



Study Expression of Fibronectin in the Samples of Oral Squamous Cell Carcinoma

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Abstract

The current study included samples of 30 sample oral squamous cell carcinoma with a ranged age 33-67 years in mean age 47 ± 9 in different sex. Regarding the size of oral squamous carcinoma case, 10 cases of each size T1, T2 and T3 appeared in the same percentage (33.3%). Concerning grade of oral squamous cell carcinoma case, majority of them (15) cases (50%) had grade I, while (10) cases (33.3%) had grade II (oral squamous carcinoma well differentiated), (3) cases (10.0%) had grade III and (2) cases (7.1%) had grade IV (differentiated poorly oral squamous carcinoma). The results of analysis showed Positive fibronectin expression using Immuno staining technique was found in all oral squamous cell carcinoma cases as brown extracellular matrix, cellular localization expression and cytoplasmic expression, fibronectin expression using immunohistochemical staining of the oral squamous carcinoma cases which reveals that (5) cases (17.3%) showed protein expression weak positive, (15) cases (50%) showed protein expression moderate positive and (10) cases (33.3%) showed protein expression strong positive.

Key words: *Fibronectin, Squamous carcinoma in oral, Immunohistochemical technique.*

Introduction

Oral Squamous cell carcinoma represented nearly 95% of mouth neoplasm's malignancies [1]. Due to late detection of advanced cancer stage mostly that lead to stratified squamous epithelium malignancy and then remains 50% of the cases in lethal form disease which diagnosed annually during the year also higher distribution frequency between the sex [2]. Oral Squamous cell carcinoma behaviors based on its site and origin. site of Oral Squamous cell carcinoma has particular pattern spread and prognosis own [3]. Floor of the mouth, lateral border of the tongue and ventral surface of the tongue consider the Main sites but generally the behavior of same disease in the lip appeared as aggressive lesion actinic [4]. Etiology have correlation with different factors specially tobacco and alcohol [5].

All forms of smoking tobacco have been strongly linked to Oral Squamous carcinoma cell causing, alcohol not consider carcinogen in direct way but may be help in the development of Oral Squamous cell carcinoma cell [6]. Fibronectin consider the Main factor lead to inhibit the Oral

Squamous cell carcinoma development and the remains prognosis poor even when the patients advances in therapy combinatorial [7]. Only 30-50% survival rate of Patients who have the advance stage disease primarily due to metastases in lymph node and also cancers with multiple primary presence [8]. In the world Oral all cancer represented percentage approximately 94% of all malignancies oral are squamous carcinoma cell [9]. oral cancer consider the Main type of malignancy and may account for more than 50% of all type of cases cancer In Asian countries and India [10]. Cancer of oral in Iraq remains a highly disfiguring disease and lethal based on report of Iraqi cancer registry ISR [11]. (Al-Talabani, 2002). The present study aimed to evaluate Fibronectin protein in the samples of oral squamous carcinoma.

Material and Method

Oral Squamous Cell Carcinoma Case and Control

The case included in the experiments of current subject included thirty blocks diagnosed as oral squamous carcinoma cell

of embedded tissue, which, dated from (2014 till 2017). The study samples were obtained from the archives of the department of oral and Maxillofacial Pathology/ Baghdad University. Hematoxylin and Eosin stain used for diagnosis confirmation. Negative control define as applied all reagents except the primary antibody while positive control defined according to data sheet of the manufacturer's Kit [12].

Principles of the Experiments

Immunohistochemistry technique based on the following process:

- The peroxidase enzyme conjugated to link-Antibody, this linking providing amplification of the antigen-antibody binding event.
- Adding Streptavidin bounded enzyme conjugate the Ab (biotinylated) will form a complex conjugates streptavidin- enzyme through adding substrate of chromogen, reaction colorimetric in the site of antigen binding [9].

Immunohistochemistry Protocol Technique

Immunohistochemistry Protocol technique in our project by following the instruction in the data sheet with some modification through the following steps:

- Slide baking 60°C through put the slides in vertical position overnight following by Deparaffinization and hydration through immersed in the following solutions:
- 15 minutes for each Xylene use Twice.
- 5 minutes for each ethanol absolute twice.
- 5minutes for ethanol 95%.
- 5minutes for ethanol 90%.
- 5minutes for ethanol 80%.
- 5minutes for ethanol 70%.
- 5minutes for Distilled water.
- 30 minutes immersed the slides with solution of 0.03 % hydrogen peroxide then slides drained with absorbent wipes to avoid the tissue disruption.
- Added 30-50 µl to cover tissue sections after draining carefully of 1% serum/PBS

solution and incubated for 30 minutes at 37°C.

- Applied primary antibodies for each slides and incubated overnight at humid chamber.
- Slides rinsed gently with stream of PBS for 5 minutes three times.
- Applied for 5 minute secondary antibody (Biotinylated) slides rinsed gently with stream of PBS for 5 minutes three times.
- Specimen covered for 30 minutes with streptavidin-HRP reagent were applied and then remain in humid chamber slides rinsed gently with stream of PBS for 5 minutes three times.
- Applied enough drops cover the sections of DAB and kept 3-5 minutes in darkness within the humid chamber then washed gently for 10 minutes with tap water.
- 1-2 minutes bathed slides in Hematoxylin counter stain then for 10minutes with tap water rinsing.
- DPX mounting medium (Distyrene-Plasticizer-Xylene) were applied cover to remove bubbles of air [13].

Evaluation of fibronectine Expression in Oral Squamous Carcinoma Sections

The scored results of each case expression scored in five fields at 40X objective representative based on the extent of staining according to the following scale:

- Fibronectin Positive brown extracellular matrix, cellular localization weak expression
- Fibronectin Positive brown extracellular matrix, cellular localization moderate expression
- Fibronectin Positive brown extracellular matrix, cellular localization strong expression And the specificity compared with negative and positive control [14].

Statistical Analysis

The studied parameters were analysis using Chi-square test based on two-sided level of probability significance was 0.05 and 0.01 in all statistical scoring [15].

Results and Discussion

Characteristics of the Studied Case

The current study included samples included of 30 sample oral squamous cell carcinoma with a ranged age 33-67 years in mean age 47±99 in different sex. The Characteristics of

studied case are summarized in Table (1). Regarding the size of oral squamous carcinoma case, 10 cases of each size T1, T2 and T3 appeared in the same percentage (33.3%).

Table 1: distribution of oral squamous carcinomacase size

oral squamous carcinomacase size	NO.	%
T1	10	33.3%
T2	10	33.3%
T3	10	33.3%
Total	30	100%

**p value ≥ 0.05

Concerning grade of oral squamous carcinoma case, majority of them (15) cases (50%) had grade I, while (10) cases (33.3%) had grade II (oral squamous carcinoma well differentiated

well), (3) cases (10.0%) had grade III and (2) cases (7.1%) had grade IV (differentiated poorly oral squamous carcinoma) as show in Table (2).

Table 2: distribution of oral squamous carcinomacase based on oral squamous carcinoma case grading

oral squamous carcinomacase grading	NO.	%
Grade I	15	50%
Grade II	10	33.3%
Grade III	3	10.0%
IV	2	7.1%
Total	30	100%

**p value ≥ 0.05

Evaluation Protein Expression of Fibronectin in Oral Squamous Carcinoma Case

The results of analysis showed Positive fibrnectinexpression using Immuno staining teqnique was found in all oral squamous carcinoma cases as brown extracellular matrix, cellular localization expression and cytoplasmic expression, fibrnectin expression

using immunohistochemichal staining of the oral squamous carcinoma cases was showed in Table (3) which reveals that (5) cases (17.3%) showed protein expression weak positive, (15) cases (50%) showed protein expression moderate positive and (10) cases (33.3%) showed protein expression strong positive. as display in Figure (1) and Figure (2).

Table 3: Fibronectin Expression in Oral squamous carcinoma studied Cases

Fibronectin Expression	No.	%
1 : weak	5	17.3%
2: moderate	15	50.0%
3: strong	10	33.3%
Total	30	100%

**p value ≥ 0.05

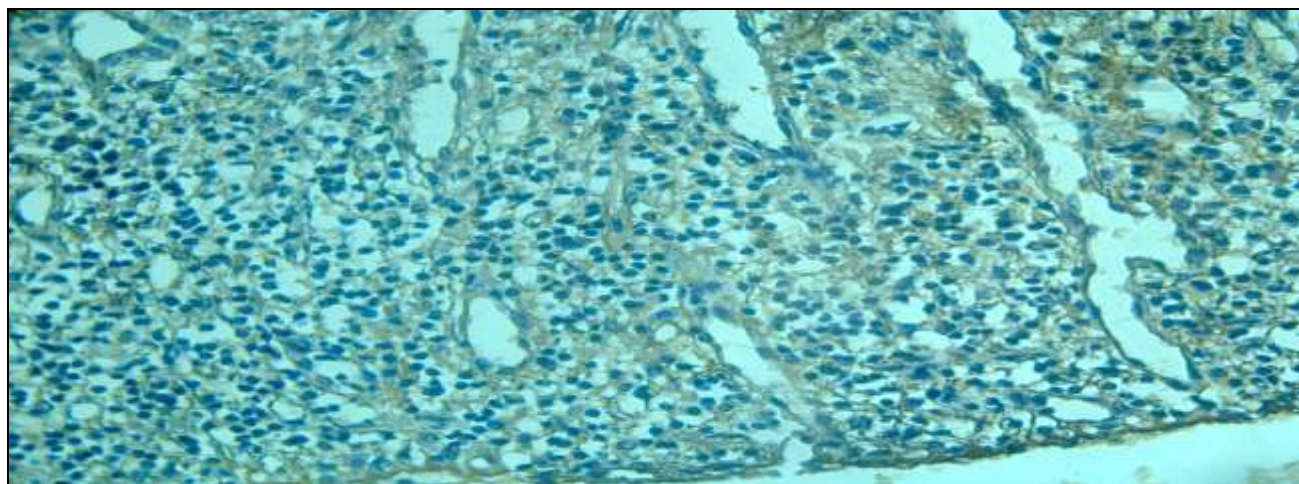


Figure1: FibronectinPositive brown extracellular matrix, cellular localization expression in the case of squamous cell carcinoma of oral

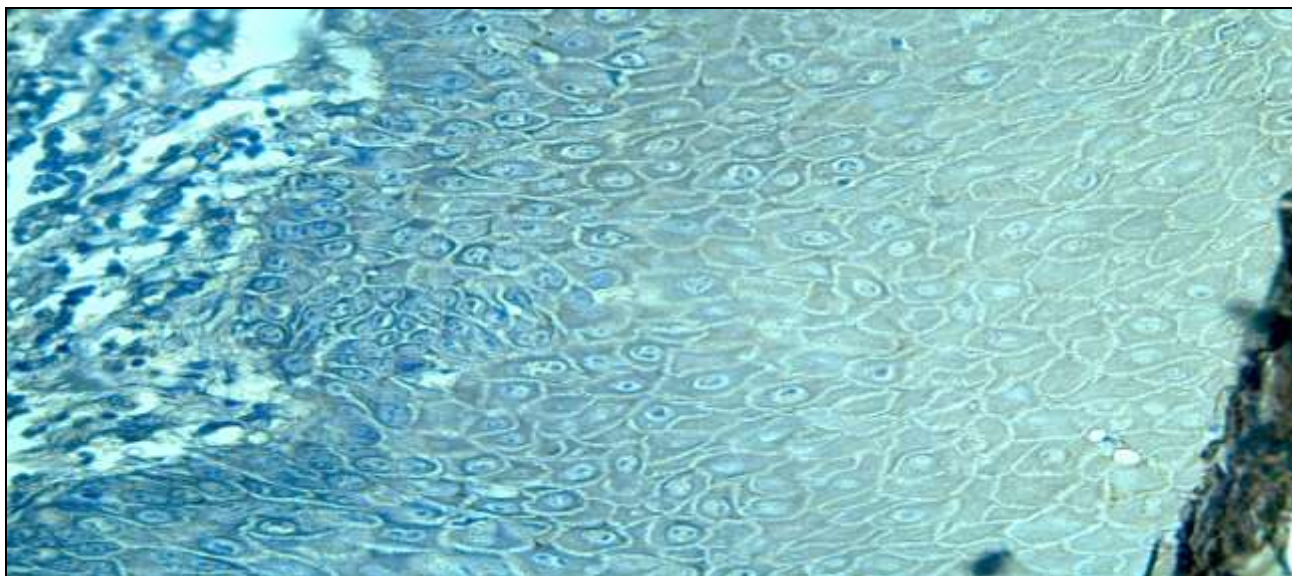


Figure 2: Fibronectin Positive brown extracellular matrix, cellular localization expression in the case of squamous cell carcinoma of oral

The Correlation between fibronectin expression and Characteristics of the studied case statistical analysis showed using chi-square test, that there was no correlation statistically significant regarding fibronectin expression in relation to age with probability value $p \geq 0.05$, regarding sex probability value $p \geq 0.05$, while based on tumor site probability value $p \leq 0.05$, and clinical presentation probability value $p \geq 0.35$. Histologic grade and systems of Grading are different based on each cancer type which used to analysis treatment plan and prognosis determination. Concerning the correlation between Histologic grade of tumor and Oral squamous carcinoma studied Cases the results of

correlation summarized statically in Table 4, and show theirs strong correlation between fibronectin expression and Histologic grade of Oral squamous carcinoma studied Cases observed strongly in grade I and IV in equal percentage 33.3% which differentiated moderately Oral squamous carcinoma, followed by Histologic grade III with percentage 19.2% which consider differentiated well and then differentiated cases poorly in Histologic grade II, with correlation statistically significant as clarified between expression of fibronectinin relation with Histologic grade based on analysis using chi-square test with probability value $p \geq 0.001$.

Table 4: Correlation of fibronectin expression with Oral squamouscarcinoma studied Cases Histologicgrade

Fibronectinexpression		Histologic grade				Total	
		I	II	III	IV		
Fibronectinexpression	1	Count	0	2	2	1	5
		weak	.0%	40.0%	40.0%	20.0%	100.0%
	2	Count	6	0	2	7	15
		moderate	40.0%	.0%	13.3%	46.7%	100.0%
	3	Count	4	2	2	2	10
		strong	40.0%	20.0%	20.0%	20.0%	100.0%
Total		Count	10	49	6	10	30
		% within Fibronectinexpression	33.3%	10.8%	19.2%	33.3%	100.0%
		Value	df	p.value			
Chi-Square		32.14	9	0.01			

Regarding the site of Oral squamous carcinoma studied cases, statistical analysis show significant difference in fibronectine expression among Oral squamous carcinoma studied cases sites, this results agreed with [16], which previously studied in Iraqi

samples. Regarding to Histological grade, majority of cases identified as differentiated moderately, result were recorded in others study in the world described differentiated Histological grade equal frequencies for poorly and well this results may related to

variety in the data collection methods prospective and retrospective cases, also determined by analysis criteria and the number of the studied cases [17]. Fibronectin consider as glycoprotein with high molecular weight and its distributed ubiquitously in the matrix of the cell [18], and plays an important role in signaling also cell adhesion, therefore used its expression in prognosis of Oral squamous carcinoma Cases [19]. Since migration of cell on matrix of fibronectin is significantly faster than those in expressing of cell lines with high levels these properties lead to invasion of cancer cell through inducing the expression markers of cell mesenchymal like fibronectin protein [20]. Through using the immunostaining evaluation the results of our current study

suggest that the specific expression facilitate adhesion and posterior migration through tumor stroma [21]. Present data from case analysis of fibronectin expression helps to better comprehend the cellular invasion process based on molecular mechanisms involved [7]. Whereas fibronectin induces adhesion of the strengthening cell substrate and tenascin spreading, has the effect on the cell promoting opposite detachment and rounding [9]. Fibronectin has strong effect on culture of cell tumor suppression and on interactions of the cell and antagonizes effects of adhesive, fibronectin and tenascin and are usually help Oral squamous carcinoma suppression and found together in order to regulate mechanisms for each of them are different quite [22].

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