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Research Article

Immunohistochemical Expression of TGF-*β3* in Oral Squamous Cell Carcinoma

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ABSTRACT

Squamous cell carcinoma characterized by poor prognosis due to aggressive tumor growth and dissemination high rate of tumor cell . age ranged of patient case included in the study 40-62 years and mean age 55±99. The sex distribution male/female ratio 1:1. Male case 15 and female 15 of the present study The results of clinical forums showed in the current study was endophytic 10(33.3%) in the same time Exophytic were presented in 20 cases (76.7%). Regarding distribution of the tumors site, the preponderance of them 19 cases 73.3% were located alveolar mucosa, followed by in the tongue 11 cases(36.7%) Tumor stage was analyzed and recorded in Oral squamous cell carcinoma included cases, the preponderance of them were Stage II 11 cases 36.7% followed by stage III 10 cases 33.3% , 9 cases 30.0% were stage I. While Concerning tumor grade, majority of them 15 cases 50% had grade II moderately differentiated SCC, while 11 cases 36.7% had grade III poorly differentiated SCC and 4 cases 13.3% had grade I well differentiated SCC Positive TGF-β3 immunostaining was detected as cell with staining brown color, all tissues sections included show Positive expression based on IHC teqnique. Positive Transforming Growth Factor TGF-β3 Immuno staining was found in all case results and display that 4 samples with percentage 13.3% expressed strong positive 87.67 ± 1.45 expression , 11cases 36.7% showed 51.33 ±0.88 positive expression moderate at the same time 15 samples 50.0% showed positive weak expression.

Keywords: TGF-β3, Oral squamous carcinoma, immunohistochemistry.

INTRODUCTION

Oral squamous cell carcinoma in clinical presentations can take many Lesions forms present in different area of the mouth and difficult to examine. The diagnosis of early squamous cell carcinoma based on the key words vigilance and suspicion[1]. Early lesions consider asymptomatic usually, the common of presentation modes white patch, erythema and small indurated ulcer, in the early stages white patch shown ulceration and consider as small exophytic growth[2]. The late lesion advance present as rough exophytic mass with nodular necrotic surface edges raised[3]. Patients with a minor oral squamous cell carcinoma are common with advanced local invasion symptoms[2] . The common presenting clinical signs of OSCC can be summarized in erythroplakia, leukoplakia, and erythroleukoplakia[4]. The size of tumor and the metastasis spread extent of of OSCC are the best indicators of patient's prognosis[5]. staging of the disease represented by Quantifying these clinical parameters[6]. The evaluation of clinical based on biopsy stage, imaging of the lymph nodes regional and distant sites[6]. The staging protocol most popular is called the tumor-node-metastasis (TNM) system based on clinical features[6]. Transforming growth factor β3 (TGF-β3) truly called because it's have the capability lead to stimulate growth of fibroblast in agar soft; at the same time work to inhibition proliferation and macrophage epithelial cell[7-8]. The current experiment's looking forward to analyses the expressed of TGF-*β3* in oral squamous cell carcinoma patient cases.

MATERIAL AND METHOD

The case of the current study included 30 paraffin blocks embedded tissue diagnosed as Oral squamous cell carcinoma, dated from (2015 till 2018). The case study samples obtained private laboratories in Baghdad. The diagnosis confirmed by specialized pathologists through examining sections by Hematoxylin and Eosin (H and E). The control obtained from information in manufacturer's antibodies data sheet based the following:

Positive Control: block tissue of breast cancer.

Negative Control: lack of specificity of the antibody to Positive staining[9].

Estimation of Transforming Growth Factor TGF-β3 by using immunohistochemical technique

It was performed in order to estimate TGF- β3 in embedded sections paraffin using Immunohistochemistry protocol (Manufacturer's data sheet).

Principle of test

The detection system using immunohistochemistry protocol based on:

detects binding of antibody an antigen in sections tissue.

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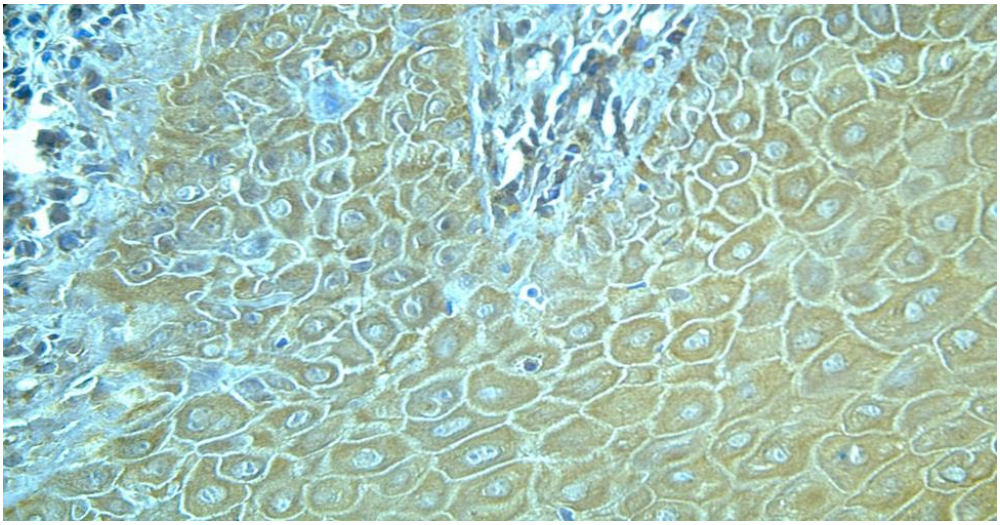

Figure 1: TGF-β3 Positive brown expression in the oral case of squamous cell carcinoma.

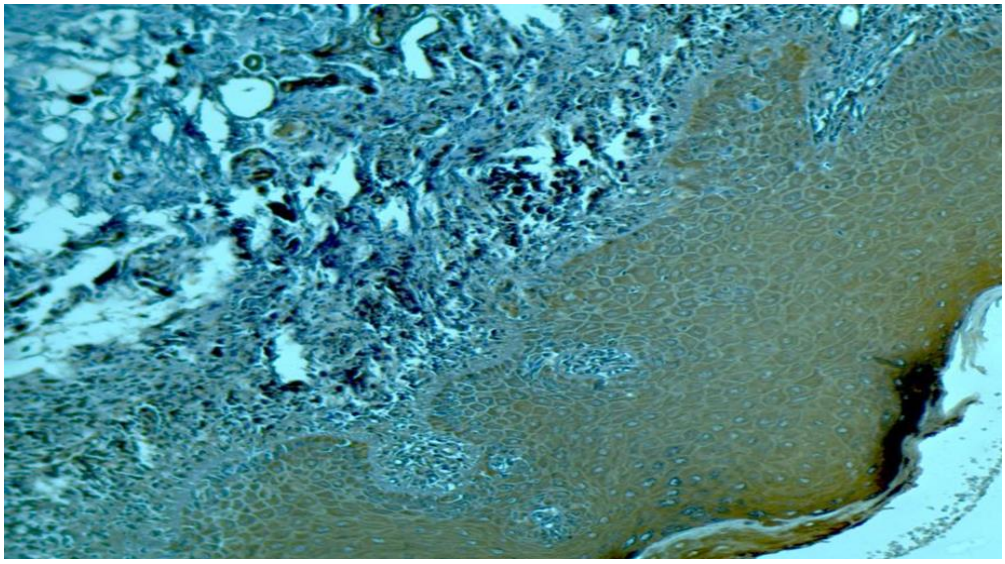

Figure 2: TGF-β3 Positive brown expression in the oral case of squamous cell carcinoma.

Table 1: Age and sex distribution of the present study.

	No.	%
Age 40-62		
>50	17	56.7%
≤ 50	13	43.3%
Total	30	100%
Sex		
Male	15	50%
Female	15	50
Total	30	100

The complex enzyme -secondary antibody detected using appropriate chromogen /substrate (Abcam, 2013) .

Preparation of the reagents

Preparation of Anti- TGF-β3 antibodies

The Anti- TGF-*β3* antibodies diluted in 1:100 manner and used in detection during the experiments.

Substrate chromogen solution

Dab chromogen was added to Substrate buffer in a ratio: (2:100 v/v) by using graduated test tube provided by the manufacturer. The prepared substrate chromogen solution stored in dark place at 2-8˚C. The substrate must be mixed well before use.

Protein-Block Buffer

Fifty µl of 20X concentrated protein block buffer was diluted with deionized water to the final volume of 1000 µl. The resulting 1-X protein-block buffer concentration was ready to use and the remaining solution was stored at 4˚ C.

Phosphate Buffer Saline

PBS prepared in section (2.2.2.1: IV) was dissolved in distilled water in a ratio 1:10 v/v.

Ethanol

To prepare Ethanol alcohol in different concentrations follow the formulae:

90% Concentration: 90 ml alcohol absolute + 5 ml D.W then completed to 100 ml.

70% Concentration: 70 ml alcohol absolute + 25 ml D.W then completed to 100 ml.

Table 2: clinical forums and tumor site in the Oral squamous cell carcinoma.

Oral squamous cell carcinoma	NO	%
Clinical forums		
Entophytic	10	33.3%
Exophytic	20	76.7%
Tumor site		
Tongue	11	36.7%
Alveolar mucosa	19	73.3%

Table 3: distribution of the Oral squamous cell carcinoma case based on staging and grading.

	No	%
Oral squamous cell carcinoma staging		
Stage I	9	30.0%
Stage II	11	36.7%
Stage III	10	33.3%
Total	30	100%
Oral squamous cell carcinoma grading		
Grade I	4	13.3%
Grade II	15	50.0%
Grade III	11	36.7%
Total	30	100%

Table 4: TGF-β3 I.H.C expression in Oral squamous cell carcinoma cases.

	TGF-β3 score*	No.	%
3 strong expression	87.67 ± 1.45	4	13.3%
2 moderate expression	51.33 ±0.88	11	36.7%
1 weak expression	42.467 ±1.3	15	50.0%
	Total	30	100%

50% Concentration: 50 ml alcohol absolute +45 ml D.W then completed to 100 ml.

Preparation of tissue sections

Paraffin embedded sections were sectioned into 4-5 µm thickness, placed on Adhesion microscope positively charged slides and left overnight to dry at room temperature.

Procedure

The exact procedures of immunohistochemistry may vary from the datasheet as they were modified to accomplish optimal results:

Slide baking: prepared slides were placed in a vertical position over night in a drying incubator (hot air incubator) at 80ºC for 70 minutes.

tissue sections deparaffinising done based on the time and solutions: a) Xylene for 30 minutes.

30 minutes using Fresh xylene.

5 minutes using ethanol Absolute.

5 minutes using 90% ethanol.

5 minutes using 70% ethanol.

5 minutes using 50% ethanol.

5 minutes using D.W.

Adding Hydrogen Peroxide(drops) then blocked to sections cover, incubated for 10 minutes then washing in buffer two times.

applied Protein Block 20µL then left it in 10 minutes at room temperature then washed one time using buffer.

placed primary antibody 40µl onto the section tissue and 30 minutes incubation at 37˚C put the slide 5 minutes in washing buffer then blotted gently.

applied secondary antibody 20 µl onto the sections and incubated at 37ºC for 10 minutes.

conjugate 20 µl of HRP Horse Radish Peroxidase was added onto each tissue section then incubated for 15minutes at 37˚C.

(100:2 v/v) DAB Substrate added to DAB Chromogen and then added to tissue and to rinsed in buffer 4 times.

placing the prepared slides for Dehydration in the following time and Solutions

5 minutes for 50% ethanol.

5 minutes for 70% ethanol.

5 minutes for 90% ethanol

Absolute ethanol for 5 minutes.

5 minutes Xylene.

Fresh Xylene for.

applied DPX drop of medium and covered with cover slips to remove air bubbles then left to dry overnight[10].

estimation of TGF-β expression

The expression of TGF-β protein using under light microscopy X40 and based on counting (DAB) cytoplasmic staining positive cells with brown. The semi quantitatively in present experiment's consider the following percentage:

(%)<10 negative.

10-30 % Weak.

31-50 % Moderate.

>50 % Strong[11].

RESULTS AND DISCUSSION

The current study samples included of 30 oral case of squamous cell carcinoma with a ranged age 40-62 years while mean age 55±99. The sex distribution male/female ratio 1:1. Male case 15 and female 15 of the present study. The age and sex distribution illustrated in this study were in table (1) and figure (1 ,2).

The results of clinical forums showed in the current study was endophytic 10(33.3%) in the same time Exophytic were presented in 20 cases 76.7%. Regarding distribution of the tumors site, the preponderance of them 19 cases 73.3% were located alveolar mucosa, followed by in the tongue 11 cases 36.7% table (2).

study sample of Oral squamous cell carcinoma based on staging and grading.

Tumor stage was analyzed and recorded in all Oral squamous cell carcinoma cases, the preponderance of them were Stage II :11 cases 36.7% followed by stage III : 10 cases 33.3% , 9 cases 30.0% were stage I. While Concerning tumor grade, majority of them 15 cases 50% had grade II moderately differentiated SCC, while 11 cases 36.7% had grade III poorly differentiated SCC and 4 cases 13.3% had grade I well differentiated SCC table (3).

Immunohistochemical Evaluation of Transforming Growth Factor TGF-β3 using immunohistochemical technique in the Oral squamous cell carcinoma case:

By applying I.H.C teqnique, the statistical analysis display

Table 5: Correlation of TGF-β3 with Oral squamous cell carcinoma stage.

			Stage			Total
			I	II	III	
TGF-β3	87.67	Count	1	2	1	4
		% within TGF-β3	25.0%	50%	25.0%	100.0%
	51.33	Count	5	3	3	11
		% within TGF-β3	33.3%	27.3%	27.3%	100.0%
	42.467	Count	5	5	5	15
		% within TGF-β3	33.3%	33.3%	33.3%	100.0%
Total	Count		9	3	8	10 30

P value = 0.02 PearsonChi-Square20.345.

significant difference with probability ≥ 0.05. Positive TGF-β3 immunostaining was detected as brown staining of the cell. Positive IHC expression appeared in all cases as display in Table 4. Positive Transforming Growth Factor TGF-β3 Immuno staining was found in all Oral squamous cell carcinoma case results reveals that 4 cases 13.3% showed 87.67 ± 1.45 strong positive expression, 11 cases 36.7% showed 51.33 ±0.88 moderate positive expression and 15 cases 50.0% showed weak positive expression.

Correlation of TGF-β3 with the Oral squamous cell carcinoma cases stage:

Regarding tumor stage statistically the level of probability (p value=0.02) using chi- square test theirs no significant correlation between them, but the TGF-β3 strong positive expression was found in stage I :33.3%, and III then II respectively as presented in table 5.

The association of Oral squamous cell carcinoma cases with age of patients may be attributed to exposure prolonged to carcinogenesis environmental such as viruses, radiation, and chemical[12]. In addition, suppression in immunity level lead to the accumulation of cellular mutations in DNA which could be a critical factor in cancer development also aging have great relationship with environmental factor since it causes cellular dysregulation in cell alteration growth and genes suppressor[13].

Regarding the sex distribution of the study samples results cleared in the present study may be attributed to the fact that the current study limited number of cases and the present finding not an epidemiological type of studies[14].

Regarding tumor stage majority of the tumors stage were stage III, while Mohammed, 2008[15] reported that most of the cases were stage II.

The present study showed positive TGF-β3 expression in all Oral squamous cell carcinoma cases which also revealed that (50.0%) showed strong positive score, these finding was in agreement with previous Iraqi study in Oral squamous cell carcinoma cases[16] and study in other part in the world in Head and Neck Cancer[17].

Many previous study find TGF-β3 have degrading ability of several human oral cancer cell lines and tissues derived from human Oral squamous cell carcinoma cases expression on mRNA and protein levels. The catalytic activities and the mRNA levels of TGF-β3 showed a good agreement among expression of TGF-β3 on the mRNA and protein levels. This imply that TGF-β3 expression is a good diagnostic factor for evaluating the metastatic properties of OSCC[18].

Previous studies showed that TGF-β3 expression levels is higher at the primary tumor site this perhaps reflect the need to break down physiological restraints like basement membrane and ECM before tumor cell can metastasize[19].

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