Journal of Research in Medical and Dental Science
2018, Volume 6, Issue 5, Page No: 165-169

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Comparative Evaluation of Density of Mast Cells and Microvessel in between Oral and Cutaneous Squamous Cell Carcinoma

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ABSTRACT

*Mast cells and microvessel play an important role in tumor development and progression through angiogenesis. Because of the more aggressive potential and poor prognosis of oral squamous cell carcinoma (OSCC) compared to cutaneous squamous cell carcinoma (CSCC), the aim of the present study was to compare of density of mast cells and microvessel between in oral and cutaneous squamous cell carcinoma. In this study cross-sectional study, we performed an immuno histochemical analysis in 100 paraffin blocks that included 40 cases of OSCC and 40 CSCC and 20 cases of normal skin and normal oral mucosa. Mast cells and microvascular density were stained by mast cell tryptase and immunohistochemistry assay using CD34. Statistical analyses were performed in SPSS 16 software. There was a significant statistical correlation (*P<0.05) between mast cell concentration and microvascular density in OSCC and CSCC compared to control groups. In OSCC the mast cell concentration and microvascular density were significantly higher (*P<0.05) compared with the CSCC. Therefore, according findings present study, mast cell concentration and microvascular density may be used as a factor for detecting the progression, malignant potential and invasion of OSCC compared to CSCC.*

Key words: Squamous cell carcinoma, Mast cell, Microvessel, Prognosis

HOW TO CITE THIS ARTICLE: Khadijeh Abdal, Mohammad Ali Roozegar, Parya Emamverdizadeh, Samira Mostafazadeh*, Comparative evaluation of density of mast cells and microvessel in between oral and cutaneous squamous cell carcinoma, J Res Med Dent Sci, 2018, 6 (5):165-169

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Received: 08/08/2018
Accepted: 17/09/2018

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and cutaneous squamous cell carcinoma (CSCC) is the second most common skin cancer [1]. Despite the advances in tumor detection methods, the afflicted patients show a low five-year survival rate [2,3]. The stage and grade of the tumor are the most important factors in determining the prognosis and treatment of OSCC. However, since the tumor shows various biological behaviors, the histopathological evaluation and the stage of the disease do not always contribute to the determining of the disease prognosis. As with normal tissue, vascularization plays an important role in the growth and development of tumoral cells, which in turn, stimulate angiogenesis which is required for the continuous progression and metastasis of the tumor [4,5]. Tumor-based angiogenesis factors are either made by tumor cells or derived from inflammatory cells that spread throughout the tumor. Interaction of the tumor with the immune system and the degree of microvascularity can affect the biological behavior of the tumor, and this can be used as a predictor of the state of the disease. It has been suggested that mast cells play a significant role in the promotion of angiogenesis [6,7]. Mast cells have been reported to behave differently in various tumors, probably due to their ability in producing various cytokines related with the tumor growth and angiogenesis, such as tryptase and kinase serine protease, interleukin (IL)-8, tumor necrosis factor (TNF)-α, and transforming

growth factor (TGF)-β, fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) [8]. It has been shown that mast cells produce both tumor resistance and simulation of tumoral cell proliferation [9,10]. In prostate and gastrointestinal cancers as well as lymphomas, the increasing of mast cell concentration has been associated with poor therapeutic results [11,12]. The protective role of mast cells against cancers has also been noted [13]. An *in vitro* study showed preventive effect of mast cells on proliferation of SCC in oral mucosa, while another animal study indicated mast cell activity was along with an increase of oral cavity tumoral cells proliferation [14,15]. Research results show malignant, metastatic potential and prognosis for OSCC and CSCC is different, so that the survival rate OSCC is less than the CSCC. For OSCC, 5- year survival rate varies between 35% and 45% that depends on tumor stage (the size of the lesion, without metastasis or with metastasis). Whereas 5-years survival of CSCC is 54%. The risk of metastasis for OSCC is about 40% and 50%, while for CSCC is 11.7% [4,13]. Although some patients die of their disease as many as 10 years after initial treatment, the great majority of deaths occur within the first 5 years [7]. Since tumor behavior and metastasis is affected by angiogenesis, it seems plausible to evaluate the possibility of determining microvessels and mast cell presence in tumoral tissue. Therefore, the aim of the present study was to compare microvessels and mast cell presence between OSCC and CSCC, to know if their presence may or may be associated with the potential malignancy of the OSCC compared with the CSCC.

MATERIALS AND METHOD

In this retrospective study, 100 paraffin embedded specimens included: 40 of OSCC grade I, 40 of CSCC grade I, 10 of normal oral mucosa (NM) and 10 of normal skins (NS). The study protocol was in accordance with WMA Declaration of Helsinki regarding ethical principles for medical research involving human subjects and approved by the Ethics Committee of Ilam University of Medical Sciences. The first set of slides were stained with hematoxylin-eosin staining and it was observed under an optical light microscope (Olympus BX41TF, Tokyo, Japan) at 400X magnification to determine the histopathologic grade of the tumor [16]. The second set of prepared slides from the same blocks were stained using anti-mast cell tryptase primary monoclonal antibody to examine the mast cell concentration. A paraffin embedded tissue section of 3-5 µm thickness. These sections were immersed in 0.3% H_2O_2 (SID 24852978, SIGMA-ALDRICH.St.louis-United States) for 15 minutes at room temperature to block the endogenous peroxidase activity. To retrieve the antigens, the slides were heated in a microwave oven and then incubated with anti-mast cell tryptase primary monoclonal antibody (Code: M7052 Dako, Glostrup, Denmark) for 30 minutes. Then, it was washed with phosphate-buffered saline (Code: 10468543, Oxoid, Loughborough-United Kingdom) for 1 hour. Finally, the sections were counterstained with Harris hematoxylin (3801560BBE, Leica Microsystems Inc., Buffalo Grove-United States) and then dehydrated and lamella placed on them. The mast cell concentration was evaluated by tryptase marker as hot spot in tumor stroma which was marked with the most intense staining under light microscope at 400X magnification [17]. Evaluations were made at 10 fields of OSCC and CSCC. Intensity of cell staining for mast cells and microvascular was categorized as following: 0=negative; 1=weak; 2=moderate; 3=severe [13]. Samples that have not enough lesion for immunohistochemical staining, were excluded from the study because sulfated proteoglycans in secretory granules of mast cells have a metachromatic property that can be stained by toluidine blue, the sections were immersed in toluidine blue (SID 24889244. SIGMA-ALDRICH, St. Louis-United States). They were then dehydrated quickly through 90% and two changes of 100% alcohol. Sections were then cleared in xylene, and mounted with DPX mounting medium. A positive comparison control (human tonsil tissue) was used to ensure accurate color. Mast cells density (MCD) and microvascular density (MVD) were determined per high power field HPF (at 400X). To determine microvascular density MVD the third set of slides were used to examine

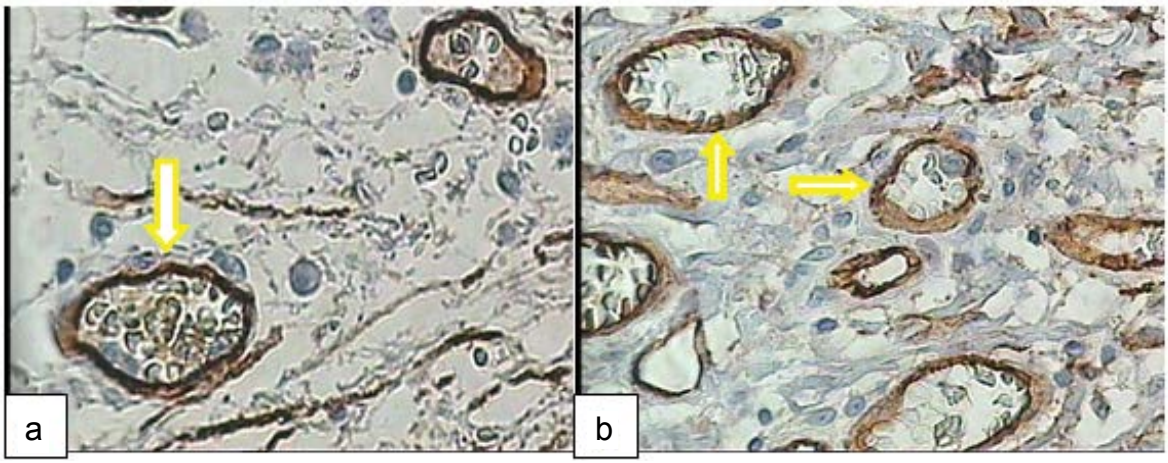

Figure 1: (a) The mild CD34-positive microvessels in skin squamous cell carcinoma; (b) The severe CD34-positive microvessels in oral squamous cell carcinoma (Arrows shows the microvessels (400X))

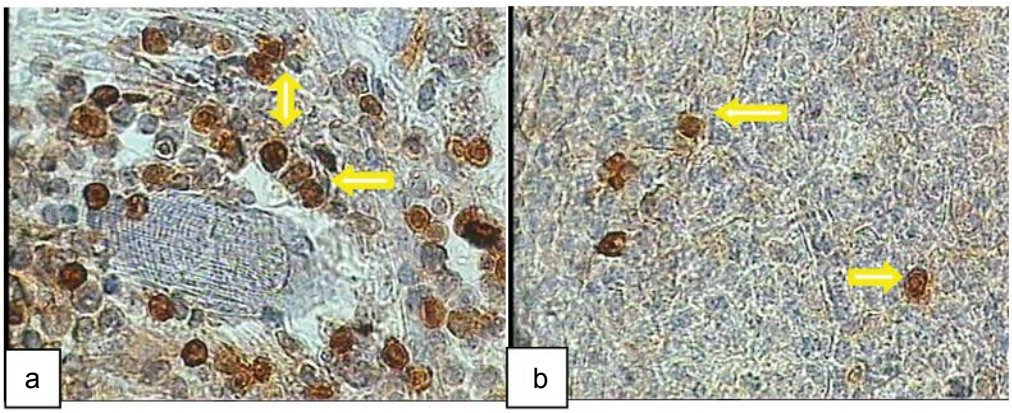

Figure 2: (a) The severe Tryptase-positive expression mast cells in oral squamous cell carcinoma; (b) The mild Tryptase-positive expression mast cells skin squamous cell carcinoma (Arrows shows the mast cells (400X))

MVD by immunohistochemistry assay using anti CD34 monoclonal antibody. Method of preparing the slides was as the same for the second set, but in this case we used a specific monoclonal antibody against CD34 [17]. A positive comparison control (hemangioma) was used to ensure accurate color. The microvascular and mast cells density was evaluated in normal mucosa and skin (20 samples), OSCC and CSCC (80 sample), in five fields under an optical light microscope (Olympus BX41TF, Tokyo, Japan) at 400X magnification per each case. The areas of the most intense vascularization (hot spot) were counted, and the average count in each case was recorded. All vessels with an endothelial covering that appeared reddish brown and clearly separated from adjacent stromal and tumor cells were considered as unique accounting microvessels (Figure 1). Three HPFs containing the largest number of mast cells adjacent to tumor invasive margins were selected and mast cells were counted and their measure that is MCD was given as the number of mast cells seen per HPF (at 400X) (Figure 2). All data were analyzed by SPSS 16 software using descriptive statistical methods (mean ± standard deviation), ANOVA, Spearman's Correlation Coefficient. In this study $^{*}P<0.05$ was considered statistically significant.

RESULTS

In total, 100 samples of: 40 OSCC, 40 CSCC, 10 normal oral mucosa (NM) and 10 normal skins (NS) were evaluated. Staining intensity of microvascular in OSCC: out of 20 samples tested for OSCC, 2 cases weak staining (10%), 3 cases moderate staining (15%) and 15 cases severe (75%) also out of 10 samples tested for NM, 7 cases negative staining (70%), 3 cases weak staining (30%). Staining intensity of mast cell in OSCC: out of 20 samples tested for OSCC, 10 cases moderate staining (50%) and 10 cases sever (50%) also, out of 10 samples tested for NM, 9 cases negative staining (90%), 1 case weak staining (10%). Staining intensity of microvascular in CSCC: Out of 20 samples tested for CSCC, 4 cases weak staining (20%), 11 cases moderate staining (55%) and 5 cases severe (25%) also, out of 10 samples tested for NS, 8 cases negative staining (80%), 2 cases weak staining (20%). Staining intensity of mast cell in CSCC: Out of 20 samples tested for CSCC, 11 cases weak staining (55%), 7 cases moderate staining (35%) and 2 cases severe (10%) also, out of 10 samples tested for NM, 7 cases negative staining (70%), 3 cases weak staining (30%). The mean percentage of mast cells and microvascular of OSCC, CSCC, NM and NS in presented in Table 1 and Table 2. OSCC samples had the highest mean percentage of mast cells and microvascular among all the samples. To compare the frequency of microvascular and mast cells, independent t-test was used. The results show that there is a significant difference between the mean percentage of mast cell and microvesseles OSCC with NM and between CSCC with OSCC ($^{*}P<0.05$). The average concentration of mast cells of was 395.60 OSCC and 1.49 CSCC. The mean number of microvascular hot spots was 12.56 OSCC and 01.37 CSCC. Density of mast cells and microvessels has been revealed in. According to Spearman's Correlation Coefficient, there was a significant statistical correlation between mast cell concentration and microvascular density OSCC and CSCC (rs=0.77, N=80, P<0.05). According to Spearman's Correlation Coefficient, there was not a significant statistical correlation between mast cell concentration and microvascular NS and NM (P>0.05). Mast cell concentration and microvascular density were significantly higher OSCC in compared to CSCC ($^{*}P<0.05$). Mast cell concentration and microvascular density in OSCC and CSCC were significantly higher compared to NS and NM ($^{*}P<0.05$). The mean percentage of mast cell and microvessels in studied groups is summarized Table 3.

Table 1: Density microvessels in all samples

Group	Negative	Weak	Moderate	Severe
Normal skin	8 (80%)	2 (20%)	0 (0%)	0 (0%)
Normal oral mucosa	7 (70%)	3 (30%)	0 (0%)	0 (0%)
Cutaneous squamous cell carcinoma	0 (0%)	4 (20%)	11 (55%)	5 (25%)
Oral squamous cell carcinoma	0 (0%)	2 (10%)	3 (15%)	15 (75%)

Table 3: The mean percentage of mast cells and microvessels in all samples

Group	Mean of mast cell density	Mean of microvessels	Std. Deviation microvessels	P- value	Std. Deviation mast cell	P- value
Normal skin	1.5	1	1.5239	-	2.2132	-
Normal oral mucosa	2.2	3.4	5.2124	-	4.1312	-
Cutaneous squamous cell carcinoma	1.49	1.37	3548.15	939	37842.11	186
Oral squamous cell carcinoma	395.6	12.56	3938.16	378	55499.14	100

Table 2: Density of mast cells in all samples

Group	Negative	Weak	Moderate	Severe
Normal skin	7 (70%)	3 (30%)	0 (0%)	0 (0%)
Normal oral mucosa	9 (90%)	1 (10%)	0 (0%)	0 (0%)
Cutaneous squamous cell carcinoma	0 (0%)	11 (55%)	7 (35%)	2 (10%)
Oral squamous cell carcinoma	0 (0%)	0 (0%)	10 (50%)	10 (50%)

DISCUSSION

Since tumor microenvironment can affect several cellular events such as growth, death, differentiation, gene expression, immigration and metastasis, it plays a key role on tumor progression [8]. While microvascular density has drastic effect on growth, development, and transformation to metastasis of tumoral cell, as tumor growth over 1-2 millimeter is almost impossible without formation and presence of new blood vessels [4,5]. Our findings in this study were similar. The role of various cells in formation of microenvironment is another effective factor in tumor growth. Various roles have been described for mast cells as part of immune system in separate studies about tumor immunity as inhibitor or inducer of tumor growth [6,7]. Recent studies of malignant solid tumors have indicated that mast cells may play role in tumor angiogenesis and their concentration would be a predictor marker [11]. Several angiogenesis factors like VEGF have been identified in mast cells, tryptase in mast cells also may be an angiogenesis factor, on the other hand there is little knowledge about mast cells role and their tryptase in OSCC and CSCC progress, therefore in this study association of mast cell concentration and microvascular density in OSCC and CSCC has been investigated [14]. The findings of this study showed significant differences microvascular density and mast cell concentration between OSCC and CSCC compare to NS and NM suggested their contributing role in tumor growth and progression, these findings are similar with the results of study by Sina et al. [18]. In the present study, there was a correlation between the mast cell concentration and microvessel density. Consistent with results of the study Iamaroon et al. have reported that there was a significant relation between tryptase positive mast cell concentration and microvascular density in OSCC and CSCC to compare NM and NS [19]. In another research by Sharma et al. there has been correlation between the mast cell concentration and microvascular density in OSCC lesions [20]. Acikalin et al. indicated that there is relation between number of blood vessels (Anti-CD34) and mast cell concentration colorectal carcinomas which was consistent with malignant damages assessment of this study [21]. Similarly, there is positive relation between mast cell concentration and tumor grade since less differentiated tumors have more mast cells than highly differentiated ones which been found in the present study. The mast cell concentration increased in colorectal, liver and gall bladder carcinoma also [22,23]. In the OSCC there was a positive correlation between mast cell concentration and microvascular density concluded to compare CSCC at this research which was in accordance with results of Sina et al. and Sharma et al. [18,20]. Astekar et al. reported that microvascular density with CD34 decreases as grade increases which led to different results compared to ours that this difference may be due to small sample size of the study Astekar due (30 SCC samples) [24]. According to Sina et al. microvascular density and mast cell concentration can be supposed as a predictor of aggressive behavior in OSCC compare to CSCC which agrees with our findings [18]. According to the findings of the present and similar studies, mast cell concentration and microvessel density can be a helpful diagnostic adjuvant in determining more progression and malignant potential in OSCC to compare CSCC while the results of the Dodani et al. studies show that different biologic behavior of OSCC compared to CSCC doesn't depend on myofibroblasts and other factors can be involved [13]. Because of the novelty of Present study and the lack of previous similar studies in the field with subject (comparison of microvascular density and mast cell concentration between OSCC and CSCC), we could not compare this study with other previous studies.

CONCLUSION

Therefore, according findings present study, mast cell concentration and microvascular density may be used as a factor for detecting the progression, malignant potential and invasion of OSCC compared to CSCC.

ACKNOWLEDGMENT

Hereby, we thank Ilam University of Medical Sciences for their support of this research.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. N Engl J Med 2001; 344:975-83.
2. Khiavi MM, Abdal K, Abbasi MM, et al. Comparison of injectable doxorubicin & its nanodrug complex chemotherapy for the treatment of 4-nitroquinoline-1-oxide induced oral squamous cell carcinoma in rats. Indian J Med Res 2017; 145:112.
3. Manfredini M, Longo C, Ferrari B, et al. Dermoscopic and reflectance confocal microscopy features of cutaneous squamous cell carcinoma. J Eur Acad Dermatol Venereol 2017; 31:1828-33.
4. Zaidi MA, Mallick AK. A study on assessment of mast cells in oral squamous cell carcinoma. Ann Med Health Sci Res 2014; 4:457–60.
5. Carlile J, Harada K, Baillie R. Vascular endothelial growth factor (VEGF) expression in oral tissues: possible relevance to angiogenesis, tumour progression and field cancerisation. J Oral Pathol Med 2013; 30:449-57.
6. Macluskey M, Chandrachud LM, Pazouki S. Apoptosis, proliferation, and angiogenesis in oral tissues. Possible relevance to tumour progression. J Pathol 2010; 191:368-75.
7. Gudiseva S, Santosh AB, Chitturi R, et al. The role of mast cells in oral squamous cell carcinoma. Contemp Oncol 2017; 21:21.
8. Cheema VS, Ramesh V, Balamurali PD. The relevance of mast cells in oral squamous cell carcinoma. J Clin Diagn Res 2012; 6:1803-07.
9. Conti P, Castellani ML, Kempuraj D, et al. Role of mast cell in tumor growth. Ann Clin Lab Sci 2007; 37:315-22.
10. Shivamallappa SM, Venkatraman NT, Shreedhar B, et al. Role of angiogenesis in oral squamous cell carcinoma development and metastasis: An immunohistochemical study. Int J Oral Sci 2011; 3:216-24.
11. Wadhwan V, Sharma P, Saxena C, et al. Grading angiogenesis in oral squamous cell carcinoma: A histomorphometric study. Indian J Dent Res 2015; 26:26-30.
12. Florence ME, Massuda JY, Bröcker EB, et al. Angiogenesis in the progression of cutaneous squamous cell carcinoma: An immunohistochemical study of endothelial markers. Clinics (Sao Paulo) 2011; 66:465-68.
13. Dodani A, Siadati S, Salehinejad J, et al. Comparative evaluation of the frequency of myofibroblasts between oral and cutaneous squamous cell carcinomas. Caspian J Dent Res 2016; 5:24-29.
14. Elezoğlu B, Tolunay S. The relationship between the stromal mast cell number, microvessel density, c-erbB-2 staining and survival and prognostic factors in colorectal carcinoma. Turk Patoloji Derg 2012; 28:110-18.
15. Aromando RF, Perez MA, Heber EM, et al. Potential role of mast cells in hamster cheek pouch carcinogenesis. Oral Oncol 2013; 44:1080-87.
16. Neville BW, Damm DD, Chi AC, et al. Oral and maxillofacial pathology. Elsevier Health Sciences 2015.
17. Mohtasham N, Babakoohi SH, Nejad JS. Mast cell density and angiogenesis in oral dysplastic epithelium and low and high grade oral squamous cell carcinoma. Acta Odontol Scand 2010; 68:300-04.
18. Sina M, Abdal K, Ghertasi S, et al. Correlation between mast cell concentration and microvascular density with grade and stage of oral squamous cell carcinoma. J Res Dent Sci 2015; 12.
19. Iamaroon A, Pongsiriwet S, Jittidecharaks S, et al. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. J Oral Pathol Med 2003; 32:195-99.
20. Sharma B, Sriram G, Sarasswathi T, et al. Immunohistochemical evaluation of mast cells and angiogenesis in oral squamous cell carcinoma. Indian J Dent Res 2010; 21:260-65.
21. Acikalin MF, Oner U, Topcu I, et al. Tumour angiogenesis and mast cell density in the prognostic assessment of colorectal carcinoma. Dig Liver Dis 2012; 37:162-69.
22. Marla V, Hegde V, Shrestha A. Relationship of Angiogenesis and Oral Squamous Cell Carcinoma. Kathmandu Univ Med J 2015; 13:178-85.
23. Kim JH, Kang YJ, Kim DS, et al. The relationship between mast cell density and tumour grade in transitional cell carcinoma of the bladder. J Int Med Res 2011; 39:1675-81.
24. Astekar M, Joshi A, Ramesh G, et al. Expression of vascular endothelial growth factor and microvessel density in oral tumorigenesis. J Oral Maxillofac Pathol 2012; 16:22-6.