Immunohistochemical Distribution of Myofibroblasts in Oral Squamous Cell Carcinoma, Verrucous Carcinoma and Oral Epithelial Dysplasia

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ABSTRACT

Background: Squamous cell carcinoma (SCC) is the most frequent type of oral malignancy that exhibits certain histological variations and is associated with a high mortality rate .Verrucous carcinoma (VC) is considered to be an uncommon exophytic distinctive low-grade well differentiated pathological variant of OSCC. Several studies have shown that the microenvironment or stroma of neoplastic tissues plays an active role in tumor progression. Concurrent with the conversion of non-diseased epithelial tissue to pre-cancerous epithelium to carcinoma, the stroma also changes from normal to primed (activated or tumor associated).

Objective: The aim of this study was to compare the distribution of myofibroblasts in normal oral mucosa, oral epithelial dysplasia, verrucous carcinoma, and different histological grades of OSCC.

Materials and methods: Twenty four formalin –fixed, paraffin -embedded tissue blocks (10 cases oral squamous cell carcinoma, 8 cases oral epithelial dysplasia and 6 cases verrucous carcinoma) were included in this study. An immunohistochemical analysis was performed using anti alpha - smooth muscle actin (a- SMA) monoclonal antibody.

Results: All cases of OSCC, intraoral dysplasia and verruous carcinoma, and normal oral mucosa showed positive reaction of actin in the stromal smooth muscles surrounding blood and lymphatic vessels. All OSCCs demonstrated stromal immunostaining for a-SMA with different scores indicating the presence of myofibroblast. There were no myofibroblasts in the stroma of normal mucosa, epithelial dysplasia or verrucuos carcinoma samples indicated by negative a-SMA expression in them.

Conclusion: The lack of myofibroblasts in normal, dysplastic oral epithelium and VC and their appearance in OSCC, suggests that the genetically altered epithelium (carcinomatous epithelium), besides the invasive behaviour of OSCC may have an inductive effect on the adjacent stroma to produce myofibroblasts.

Key words: Dysplasia,Verrucous Carcuinoma, Oral Squamous Carcinoma, a-SMA

INTRODUCTION

Squamous cell carcinoma (SCC) is the most common head and neck cancer and holds the sixth position worldwide and it is the frequent type of oral malignancy that exhibits certain histological variations and is associated with a high mortality rate [1&2]. Verrucous carcinoma (VC) is considered to be an uncommon exophytic distinctive low-grade well differentiated pathological variant of OSCC [3].

Cancer is a multifactorial, multifaceted, and multimechanistic disease requiring a multidimensional approach for its diagnosis, treatment, and prevention [4]. Over the past decade, several studies have shown that the microenvironment or stroma of neoplastic tissues plays an active role in tumor progression. Concurrent with the conversion of non-diseased epithelial tissue to pre-cancerous epithelium to carcinoma, the stroma also changes from normal to primed (to activated or tumor associated) [5, 6].

Remodeling of the extracellular matrix or stromagenesis' is initiated by tumor cells, while stromal cells are responsible for the organization of this process [7]. Fibroblasts are considered as one of the most important mesenchymal cells involved in tumor progression [5, 7].

Trans-differentiation of fibroblasts to myofibroblasts is a crucial and early event in tumorigenesis, which is mediated by growth factors and cytokines expressed by tumor cells [5, 6, 7, 8]. Myofibroblasts secrete numerous growth factors and inflammatory mediators that stimulate epithelial cell proliferation [9]. Therefore, these cellular elements play an important role in tumoral invasion and use a combination of different factors in the course of neoplastic growth and development. An invasion promoting role of myofibroblasts has been shown in numerous aggressive and malignant lesions [10, 11, 12, 13, 14]. In addition, decreased CD34+ fibrocytes along with an increase in smooth muscle actin (SMA)-positive myofibroblasts has been observed in invasive oral squamous cell carcinomas (OSCCs) [15]. However, the number of studies evaluating the role of myofibroblasts in OSCC remains limited.

The aim of this study was to compare the distribution of myofibroblasts in normal oral mucosa, oral epithelial dysplasia, verrucous carcinoma, and different histological grades of OSCC.

MATERIALS AND METHODS

The study sample consisted of twenty four formalin–fixed, paraffin-embedded tissue blocks (10 cases were diagnosed as oral squamous cell carcinoma, 8 cases were diagnosed as oral epithelial dysplasia and 6 cases were diagnosed as verrucous carcinoma). The blocks were obtained from the archives of the Department of Oral Pathology/ College of Dentistry / Baghdad University.

Normal oral mucosa was obtained from patients undergoing tooth extraction for orthodontic purposes who had no signs of gingival inflammation or periodontal disease.

Histopathological and immunohistochemical evaluation

The studied cases were reviewed for histopathological reassessment and the suitable cases were selected (graded as well, moderately, poorly differentiated for OSCC and mild, moderate and sever for oral epithelial dysplasia). Data concerning the clinicopathological parameters were obtained from the associated surgical reports.

From each tissue block a 4µm section was obtained for immunohistochemical analysis using anti a- SMA monoclonal antibody (US Biological/ Catalogue No A0760-26).

Negative and positive control slides were included in each IHC run. Colon tissue presented acute appendicitis was used as a positive control for SMA, according to the manufacturer (Fig.1).

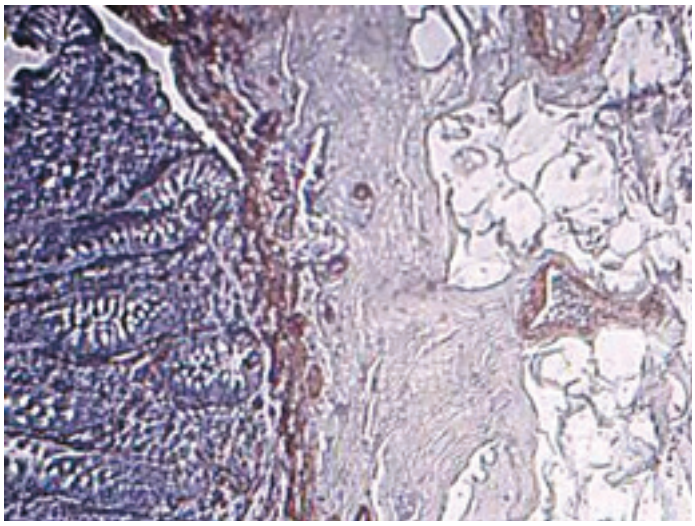

Figure 1: Positive actin immunostaining in acute appendicitis (positive control) (x400)

Slides were baked in hot air oven at 65°C overnight. Sections were sequentially dewaxed and rehydrated through a series of xylene, graded alcohol and water immersion steps. Then endogenous peroxidase activity was blocked followed by blocking the non-specific staining. Anti SMA monoclonal antibody (100 ml) at a dilution (1-200) was applied for each section. The samples were then incubated at 4°C overnight in a humid chamber. After washing with PBS, secondary Ab was applied to the sections, incubated and rinsed with a stream of PBS. Primary Ab was visualized with DAB chromogen. Sections were counterstained with Mayer's hematoxyline for 30 seconds, dehydrated and mounted.

Immunostaining was scored, according to the extent of stromal positivity, as follows (16)

0: Negative or non-reactive.
1- +: Scattered spotty staining.
2- ++: 25% positive cells.
3- +++: 25-50% positive cells.
4- ++++: More than 50% positive cells

RESULTS

A total of 24 cases enrolled in this study, of which 10 cases were oral squamous cell carcinoma, 3 of them were females and 7 were males with an age range from (35-75) years. Four of the cases were located on the tongue, 3 in the mandibular area, 2 at the maxilla and one case on the buccal mucosa. Regarding the histological grading of the OSCC cases 6 of them were diagnosed as well differentiated SCC, 3 were moderately differentiated SCC and only one case was poorly differentiated SCC.

From the total sample 6 cases were verrucous carcinoma, four females and two males, with an age range from (50-70) years. All the cases were located on the mucous membrane of the buccal mucosa and appeared clinically as cauliflower, gray to whitish raised lesions.

Oral epithelial dysplasia represented in 8 cases, three males and five females with an age range from (34-60) years, located at different sites through the mucous membrane of the oral cavity including the buccal mucosa, floor of the mouth and tongue. Intraepithelial dysplasias were recorded as mild (3 cases), moderate (4 cases), and severe (one case).

Blood vessels present within the connective tissue of the immunostained sections served as positive internal control. All cases of SCC, intraoral dysplasia and verruous carcinoma, and normal oral mucosa showed positive reaction of actin in the stromal smooth muscles surrounding blood and lymphatic vessels (Fig.2,3, 4&5).

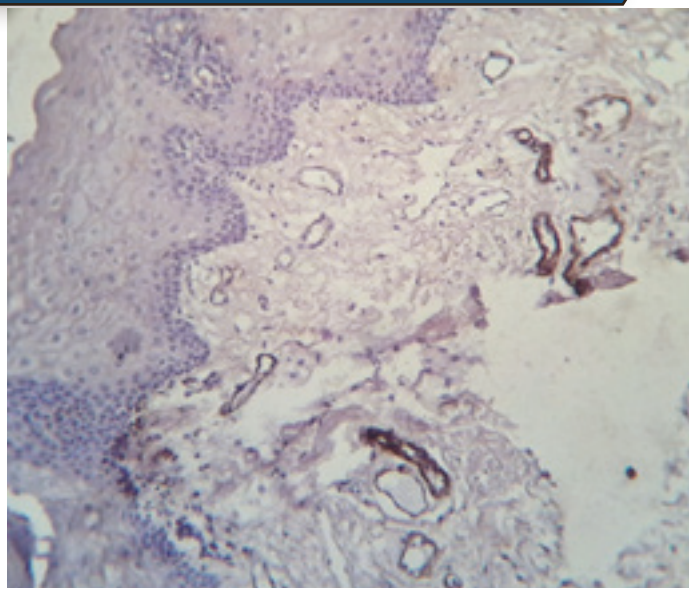

Figure2: Positive actin immunostaining of the smooth muscles surrounding the blood vessels and lymphatic vessels in normal oral mucosa (x400)

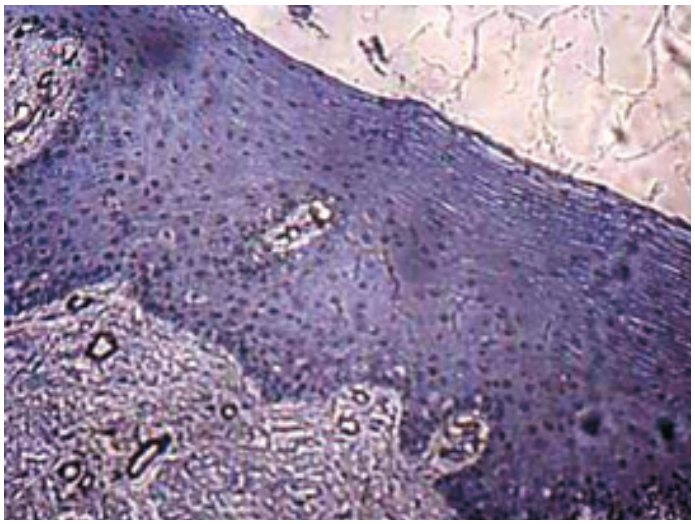

Figure 3: Positive actin immunostaining of the smooth muscles surrounding the blood vessels and lymphatic vessels in dysplasia (x200)

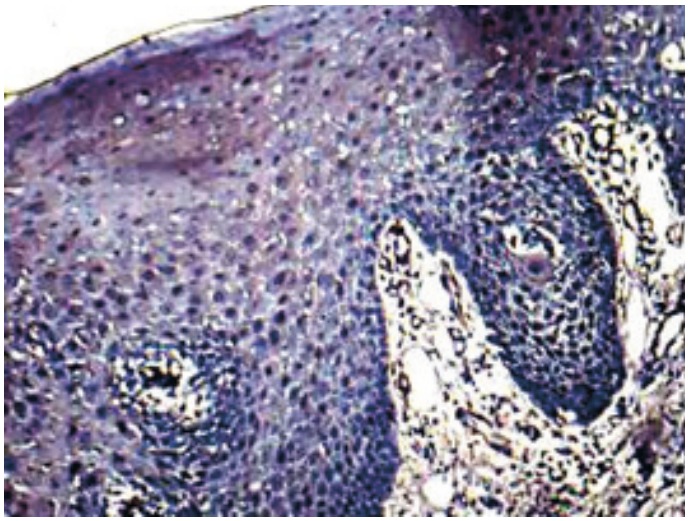

Figure4: Positive actin immunostaining of the smooth muscles surrounding the blood vessels and lymphatic vessels in VC (x200)

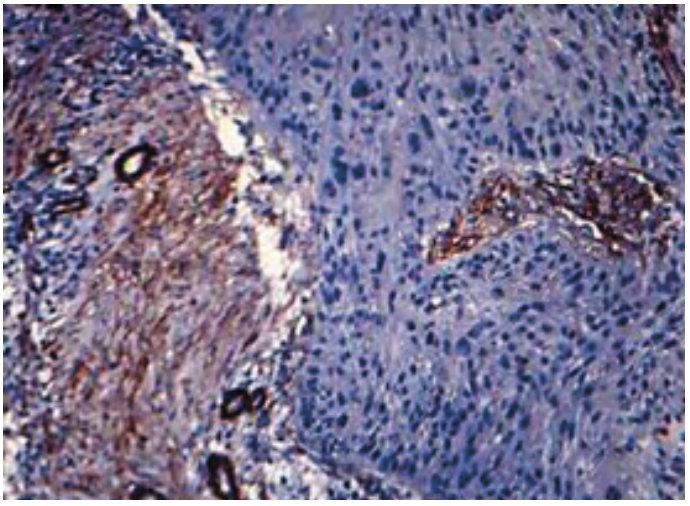

Figure5: Positive actin immunostaining of the smooth muscles surrounding the blood vessels and lymphatic vessels as well as the stroma in well differentiated SCC (x200)

All OSCCs demonstrated stromal alpha-SMA immunostaining with different scores, indicating the presence of myofibroblasts (Fig.5&6). There were no myofibroblasts in the stroma of normal mucosa, epithelial dysplasia or verrucuos carcinoma samples indicated by negative a-SMA expression in them.

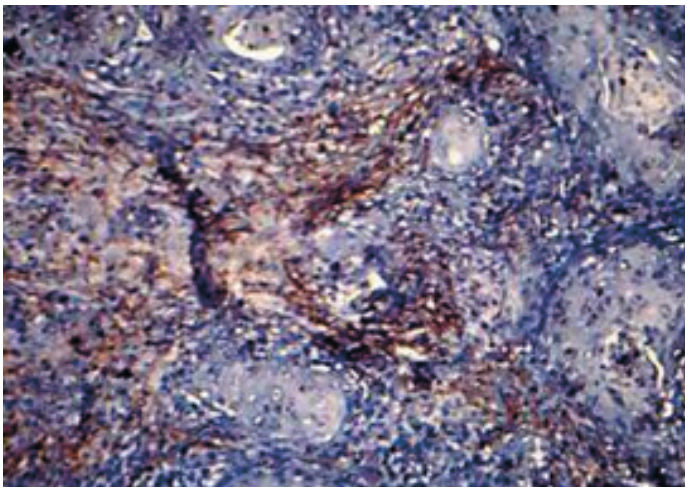

Figure 6: Positive brown stromal actin immunostaining in well differentiated SCC (x200)

Regarding the immunostaining of the tumor cell itself, Out of ten cases of OSCC, only two cases showed (score 1) nuclear actin staining (Fig.7).

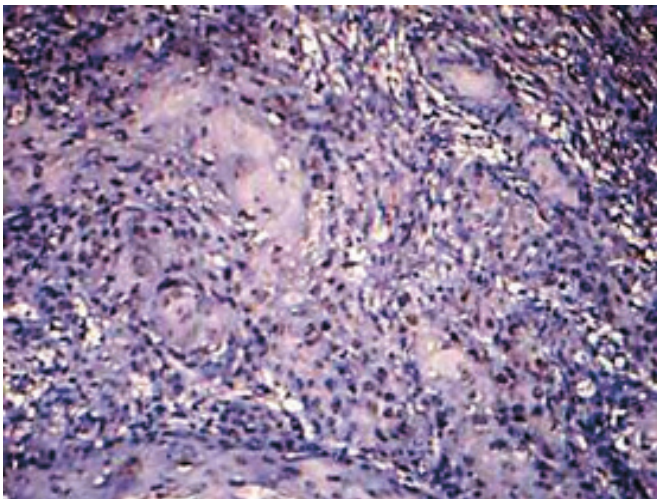

Figure7: Positive nuclear actin immunostaining of tumor cells (well differentiated SCC) (x200)

Out of 8 cases of verrucous carcinoma, 4 cases showed positive immunoreactivity, which appeared as nuclear staining. Two out of these four cases had (score 1) and two had (score 3) actin positive immunostaining (Fig.8).

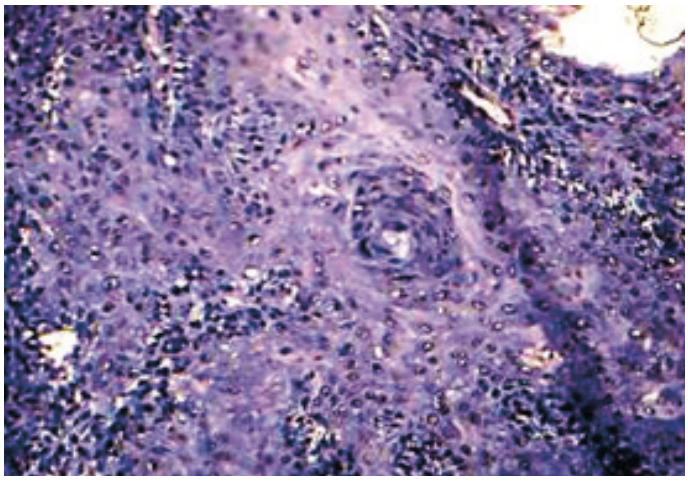

Figure 8: Positive nuclear actin immunostaining of tumor cells in VC (x400)

Out of 6 cases of epithelial dysplasia, three cases showed positive nuclear immunoreactivity in dysplastic cells with (score 2), while the adjacent intact or normal basal cells showed negative reaction (Fig. 9&10).

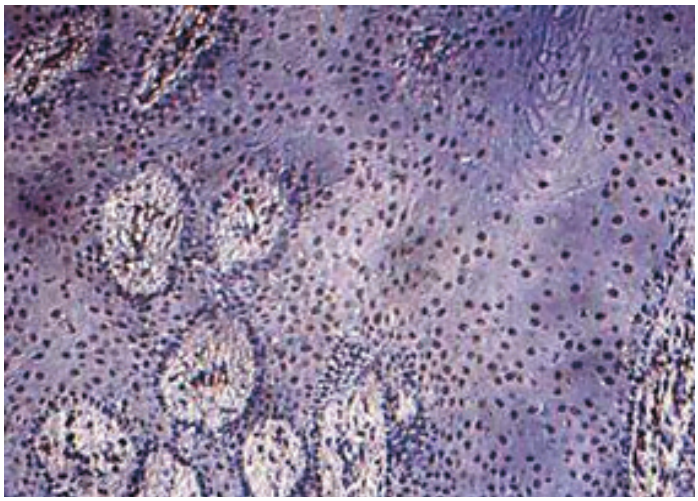

Figure 9: Positive nuclear actin immunostaining of dysplastic cells(x200)

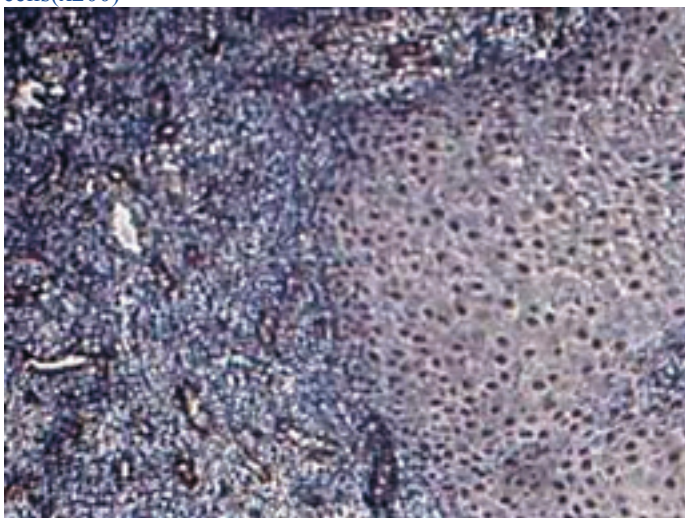

Figure10: Positive actin immunostaining of dysplastic cells, vascular and lymphatic smooth muscles in dysplasia(x400)

DISCUSSION:

It is assumed that carcinogenesis and tumor progression result from a defective response of epithelium and lamina propria due to genetic and epigenetic factors [17]. Several studies have confirmed the important role of the carcinomatous stroma in tumorigenesis, invasion and metastasis [5, 7, 8]. However, the exact mechanism by which different stromal cell types such as myofibroblasts can influence neoplastic cells remains unclear. The altered epithelial cells of SCC would not be solely responsible for carcinogenesis, and different stromal factors participate in its development via communication with the epithelial elements [17]. Transdifferentiation of fibroblasts to myofibroblasts is considered an important event that occurs in the stroma of several invasive carcinomas [10, 11, 14, 18]. According to the results obtained in this study, myofibroblasts were demonstrated in OSCC compared to normal, dysplastic epithelium, and verroucus carcinoma which were devoid of myofibroblasts. These findings are in agreement with those reported by Zidar *et al*. (2002) who also found a lack of myofibroblasts in normal and dysplastic laryngeal epithelium. The presence of myofibroblasts in dysplastic and carcinomatous oral epithelium has not been extensively investigated.

In studies that evaluated the transdifferentiation of fibroblasts to myofibroblasts in OSCC [19, 20, 21, 22], in two of them, the non-neoplastic epithelium adjacent to invasive SCCs was considered as normal tissue and both studies failed to demonstrate myofibroblasts in the approximal connective tissue,while these investigations showed an increased amount of myofibroblasts in the stroma of SCCs, which is in accordance with the results obtained in this study. None of the two studies had included oral epithelial dysplasia in their samples [19, 21].Another study conducted by Lewis *et al*. [22] demonstrated the presence of myofibroblasts in the vicinity of invasive SCC but not in benign mucosal polyps. These cells were also absent in the stroma distant from carcinomatous epithelial islands. Dysplasia was not included in their study sample. Kellermann *et al*. (2007) in a correspondence article reported the prognostic significance of myofibroblasts in SCC of the tongue, normal controls and premalignant leukoplakias with histological dysplasia. Similar to the current study, no myofibroblasts were found in the stroma of normal mucosa and epithelial dysplasia; however they were detected at the invasive front of the SCCs. It is noteworthy that the increase in myofibroblasts found in SCCs may be due to an inducing effect of the carcinomatous component. Epithelial – stromal interactions, different growth factors released by malignant epithelial cells or numerous other

processes may be responsible for the appearance of myofibroblasts.

In this study, the three histological grades showed the presence of myofibroblasts, similar to the finding of Kellermann *et al*. (2007). These findings may suggest that the transdifferentiation of myofibroblasts is induced somewhere in the invasive stage of SCC, and further loss of tumoral differentiation (increased grade) would not affect the number of these cells. According to the tissue organization field theory, cells are normally in a proliferative state and do not tend to be quiescent. Thus mutated epithelial/stromal cells and disturbed stromal – epithelial interactions may be equally responsible for the induction of carcinogenesis [5, 23], emphasizing the importance of the neoplastic microenvironment in oncogenesis.

The nuclear actin immunostaining of the dysplastic and neoplastic epithelial cells observed in this study may reflect its inactive, non-functioning role (prosynthetic state) in these cells since actin expresseion is cytoplasmic and it stains only smooth muscle cells in vessel walls, gut wall and myometrium, myoepithlial cells in breast and salivary glands as well as it reacts with tumor cells arising from smooth muscles and myoepithelial cells (according to the manufacturer's data sheet)

Statistical evaluation was not performed for the different groups in this study due to the small number of cases.

Additional investigations on these myofibroblasts in different stages of carcinogenesis may help to clarify how and to what extent these cells contribute to carcinogenesis.

In summary, considering the lack of myofibroblasts in normal, dysplastic oral epithelium and VC and their appearance in OSCC, it seems that the genetically altered epithelium (carcinomatous epithelium), besides the invasive behaviour of OSCC may have an inductive effect on the adjacent stroma to produce myofibroblasts. However, more sophisticated techniques are suggested to further clarify the exact mechanism by which these important cellular elements exert their effects on stromal and epithelial tissue compartments.

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