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Evaluation of CD31 Expression and Mast Cell Count in Dysplastic Lesions and Squamous Cell Carcinoma of the Oral Cavity

Z Jaafari Ashkavandi[1], M Moshref[2*], F Mashhadi-Abbas[2], S Sargolzaie[2], N Taghavi[2]

[1]Department of Oral and maxillofacial Pathology, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, [2]Department of Oral Pathology, School of Dentistry, Shahid Beheshti of Medical Sciences, Tehran, Iran

Abstract

Background: Squamous cell carcinoma (SCC) is the most common malignancy in the oral cavity. Angiogenesis is essential for development and progression of SCC. Recently, some studies have reported that mast cells play a role in tumor progression, via promoting angiogenesis. The aim of this study was to determine CD31 (an endothelial marker) expression and mast cell count in oral dysplastic lesions and SCC.

Methods: The CD31 expression and mast cell count were investigated in paraffin-embedded specimens of 10 cases of fibroma (control group), 10 cases of epithelial dysplasia, and 20 cases of SCC. CD31 expression was examined by IHC and mast cell count was evaluated by Giemsa staining.

Results: The mean of CD31 expression did not show any significant difference between groups, but in the tumors, peritumoral stroma revealed a significantly higher CD31 expression than intratumoral stroma. A significant difference in the mast cell count was observed between the groups and between peri- and intratumoral stroma of SCCs.

Conclusion: The mean of CD31 expression and mast cell count did not show any correlation. Pre- and post-treatment studies and double staining methods are suggested for more definitive results.

Keywords: SCC; CD31; Mast cell; Oral cavity

Introduction

Squamous cell carcinoma (SCC) is the most common malignancy in the oral cavity. Despite the attempts and approaches to detect the premalignant lesions and SCC at the early stage and treating patients with several modalities, the survival rate of patients has been rather disappointing during the past several decades.[1]

Angiogenesis or neovascularization is essential for development and progression of malignant tumors.[2] This biologic process is regulated by angiogenic factors, like basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), and angiogenic inhibitors, such as angiostatin, platelet factor IV (PF4) and thrombospondin-1 (TSP-1).[3] These factors are released by tumoral and stromal cells.[4] Among the host immune cells, some roles have been implicated for mast cells (MCs) in tumor progression via promoting angiogenesis, mitogenic effects and degradation of extracellular matrix in some malignant tumors.[5] Mast cells originate from bone marrow and migrate to the peripheral tissues where they mature in-situ.[6] Several angiogenic factors mentioned above, and some other factors such as tryptase and chymase have been found in MCs.[3,7] Some authors have reported that assessment of microvessel density and MC count could serve as useful prognostic tool for patients after surgical surgery.[8,9] The aim of the present study was to

***Correspondence:** Mohammad Moshref, MD, Associate professor of Department of Oral Pathology, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran Tel: +98-21-22403194, Fax: +98-21-22990302, e-mail: moshrefdds@yahoo.com
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evaluate the number of MCs and microvessels in the oral dysplastic epithelium and SCC (OSCC).

Materials and Methods

Twenty histologically confirmed cases of oral SCC including invasive front, 10 cases of oral dysplastic epithelium with various differentiated features, and 10 cases of irritation fibroma (as control group) were retrieved from the files of the oral pathology department of Shahid Beheshti dental faculty.

The specimens from the paraffin-embedded blocks were cut into 5-µm sections. Standard immunohistochemistry staining was performed. Monoclonal mouse anti-human CD31-antibody (Dako, JC70A) was used for detection of endothelial cells. Immunostaining was performed with the avidin-biotin-proxidase complex detection kit (Dako, Danmark). Briefly, the sections were placed on silicon coated slides and were dewaxed. Endogenous proxidase activity was blocked by incubation with 0.3% hydrogen peroxide in absolute methanol for 30 min. To retrieve the antigenicity, the sections were treated with microwaves in 10 mM citrate buffer (pH=6) for 15 min. After treatment with non-immune serum, to block non-specific binding, the specimens were incubated in a moist chamber with monoclonal mouse anti-human primary antibody for CD31 at 4° C overnight. Following incubation with the primary antibody, the sections were washed twice in phosphate buffered saline (PBS), and incubated with secondary antibody (anti-mouse and anti-rabbit Ig, Dako, K0637) and treated with streptoavidin-biotin complex. The sections were visualized with diaminobenzidine (Dako, LSAB2, K0673). Finally, they were counterstained with Mayer's hematoxylin. Blood vessels in the hemangioma were used as internal positive control. After dewaxation and dehydration, the sections were stained by Giemsa solution (1:20 dilution) for 20 min.

The methods of microvessel staining and counting were performed according to previous studies.[10] The stained sections were screened at ×40 magnifications to identify the areas of the highest vascular density (hot spots). The stained vessels were counted in the five areas of hot spot at ×400 magnification. The selected areas were the connective tissue subjacent to the epithelium in the tissues of fibroma and dysplastic epithelium. In the sections of OSCCs, the hot spots were selected in intratumoral and peritumoral stroma separately. The average of microvessels in selected areas was determined as microvessel density. Mast cells were counted at five similar areas (hot spots) and the average was determined as MC count.

Comparison of data between groups was done by Kruskal-Wallis analysis. In SCCs samples, peri- and intra-tumoral areas were compared by Mann-Whitney test. Statistical significance was set at $p<0.05$ for all tests.

Results

Vessels stained brown with CD31-antibody (including most of mature vessels with thick muscular layer, small vessels with lumen formation and less common clusters of endothelial cells without lumen formation). In fibroma, most of the stained vessels were seen from subepithelial area to deep connective tissue, but in dysplastic epithelium and OSCCs, staining was not seen in some vessels (Figures 1A-C). Three

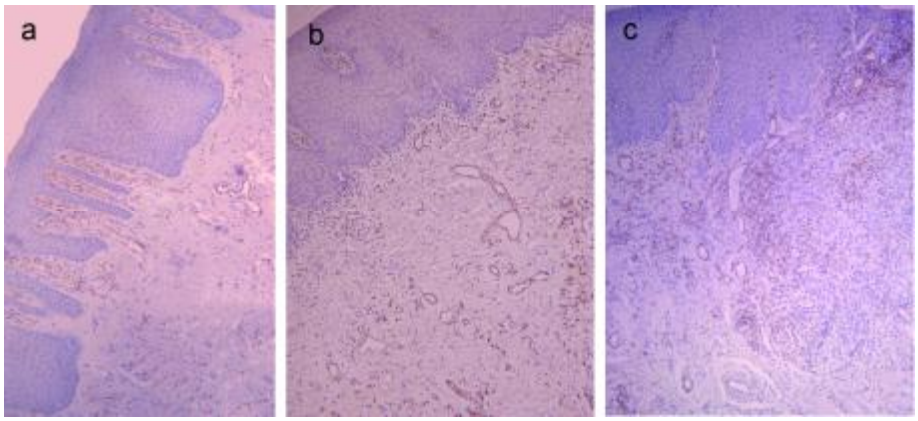

Fig. 1: CD31- staining in Fibroma (a), Dysplasia (b), Squamous cell carcinoma (SCC) (c) at ×100 magnification

samples of SCCs did not show any staining. Some inflammatory cells were co-stained by this antibody. The average of the CD31 staining in fibroma, dysplasia and SCC is shown in Table 1. The difference of staining between these groups was not significant (p>0.05). The peritumoral area showed significantly more stained vessels than intra-tumoral stroma (p=0.018).

Table 1: CD-31 staining in three groups

Specimen	CD-31 staining (mean±SD)	*P* value
Fibroma	28±12.2	0.738
Dysplasia	26.1±12.2	
peritumoral sroma of SCC	21.6±11	0.018
Intratumoral stroma of SCC	9.7±7	

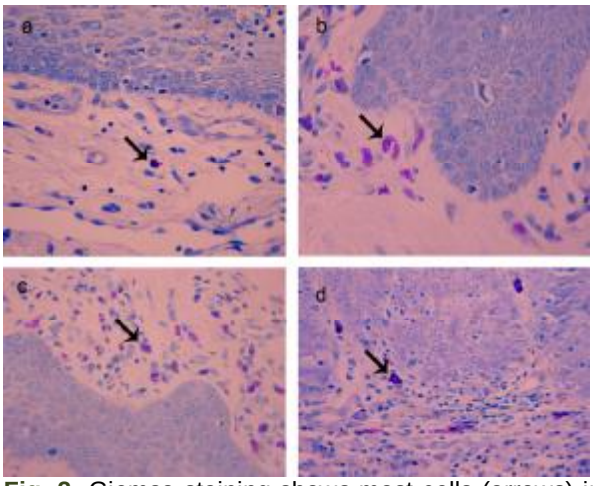

Fig. 2: Giemsa staining shows mast cells (arrows) in Fibroma (a), Dysplasia (b), peritumoral stroma of squamous cell carcinoma (SCC) (c), intratumoral stroma of SCC (d)

Mast cells were oval, short spindled or stellate cells with basophilic cytoplasmic granules (Figures 2A-D). Most of them appeared to accumulate in association to vessels and some of them were in the state of degranulation. The average of MC density in fibroma, dysplasia and SCC is shown in Table 2. The MC count was significantly higher in SCC than dysplasia and fibroma (p<0.05). Peritumoral MC count revealed a significant increase when compared with intra-tumoral stroma (p<0.001).

The MC count and CD31-staining in all of the lesions are compared in Figure 3. Statistical analysis did not reveal any significant correlation between them. Despite the absence of correlation between the three groups, in each group, the samples with higher MC count showed higher CD31-staining. The average of CD31-staining and MCs was compared among various grades of dysplastic epithelium and SCC and no significant difference was seen (p>0.05).

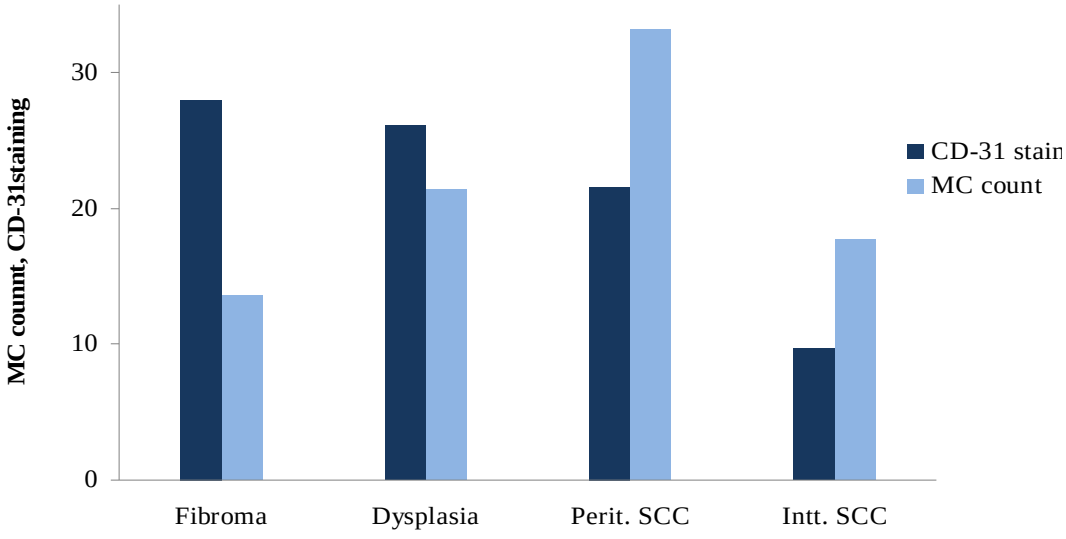

Fig. 3: Mast cell (MC) count and CD-31 staining in three groups.
(Perit: peritumoral stroma of squamous cell carcinoma (SCC), Intt: intratumoral stroma of SCC)

Table 2: Mast cell (MC) count in three groups

Specimen	MC count (mean±SD)	*P* value
Fibroma	13.6±8.5	0.049
Dysplasia	21.4±7.8	
peritumoral sroma of SCC	33.2±14	<0.001
Intratumoral stroma of SCC	17.7±7.1	

Discussion

Tumoral blood vessels in SCC have been investigated in several recent studies by different vascular markers. The most common markers were used including VEGF, VWF (Von-Willebrond Factor), CD31, CD34 and CD105. In the present study, we used CD31-antibody to identify endothelial cells in oral SCC. CD31-staining was seen in the endothelial and inflammatory cells, as previously reported.[11] Shieh *et al*. reported that CD31 and CD34 were more sensitive than factor VIII-RA for evaluating the tumor blood vessels of oral SCC and CD31 or CD34 are generally a choice for paraffin-embedded sections.[11]

According to Alessandri *et al*., CD31 is detected in the endothelial cells ranging from non-lumen-forming cells to lumen-forming ones.[12] The present study showed that CD31-antibody stained mature vessels, smaller lumen-forming vessels and less common endothelial cells without lumen formation.

In the peritumoral and intratumoral stroma of the SCCs, non-stained vessels were more than the control group and CD31-staining did not show any significant difference during transition from control group through the dysplasia to cancer. Minhajat *et al*. also reported that CD31-antibody is expressed in 80-95% of the normal mucosa, adenoma and adenocarcinoma of colon; no significant difference was noted among these groups.[13] Their results also revealed that CD105 did not stain mature vessels. Rainier et al. and Pazouki demonstrated that expression of CD34 and VWF markers did not differ significantly between dysplasia and SCC.[14,15] Turner *et al.* reported that CD31 expression in pituitary adenoma was less than that in normal tissue, but others have revealed that expression of endothelial markers such as VWF, CD31 and CD34 increases in agreement with progression of histological abnormality.[3,11,16]

According to the above-mentioned points, there are no consistent results about vascular density in various studies. It has been proposed that these discordant results could be attributed to the use of different vascular markers, different methods in the microvessel counting (in hot spots area or the mean of total vascularity), antigenic retrieval and inter-observer variations.[17] Schor *et al.* demonstrated that all of these factors affect the assessment of vascularity.[18] So, these factors pose potential limitation for comparison in these studies.

Our results demonstrated a significant increase in peritumoral vascular staining versus intra-tumoral area. Our findings are in agreement with those reported by previous authors that found that vascular hot spots were encountered predominantly at the peripheral tumor margins.[13,19]

Shieh *et al.* suggested that at the initiation of oral SCC, increasing vascularity is observed at the periphery of the tumor. As the tumor progresses, intratumoral vascularity increases more. They demonstrated that peritumoral vascular density was not consistent with intra-tumoral area and explained, in part, why angiogenesis in oral SCC remains controversial.[11]

This study showed that one endothelial marker does not show a useful angiogenesis index and for determining a correct vascular density, employing several markers is necessary. Minhajat *et al.* also reported that CD105 did not detect some of the vessels in the tumoral tissues of the colon.[13]

The presence of MCs in the periphery of the tumor was first reported by Westple *et al.* in 1981 and then confirmed by several investigators.[20,21] This study demonstrated the increase of MCs in oral SCC when compared with dysplasia and control group. These findings confirmed previous reports which showed an increase in MC count in SCC than normal mucosa,[3,7] but Oliveira *et al*. reported a decrease in MCs in OSCC when compared with leukoplakia and normal oral mucosa.[22]

The accumulation of mast cells in the periphery of the tumors might explain more vascular density in this area. Mast cell's chymase is associated with matrix degeneration, angiogenesis and tumor growth.[23] MCs also contained other potent angiogenic factors.[3]

Our results did not reveal a positive significant correlation between CD31-staining and MC count when the three groups were compared while in each group, the specimens with high CD31-staining showed a higher MC count. According to our results, to achieve a reliable correlation between vascular density and mast cells, a pre- and post -treatment study should be designed and double-staining methods with suitable markers for detecting endothelial cells should be used.

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Conflict of interest: None declared.

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