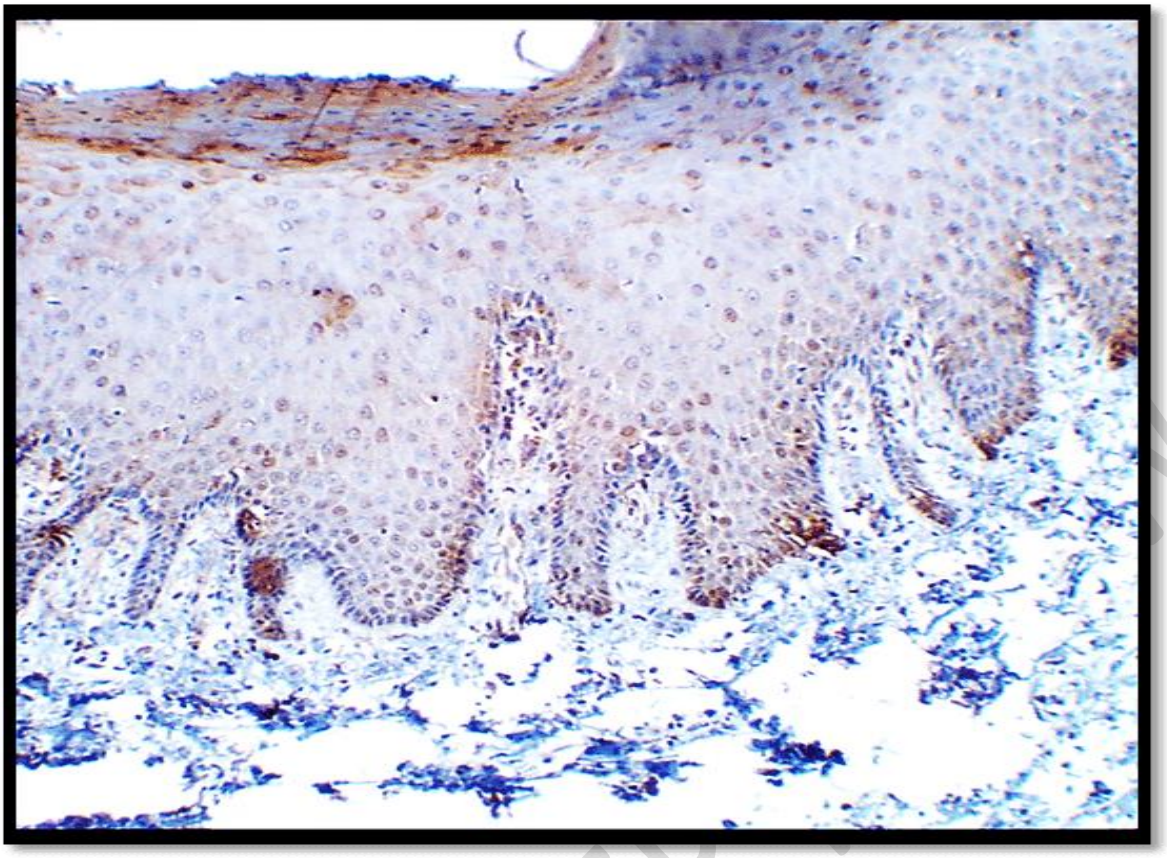

Figure 7: Photomicrograph reveals weak h-TERT expression in normal oral mucosa (the control group, (ABC/DAB, x200).

Table 6: Comparison between h-TERT expression in OSCC group withthe control group.

The studied groups	**H-score for h-TERT immune expression**								Total	*P*
	Negative		**Weak**		**Moderate**		**Strong**			
	no.	%	no.	%	no.	%	no.	%	no. (%)	
OSCC group	0	0.0%	5	16.7%	20	66.7%	5	16.7%	30(100%)	*
Control group	4	80%	1	20%	0	0.0%	0	0.0%	5(100%)	**0.000**

*Chi-square test *Significant difference, (p values ≤ 0.05)*

4. DISCUSSION

The present study was a trial performed to through a beam of light on the possible role of h-TERT in oral carcinogenesis. Human TERT is well known to maintain the replicative immortality in various tissues including OSCC as well as normal oral epithelium[18]. This was a suggested model to reveal the potential role

of h-TERT in oral cancer, and how h-TERT immunoexpression could predict malignant transformation in oral tissue. Furthermore, correlations of h-TERT expression with different clinicopathological parameters were accomplished to investigate if h-TERT expression could predict the progression of cancer.

In the present work, the immunohistochemical results of h-TERT were compared with the patients` clinical parameters. The results revealed no correlation between age, sex, and tumor site concerning h-TERT expression. This was in agreement with previous reports on oral cancer which revealed no significant association between TERT expression and various clinicopathological parameters[19, 20].This might sign that h-TERT has no impact on clinical parameters.

Concerning the TNM categories as well as staging,there was no statisticaly significant difference of H-score for h-TERT reaction among different stages. This was in agreement with others using polymerase chain reaction- based telomeric repeat amplification protocol assay in OSCC. They stated that telomerase reactivation has been observed as a necessary and rate- limiting step of tumor progression and might deliver useful diagnostic information about clinical tumor behavior[21].Meanwhile, others reported that h-TERT expression was significantly associated with T (tumor size) and N (nodal stage), in addition to significant h-TERT expression among advanced clinical stages (III and IV) thus, associated with aggressive clinical parameters[22]. Our results might confirm a limited role of h-TERT for EMT activation and tumor progression.

The current IHC expression of h-TERT revealed that most of the normal oral mucosa specimens showed negativity for h-TERT and sporadic weak expression in few of the basal cells. Similar results were reported by other investigators who explained that the telomerase-negative samples might exhibit alternative lengthening mechanisms of telomere maintenance in the form of chromosome maintenance or other uncharacterized mechanisms. Telomerase positivity in oral mucosal samples might be due to stratification and differentiation patterns of squamous epithelium, which has a high cell turnover rate, resulting in more stem cells for active self-renewal [21,23].

The current studied OSCCs, showed significant increased h-TERT activity from well to poorly differentiated OSCC grades. This finding was in agreement with other reports which revealed that h-TERT expression gradually increased as the degree of malignancy of OSCCs increased [19,22]. It has been reported that well-differentiated OSCCs were slow-growing, compared with moderate and poorly differentiated OSCCs, which were also more aggressive. Less differentiated OSCCs might likely contain more immortal cells than the more differentiated histological types; hence, h-TERT would also be high[22]. Currently, the distribution of the

h-TERT stain was typically heterogeneous among neoplastic cells with different expression intensities between tumor cells. This might be due to intratumoral cell heterogeneity, which had been linked to different tumor subpopulations, and might be linked to resistance to systemic treatments[24,25]. The gradual increase in h-TERT expression from control to different grades of OSCC groups might suggest its involvement in a modified way during malignant transformation.

Regarding Anneroth`s criteria, there was a significant association of increased expression of h-TERT with the DOI as well as pattern of invasion scores. This could be associated with expression levels of several members of the matrix metalloprotease (MMPs) to promote cell adhesion and migration besides induction of EMT [26,27]. Additionally, h-TERT can use other extracellular proteases thereby promoting the invasiveness of cells[26]. There was a significant direct relation between LPI scores and h-TERT expression among the presented OSCCs. It has been reported that an inverse correlation was revealed between TERT expression and adaptive immune cells in cancers other than OSCC. Studies reported that high h-TERT showed down regulation of genes and pathways related to B and T cell activation, proliferation, migration, and cytotoxicity, while factors associated with immunosuppression and cancer cell invasiveness were up-regulated[28].

5. CONCLUSION

Increased h-TERT immunoexpression from normal to OSCC groups suggests its involvement in malignant transformation. Additionally, the significant differential expression of h-TERT among different OSCC histologic grades signaling its valuable use as a biomarker for assessing the cellular malignant progression of oral carcinomas, but unreliable as a clinical tumor progression marker. Moreover, the significant correlation of h-TERT expression with LPI, DOI and pattern of invasion indicating its possible reliable role in tumor host relation during multistage carcinogenesis.

CONSENT

Informed written consent to participate in the study was obtained from each individual.

ETHICAL APPROVAL

The current study was approved by the research ethics committee of Faculty of Dentistry, Mansoura University. (Code Number: A10060421).

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ABBREVIATIONS

h-TERT: human telomerase reverse transcriptase.

OSCC: Oral squamous cell carcinoma.

OC: Oral Cancer.

LPI: Lymphoplasmacytic infiltration.

DOI: Depth of invasion.