DOI: 10.5958/2319-5886.2015.00008.9

International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 4 Issue 1 Coden: IJMRHS Copyright @2014 ISSN: 2319-5886

Received: 5th June 2014 Revised: 28th July 2014 Accepted: 2nd Sep 2014

Research article

MAST CELLS AND ANGIOGENESIS IN ORAL EPITHELIAL DYSPLASTIC LESIONS AND ORAL SQUAMOUS CELL CARCINOMA

*Hegde Veda[1], Marla Vinay[2]

[1]Associate Professor, Department of Oral Pathology, SDM College of Dental Sciences and Hospital, Sattur, Dharwad, Karnataka, India.

[2]Post Graduate Student, SDM College of Dental Sciences and Hospital, Sattur, Dharwad, Karnataka, India

*Corresponding author email: hegdeveda6@rediffmail.com

ABSTRACT

Background: The progression of oral epithelial dysplastic lesions into oral squamous cell carcinoma is characterized by an 'angiogenic switch' which is characterized by an increase in neo-vascularization in the sub-epithelial lamina propria which can be considered an indicator of malignant transformation. Mast cells are a rich source of various angiogenic factors. Moreover mast cells secrete various proteolytic enzymes which degrade the extracellular matrix and create space for the developing blood vessels. **Aims:** This study was undertaken to determine the relationship between mast cell density and microvessel density in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma and to find out whether any correlation exists between these two parameters. **Material and Methods:** This retrospective study was performed using formalin fixed, paraffin embedded tissues of previously diagnosed cases of oral epithelial dysplasia and oral squamous cell carcinoma. Mast cells were stained using toluidine blue, whereas in the capillaries, immunohistochemical staining technique was performed using mouse monoclonal antibody against CD34. **Results:** Mast cell density and microvessel density were higher in oral epithelial dysplasia and in oral squamous cell carcinoma compared to the normal mucosa. However, statistically significant positive correlation was noted only in oral epithelial dysplasia **Conclusion:** The above results probably indicate a role of mast cells in 'angiogenic switch'. These angiogenic factors secreted by mast cells promote angiogenesis either directly by stimulating the migration and/or proliferation of mast cells or indirectly through degradation of extracellular matrix. Targeting the mast cells may contribute in preventing the progression of the lesion.

Key words: Angiogenesis, CD34, Epithelial dysplasia, Mast cells, Microvessel density and Oral squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma is the sixth most common cancer in the world and is the leading cause of death in India accounting to more than 90% of all oral cancers [1]. Oral squamous cell carcinoma arises de novo or from pre-existing lesions. The progression of oral epithelial dysplastic lesions to squamous cell carcinoma is characterized by an increase in neo-vascularization in the sub-epithelial lamina propria and can be considered as an indicator of malignant transformation.[2]It is also well known that tumour growth is limited to 1-2mm in the absence of adequate perfusion and require adequate vascularity to grow.[3] Angiogenesis, the growth of new vessels from existing ones is a complex phenomenon,

required for the absolute growth and survival of neoplasm Pain is the characteristic feature which may be aggravated on eating or swallowing. This angiogenesis are triggered by hypoxia resulting from an increasing distance of the growing tumour to the capillaries. Tumour angiogenesis is a complex event, mediated by angiogenic factors released by cancer cells and or by host immune cells.[1] Among the host immune cells, mast cells have been implicated in tumour progression via promoting angiogenesis.[1] Mast cells are recruited early in tumour development and play a key role in both angiogenesis and tissue remodeling. Mast cells are a source of several proangiogenic and angiogenic factors such as histamine, heparin, chymase, basic fibroblastic growth factor, vascular endothelial growth factor, transforming growth factor.[4]

Microvessel density has been used as a method for assessment of angiogenesis. The technique involves the immunohistochemical staining of endothelial cells of the capillaries by using monoclonal antibodies against CD34. CD34 is a transmembrane glycoprotein expressed by the endothelial cells. Although expression of CD34 is evident in other cells, these do not have any effect on the assessment of microvessel density, unlike CD31 which in addition to being expressed by endothelial cells is also localized in macrophages [5]. The accumulation of mast cells is usually estimated by counting the mast cell density, which is the number of mast cells per optical field in tissue sections. Mast cells are easily recognized in light microscopy by toluidine blue staining because of the metachromatic granules that fill the cytoplasm.[6]

The objectives of the present investigations were to compare the mast cell density and microvessel density in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma and correlate the microvessel and mast cell density in each of the above groups in order to know the function of mast cells in tumour invasion, in promoting angiogenesis or its role in anti-tumour activity.

MATERIAL AND METHODS

This After obtaining the ethical clearance, this retrospective study involved the use of buffered formalin fixed, paraffin embedded tissues of previously diagnosed cases of oral epithelial dysplasia and oral squamous cell carcinoma, retrieved from the archives of Department of Oral & Maxillofacial Pathology in SDM College of Dental College and Hospital, Dharwad, India from 2010-2013. A total of 50 cases, 10 cases of normal oral mucosa, 20 cases each of oral epithelial dysplasia and oral squamous cell carcinoma were selected. Ten cases of clinically normal oral mucosa obtained from non-inflamed third molar extraction sites were used as control samples Haematoxylin & Eosin (H&E) stained sections were evaluated for the presence of the lesion and also for the adequacy of the connective tissue depth. Only OSCC biopsy specimens which contained sufficient stroma for evaluation were selected. The exclusion criterion constituted recurrent cases with or without radiotherapy.

Examination of slides:

Staining of endothelial cells:

I. Interpretation of staining: The antibody used was antihuman CD34 [Biogenex Life Science limited (CA, USA)]. The presence of brown coloured blood vessel with lumen or cluster of endothelial cells without evidence of lumen formation was considered as a single microvessel unit.

II. Selection of field for counting cells: The stained sections were scanned under low power magnification to determine the epithelial connective tissue junctions in case of epithelial dysplasia and presence of tumour islands in case of oral squamous cell carcinoma. Such representative fields were selected carefully in each slide by scanning the slides form left to right of every slide to avoid recounting of the same areas.

III. Counting of cells: The microvessel density counting was performed with a binocular light microscope under high power magnification (400x) Counting in cases of oral epithelial dysplasia was done in the connective tissue adjacent to the epithelial basement membrane in 10 successive fields (400x). The total count was divided by the 10 (number of fields studied) to get the average microvessel density in each field. In each slide, cells were counted in consecutive fields to avoid recounting the same areas.

Staining of mast cells**:** The most striking morphological feature of mast cells is the large number of strongly stained metachromatic granules present in the cytoplasm. Similar method as used for counting microvessel density was used for counting mast cell density.

Statistical analysis: Statistical analysis was performed using Kruskal- Wallis, Mann-Whitney U test and Pearson's correlation test. The software used

for statistical analysis was SPSS version 10. A significance level of $p < 0.05$ was used for all tests and comparison.

RESULTS

The age of presentation of the study group ranged between 25-72 years of age. Nineteen [95%] were males and one was female [5%] with oral epithelial dysplasia. A history of either smoking –smoking is associated with combustion of tobacco which releases various types of carcinogenic elements or eating tobacco was noted in all males. Buccal mucosa was the most predominant site of involvement [84.19%]. Lesions presented most commonly as speckled leukoplakia [68.42] with most lesions more than 2 cms in dimension with no clinical evidence of nodal involvement. In the single female, the lesion presented as an ulcer of less than 2cms without habits and no clinical evidence of lymph node involvement. There was no information regarding the location of the lesion.

Eighteen were males [90%] and two were females [10%] with oral squamous cell carcinoma. In contrast to oral epithelial dysplasia, lesions of oral squamous cell carcinoma in males were located predominantly in the lateral border of the tongue [38.88%] with buccal mucosa being the next most common site [27.77%]. History of tobacco habit was noted in most of them [83.33%]. There was no information regarding the habit history in one patient. Tobacco consumption in smoke or smokeless form, alcohol consumption, source of chronic irritation, viruses and other lesser known risk factors are associated with squamous cell carcinoma. If a known factor is elucidated, it has to be eliminated to reduce the risk. Most lesions presented as ulcers [66.66%] with only 5.5% cases exhibiting the appearance of speckled leukoplakia. Lesions were between 2-4 cms [77.77%] with no information in three patients. Clinical evidence of lymph node involvement was noted in more than half the number of patience [61.11%]. In females, the lesions were located in the buccal mucosa and gingiva. There was no history of tobacco use in any form. In both the cases the lesion presented as an ulcer between 2-4 cms in dimension. There was no clinical evidence of nodal involvement. The presence of distant metastasis could not be assessed in any of the above cases.

In normal oral mucosa mast cells were localized near the basement membrane, near the capillaries and in the sub mucosa. Endothelial cell lined capillaries were observed in the lamina propria which were evenly distributed.

In oral epithelial dysplastic lesions, numerous mast cells were localized close to the basement membrane and around the capillaries (Fig 1). Degranulated mast cells were also observed Metachromatic granules clustered outside the mast cell were considered as single mast cells for counting. Numerous endothelial cell lined capillaries were evident in the stroma of oral epithelial dysplastic lesions. The capillaries were lined by flattened to plump endothelial cells (Fig 2).

In oral squamous cell carcinoma, numerous granulated and degranulated mast cells were observed close to the epithelium, around the capillaries, around the tumour islands (Fig 3) and in the intra-tumoural region. Numerous plump to flattened endothelial lined capillaries and clusters of endothelial cells were observed around the tumour islands (Fig.4).

Inter-observer bias: Counting the parameters was done by observers (observer one and two) to reduce the observer bias. Mann-Whitney U test was done to test the consistency in the values obtained between the two observers. As no statistically significant difference in the mast cell density and microvessel density were observed between the two observers, it was decided to use the values of the first observer for further analysis.

On analysis, there was a statistically significant increase in above values from normal oral mucosa to oral epithelial dysplasia and to oral squamous cell carcinoma ($p=0.00$). However, when the mast cell density and microvessel density were correlated in each of the study samples, group I and III showed a positive correlation which was not statistically significant. While in group II, a statistically significant correlation was noted between mast cell density and microvessel density ($p=0.002$).

Table: 1. Comparison of the three study groups with mast cell density values by Kruskal-Wallis test.

Groups	Sample size	Mean	Std. Dev.	Mean rank	p-value
Normal oral mucosa	10	2.970±1.679		5.550	**0.000**
Oral epithelial dysplasia	20	11.235±2.866		23.98	
Oral squamous cell carcinoma	20	16.165±3.838		37.03	

Table:2.Comparison of the three study groups with microvessel density values by Kruskal-Wallis test:

Groups	Sample size	Mean	Std. Dev.	Mean rank	p-value
Normal oral mucosa	10	7.460±1.223		6.00	**0.00**
Oral epithelial dysplasia	20	12.215±2.013		23.98	
Oral squamous cell carcinoma	20	17.275±4.520		36.78	

Table: 3. Pearson's correlation test for determining the relationship between microvessel density and mast cell density in normal oral mucosa, oral epithelial dysplasia & oral squamous cell carcinoma

Groups	Sample size	r-value	p-value
Normal oral mucosa	10	0.264	0.460
Oral epithelial dysplasia	20	0.651	0.002
Oral squamous cell carcinoma	20	0.341	0.142

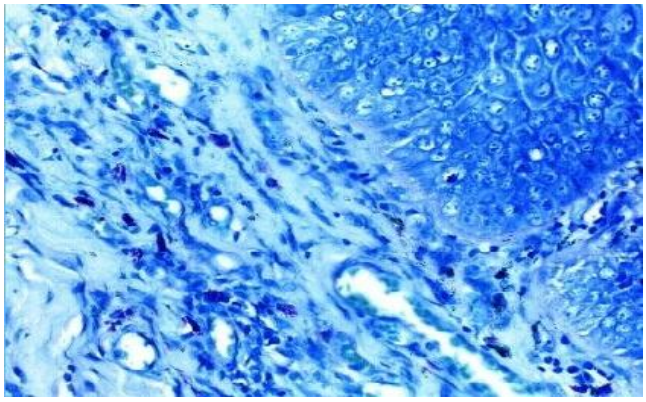

Fig 1: Oral epithelial dysplasia shows mast cells in the lamina propria adjacent to the capillaries. (Toluidine blue stain, original magnification, 400x).

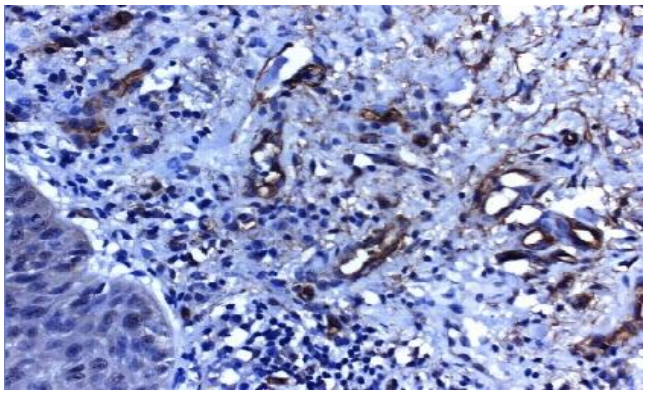

Fig 2: Endothelial lined capillaries in the lamina propria of oral epithelial dysplasia. (Immunostainiing, DAB chromogen- CD34 monoclonal antibodies, original magnification, 400x)

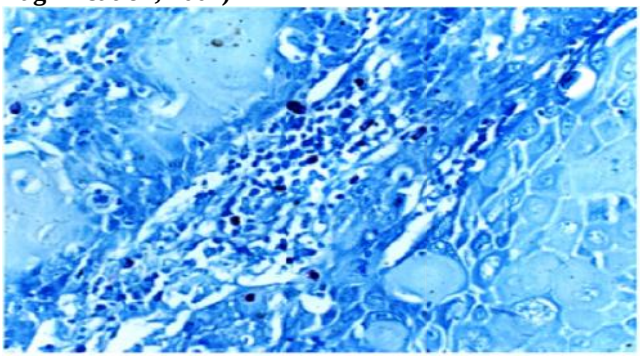

Fig 3: Oral squamous cell carcinoma shows mast cells around tumour epithelial islands. (Toluidine blue stain, original magnification, 400x)

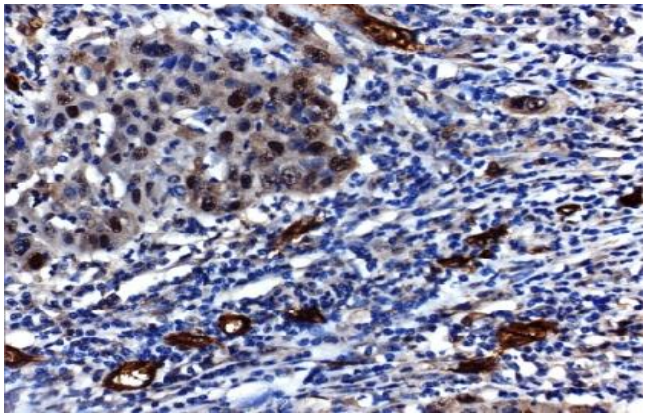

Fig 4: Oral squamous cell carcinoma shows endothelial lined capillaries in the lamina propria. (Immunostaining, DAB chromogen- CD34 monoclonal antibodies, original magnification, 400x)

DISCUSSION

The oral mucosa undergoes various reversible and irreversible changes to carcinogens. These changes have been observed in the epithelial cells as well as in the connective tissue.[7] The changes in the connective tissue have been evident by the occurrence of an inflammatory component as well as an increase in vascularity.[2] Mast cells are one of the inflammatory components involved in the process of angiogenesis. Although several methods, including tryptase exist in accurately detecting mast cells, we used toluidine blue as this method is very simple, less time consuming, and inexpensive. Moreover, mast cells are easily recognized in light microscopy by toluidine blue staining because of the metachromatic granules that fill the cytoplasm.[6]

In normal oral tissues, mast cells have been found to be present in the connective tissue of gingiva, tongue and lining mucosa. Mast cells have also been reported in normal periodontal ligament and pulp although in very low densities.[9]

Bivji showed an increase in the number of mast cells/unit microscopic field in oral leukoplakia (which represents an increase in the thickness of the epithelium and its keratinization without dysplasia in this study) compared to normal mucosa. The authors concluded that pharmacologically active agents in the mast cells might contribute to inflammatory reaction seen in leukoplakia.[10] Mast cells may release interleukin -1 which causes increased epithelial proliferation that is seen in leukoplakia. Histamine may also cause increased mucosal permeability, which could facilitate increased access for the antigen to the connective tissue.[10] Mohtasham et al., Pazouki et al observed an increase in vascularization during transformation from normal oral mucosa, through dysplasia, to in-situ and infiltrating carcinoma supporting the pivtal role of angiogenesis in malignancy progression.[11,12] Flynn E et al, demonstrated a direct co-relation between sequential mast cell infiltration, activation and distinct stages of hyperkeratosis, dysplasia, carcinoma in-situ in the oral cavity and implicated the role of mast cells in configuring the angiogenic phenotype in premalignant lesions.[13] Similarly Iamaroon et al also observed a linear increase from normal oral mucosa, hyperkeratosis, premalignant dysplasia to squamous cell carcinoma suggesting the role of mast cells in up-regulation of angiogenic process.[14] Michailidou et.al. observed that the mast cell density & microvessel density did increase significantly between normal oral mucosa and oral leukoplakia without dysplasia and oral leukoplakia with mild, moderate or severe dysplasia. [4] They concluded that an angiogenic switch seemed to be turned on in the later stages of dysplasia indicating a transformation into malignancy. Also a possible role of mast cells during the progression from normal oral tissue to oral epithelial dysplasia & subsequently to oral squamous cell carcinoma was elucidated. [4]

In contrast to the above studies, Oliverira- Neto et al. observed a decrease in mast cell numbers in premalignant and malignant oral lesions which was attributed to the failure of mast cell migration.[15]

Our study showed an increase in mast cell density (mast cells are activated in allergic, inflammatory, autoimmune conditions, in response to factors released by the tumour cells, and in response to infections) & microvessel density from normal oral mucosa to oral epithelial dysplasia to oral squamous cell carcinoma (Table 1and 2). A hypothesis can be suggested that hypoxia might induce tumour cells to release angiogenic factors which in turn could chemo attract mast cells to migrate into the hypoxic areas of the tumour. After migration into the hypoxic areas, mast cells might produce stimulating factors that help in further angiogenesis. Mast cells are also an important source of several pro-angiogenic and angiogenic factors, such as histamine, heparin, chymase, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β) etc.[1] These angiogenic factors secreted by mast cells either directly promote angiogenesis by stimulating the migration and/or proliferation of endothelial cells or indirectly through degradation of extracellular matrix.[4] Tryptase is a serine endopeptidase that is released in abundant quantities from mast cells in a bound form with heparin. Both heparin and tryptase are potent angiogenic factors. Tryptase also activates latent metalloproteinases and plasminogen activator, which degrades the extracellular matrix, is important in the initial stages of angiogenesis. [8]

The mast cell density and microvessel density in our study showed a positive correlation in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma. However, a statistically significant correlation between them with an increase in the mast cell density and microvessel density was observed only in oral epithelial dysplasia. All the cases of oral squamous cell carcinoma used in our study were well differentiated squamous cell carcinoma. From the above, we can probably conclude that tumours that are rapidly growing may have a high nutritive demand that is provided by the vasculature. When the epithelium is altered as in oral epithelial dysplasia, recruitment of inflammatory cells is noted. The inflammatory and mast cells that migrate to areas of altered epithelium may stimulate angiogenesis by secreting proangiogenic and angiogenic components either directly or indirectly prior to the invasion. However, once invasion is established as in oral squamous cell carcinoma, the role of mast cells is probably shifted from angiogenesis to further promoting invasion as seen in our case. Another hypothesis why such a finding was observed in oral squamous cell carcinoma is probably that mast cells

are involved in cytotoxic function corresponding to the invasion of dysplastic tumour epithelial cells rather than supporting angiogenesis which would take place once invasion is established. Cell-mediated cytotoxic effects of mast cells have also been reported, with mast cell: tumour ratio greater than 20:1.[16] Conversely, cytotoxic effects of mast cells were nullified and tumour progression was found to be enhanced when the mast cell-tumour ratios were increased from 20:1 to 1:100.[16] Hence, the effect of mast cells against cancer cells might depend on the concentration of mast cell products in the microenvironment. Tomita M et. al. hypothesized that reversing this process, i.e., enhancing the cytotoxic functions of mast cells and suppressing their angiogenic functions, could lead to a new anti-cancer treatment strategy.[16]

Kalra et al observed an increase in angiogenesis in different histological grades of oral squamous cell carcinoma.[1] Poorly differentiated and moderately differentiated oral squamous cell carcinoma attained a highly angiogenic phenotype as compared to well differentiated.[1] Similarly Sharma et al observed that mast cell density and microvessel density to be higher in moderately differentiated compared to well differentiated squamous cell carcinoma supporting our hypothesis. [17] Thus when different grades of oral squamous cell carcinoma are compared, poorly differentiated carcinoma and moderately differentiated carcinomas are known to be more proliferative and invasive thus in such cases mast cells may have a dual role of promoting angiogenesis and invasion and the cytotoxic function of mast cells may be too ineffective in such situations. The treatment of oral squamous cell carcinoma is surgical, radiotherapy or chemotherapy or a combination depending whether it is a primary or a recurrent tumour. However, research is being done to promote targeted therapy against angiogenesis, mast cells or other factors that promote tumour growth

CONCLUSION

To conclude, there was a statistically significant increase in mast cells and microvessel density from normal mucosa to oral epithelial dysplasia and squamous cell carcinoma consistent with the pathogenesis. However, a statistical significance in the ratio of the mast cells to microvessel density was noted only in oral epithelial dysplasia. Thus mast cells either promote or inhibit tumour growth either alone or in association with other cells in the microenvironment and exert an effect on the altered tissue. Thus the present study has been valuable in defining the role of mast cells in dysplastic epithelium, while highlighting the role of mast cells in modifying the stroma for invasion in oral squamous cell carcinoma. Thus thorough understanding the role of mast cells in premalignant lesions and oral squamous cell carcinoma would help in the development of anti- cancer therapies utilizing angiogenesis as a target for the drugs.

Conflict of Interest: Nil

REFERENCES

1. Karla M, Rao N, Nanda K, Rehman F, Girish KL, Tippu S et al. The role of mast cells on angiogenesis in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2012; 17(2): 190-6.
2. Raica M, Cimpean AM, Ribatti D. Angiogenesis in premalignant conditions. Eur J Cancer. 2009; 45: 1924-34
3. Weidner N, Semole JP, Welch WR, Folkman J. Tumour angiogenesis and metastasis- a correlation in invasive breast cancer. N Engl J Med. 1991; 324(1): 1-8.
4. Michailidou E Z, Markopoulos A K, Antoniades D Z. Mast cells and angiogenesis in oral malignant and premalignant lesions**.** The Open Dent J. 2008; 2: 126-32.
5. Hung P H, Chou K C, Hsieh S H, Shieh Y S. Tumour angiogenesis in oral squamous cell carcinomas: the significance of endothelial markers and hotspot selection. J Med Sci. 2009; 29(2):67-74.
6. Elpek G O, Gelen T, Aksoy N H, Erdogan A, Dertsiz L, Demircan A, et al. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol. 2001; 54:940–44
7. Trapero J C, Sanchez J C, Sanchez B P, Gutierrez J S, Gonzalez M A, Martinez A Update on molecular pathology in oral cancer and precancer. Anticancer Res. 2008; 28 :1197-06.

8. Leon C, Kempen L V, Rhee J S, Dehne K Epithelial carcinogenesis: Dynamic interplay between neoplastic cells and their microenvironment. Differentiation. 2002; 70: 610-23.
9. Madhuri A R, Alka K D, Ramakant N. Mast cells are increased in leukoplakia, oral submucous fibrosis, oral lichen planus and oral squamous cell carcinoma. J Oral Maxillofac Pathol. 2007; 11(1): 18-22.
10. Bivji AT. Mast cells in normal and leukoplakic buccal mucosa J Ind Dent Assoc. 1973; 45: 189-91.
11. Mohtasham N, Babakoohi S, Salehinejad J, Montaser- Kouhsari L, Shakeri MT, Shojaee S et al. Mast cells density and angiogenesis in oral dysplastic epithelium and low and high grade oral squamous cell carcinoma. Acta Odontol Scand. 2010; 68(5)**:** 300-04.
12. Pazouki S, Chisholm DM, Adi MM, Carmichael G, Farquharson M, Ogden GR et al. The association between tumour progression and vascularity in the oral mucosa. J Pathol. 1997; 183:39-43.
13. Flynn E, Schwartz j, Shklar G. Sequential mast cell infilteration and degranulation during experimental carcinogenesis. J Cancer Res Clin Oncol. 1991; 117:115- 22.
14. Iamaroon A, Pongsiriwet S, Jittidecharaks S, Pattanaporn K, Prapayasatok S, Wanachatararak S. Increase of mast cells and tumour angiogenesis in oral squamous cell carcinoma. J Oral Pathol Med. 2003; 32: 195-99.
15. Oliveira Neto HH, Leite AF, Costa NL, Alencar RC, Lara VS, Silva TS et al. Decrease in mast cells in oral squamous cell carcinoma: possible failure in the migration of these cells. Oral Oncol.2007; 43 4:84-90.
16. Tomita M, Matsuzaki Y, Onitsuka T. Effect of mast cells on tumor angiogenesis in lung cancer. Ann Thorac Surg. 2000; 69(6): 1686-90.
17. Sharma B, Sriram G, Saraswathi TR, Sivapathasundaram B Immunohistochemical evaluation of mast cells and angiogenesis in oral squamous cell carcinoma Indian Dent Res. 2010; 21(2**)**: 260-64.