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Modulating the role of mast cell stabilizers in the treatment of gingival enlargements. Running title- Mast cell stabilizers in gingival enlargements

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Abstract

Aim: To histo-morphologically compare the mast cell density, epithelium thickness, mean vessel density in the gingiva before and after giving mast cell stabilizers.

Settings and Design: The present cross-sectional study was conducted on 45 patients diagnosed with chronic periodontitis drawn from the Departments of Periodontics and Oral and Maxillofacial Pathology.

Methods and Material: Gingival biopsies were obtained from 45 patients categorized into 2 groups: group 1 before giving mast cell stabilizers and group 2 after giving mast cell stabilizers. The groups were sub-categorized as smokers and non-smokers. Three sections were obtained and were stained with Haematoxylin & Eosin, Toluidine blue and immunohistochemistry was performed with antibody Factor VIII. The epithelial thickness, mean vessel density and number of mast cells were assessed using histo-morphometric analysis.

Statistical analysis used: Data was analysed using Statistical Package for Social Science (SPSS) version

20.0.0.0. P<0.05 was considered to be statistically significant

Results: Results of this study revealed that the mean mast cell density and mean vessel density decreased after giving mast cell stabilizers in group 2 compared to group 1. There was no significant difference in the epithelium thickness between both the groups.

Conclusion: Mast cells play a crucial role in the sustenance and progression of periodontal disease as mean mast cell density was high before giving mast cell stabilizers.

Keywords: Mast cell stabilizer, Immunohistochemistry, morphometry, Gingiva, epithelium thickness, mast cell density, mean vessel density, inflammation

Introduction

Periodontal disease initiation and progression occurs as a consequence of the host immune inflammatory response to microbes and their by-products present in the dental plaque. These factors act in unison to alter the gingival and periodontal tissue homeostasis [1]. Smoking has been established as a major risk factor of periodontal disease and has an astute effect on the prevalence, extent and severity of periodontitis. Periodontal disease is highly progressive in smokers and their response to periodontal therapy is significantly reduced than non-smokers. Nicotine plays a major role in promoting the rate of proliferation of gingival epithelium, thereby increasing the epithelial thickness [2]. Altered microvasculature of the gingival connective tissue and increased thickness of the epithelium among smokers masks the signs of inflammation [3].

The tobacco products affect various aspects of the host response and are capable of causing alterations in the cells of the periodontium. It is well established that smoking increases the proliferation of epithelial cells in healthy and dysplastic oral mucosa, the effect of smoking on the proliferative activity of gingival keratinocytes has not been extensively evaluated in periodontally diseased patients [4]. Among the cells found in periodontal tissues, mast cells have been detected in both healthy and inflamed gingiva, in different numbers at various sites. They play a pivotal role in the immune response to infection by pathogenic parasites and in inflammatory reactions; at the same time, MCs are the main effector cells in allergic diseases [5].

Under normal conditions, mature mast cells do not circulate in the bloodstream. However, mast cell progenitors migrate into tissues and differentiate into mast cells under the influence of stem cell factor and various cytokines. Mast cells are present throughout the body and they play important roles in the maintenance of many physiological functions as well as in the pathophysiology of diseases [2].

Mast cells are involved in abundant activities ranging from control of vasculature, host defence, tissue injury and repair and allergic inflammation [2]. Huang S et al. suggested that mast cell degranulation may contribute to the progression of periodontal disease and there is strong correlation between the density of mast cells, degranulation and severity of periodontitis [6]. Therefore, the present study was designed to assess the role of mast cell stabilizers in modulating mast cell activity and its mediators and its correlation with various gingival histomorphological parameters in chronic smokers and non-smokers.

Subjects and methods

A sample of 45 patients diagnosed with chronic gingivitis and/or periodontitis were selected and categorized into two groups as; Group 1: Before mast cell stabilizer therapy and Group 2: After mast cell stabilizer therapy.

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Sample collection and tissue preparation

Incisional biopsies of approximately 5 mm thickness were attained with a scalpel blade (no. 15) from suitable sites at the time of extraction. The biopsied specimens were fixed in N/10 formalin solution, processed, embedded in fresh paraffin wax and serially sectioned at 4 µm thickness. Three sections were obtained and stained with H&E and toluidine blue and immunohistochemistry was performed using antibody FactorVIII respectively. Sections stained with H&E were used to assess the epithelial thickness and inflammation and sections stained with toluidine blue were used to assess the number of mast cells and sections immunostained with antibody FactorVIII were used to assess mean vessel density.

Quantitative evaluation of mast cells density

The number of mast cells were counted at 5 high power field at X400 magnification in superficial and in 3 high power field at X400 magnification in deeper connective tissue stroma. Mast cell density was determined as mean number/per high power field.

Assessment of epithelial thickness

The images for assessing the epithelial thickness were captured under X100 magnification. Epithelial thickness was measured from the surface of epithelium to the basement membrane in µm using Magnus pro image analysing software, in a chosen field and the average of 5 readings were obtained and the mean thickness was considered as the epithelial thickness per case in the study groups.

Assessment of inflammation

Assessment of inflammation was carried out considering the parameters such as density, distribution and nature of inflammatory infiltrate.

Distribution of inflammation was graded as diffuse and dense.

Nature of inflammatory infiltrate was graded as chronic and mixed.

Density of inflammation was graded according to Huang S et al [6] as 0,1,2 and 3 grading with absence of inflammation, mild, moderate and severe respectively.

Assessment of microvasculature

Immunoexpression of factorVIII antibody was seen in the blood vessels and images were captured at 40x magnification. Mean vessel density was calculated using Magnus pro image analysing software and blood vessels were classified into 3 groups as small (≤33 µm2), medium (34-64 µm2) and large (≥67 µm2).

Statistical expression

Independent sample t-test was used to compare the epithelium thickness, mean vessel density, mast cell density in the study groups. $P<0.05$ was considered to be statistically significant. Data was analysed using Statistical Package for Social Science (SPSS) version 20.0.0.0.

Results

The data from the participants of the study groups were analysed as stated in the methods. Correlation of mean mast cell density count and its topographical distribution with epithelium thickness in study groups. The mean mast cell density (total, superficial, deep) with mean epithelium thickness (1675.32 µm) in 40 cases of group 1 was greater when compared to mean mast cell density with mean epithelium thickness (1682.22 µm) in 5 cases of group 2 [Table 1] [Figure 1].

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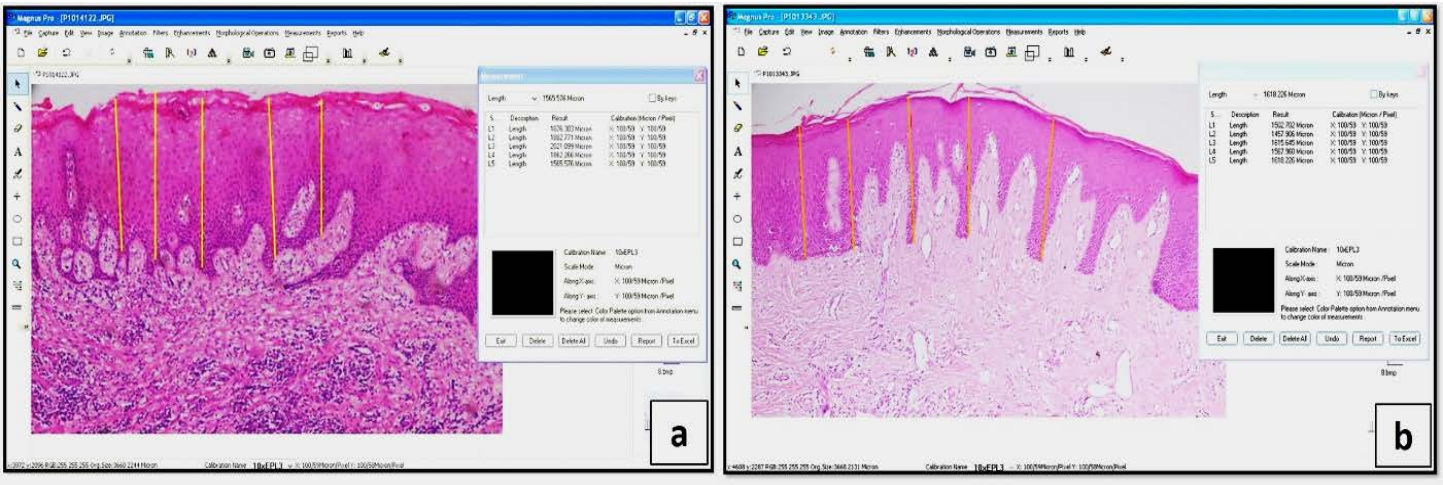
Table 1: Correlation of mean mast cell density count and its topographical distribution with epithelium thickness in study groups.

Study Groups	Mean Epithelium thickness (µm)	Mean Mast Cell Density (Mean± SD)			P-value
		Total	Superficial connective tissue	Deeper connective tissue	
Group 1	1675.32	3.34±3.2	3.24±3	2.00±3.3	0.562
Group 2	1682.22	1.46±1.2	1.96±1.9	0.62±0.1	

The above table depicts correlation of mean mast cell density (total, superficial, deep) with mean epithelium thickness (1675.32 µm) in 40 cases of group 1 was greater when compared to mean mast cell density with mean epithelium thickness (1682.22 µm) in 5 cases of group 2. [s)]Statistically significant difference.

Figure 1: (a) Histomorphometric analysis of epithelium thickness in patients before giving mast cell stabilizers (group 1), (b) Histomorphometric analysis of epithelium thickness in patients after giving mast cell stabilizers (group 2).



Correlation of Mean mast cell density with inflammation in study groups shows that the mean mast cell density was higher in cases of chronic as well as mixed inflammation in group 1 compared to group 2. The mean mast cell density increased from mild to severe inflammation density in group 1 compared to group 2. In cases of both dense and diffuse inflammation mean mast cell density was higher in group 1 compared to group 2 [Table 2] [Figure 2].

Table 2: Correlation of Mean mast cell density with inflammation in study groups.

Inflammation		Group 1		Group 2	
		Number of cases (n)	Mean Mast Cell Density (Mean± SD)	Number of cases (n)	Mean Mast Cell Density (Mean± SD)
Type	Chronic	33	3.01±2.8	4	1.70±1.3
	Mixed	7	2.94±2.8	1	0.5
	P-value	0.31		0.48	
Density	Mild	25	2.35±1.9	2	2.40±1.6

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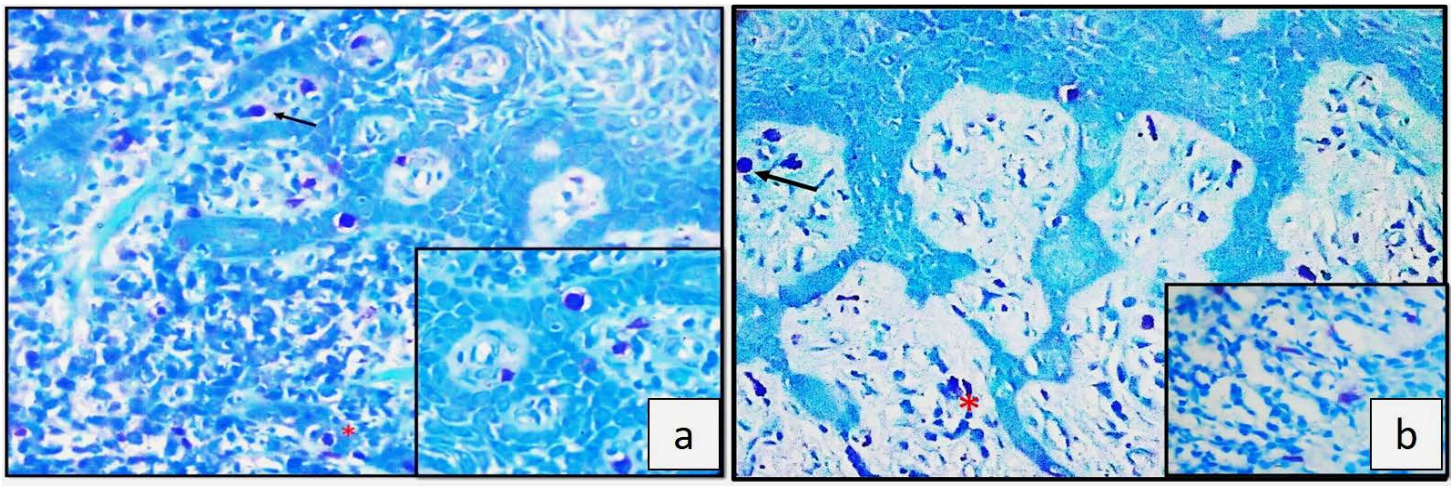
	Moderate	7	4.32±2.0	1	0.5
	Severe	8	5.62±5.5	2	1.00±0.7
	P-value	0.02[s)]		0.52	
Distribution	Dense	9	6.05±5.1	2	1.00±0.7
	Diffuse	31	2.57±1.9	3	1.76±1.6
	P-value	0.00[s)]		0.22	

The above table depicts mean mast cell density was higher in cases of chronic as well as mixed inflammation in group 1 compared to group 2. The mean mast cell density increased from mild to severe inflammation density in group 1 compared to group 2. In cases of both dense and diffuse inflammation mean mast cell density was higher in group 1 compared to group 2. [s)]Statistically significant difference.

Figure 2: (a) Quantitative Evaluation of mast cells density using Toluidine blue stain in patients before giving mast cell stabilizers (group 1),

b) Quantitative Evaluation of mast cells density using Toluidine blue stain in patients after giving mast cell stabilizers (group 2).



Comparison of mean vessel density between the study groups revealed that the mean vessel density (small, medium and large) was higher in group 1 when compared to group 2 [Table 3] [Figure 3].

Table 3: Showing comparison of mean vessel density between the study groups.

Mean vessel density	Small ≤33 μm^2	Medium 34-64 μm^2	Large ≥67 μm^2
Group 1	22.37±8.45	47.96±11.73	141.36±22.09
Group 2	17.6±2.34	46.39±2.11	110.09±17.33
p-value	0.56	0.98	0.23

The above table depicts mean vessel density (small, medium and large) was higher in group 1 when compared to group 2. [s)] Statistically significant difference.

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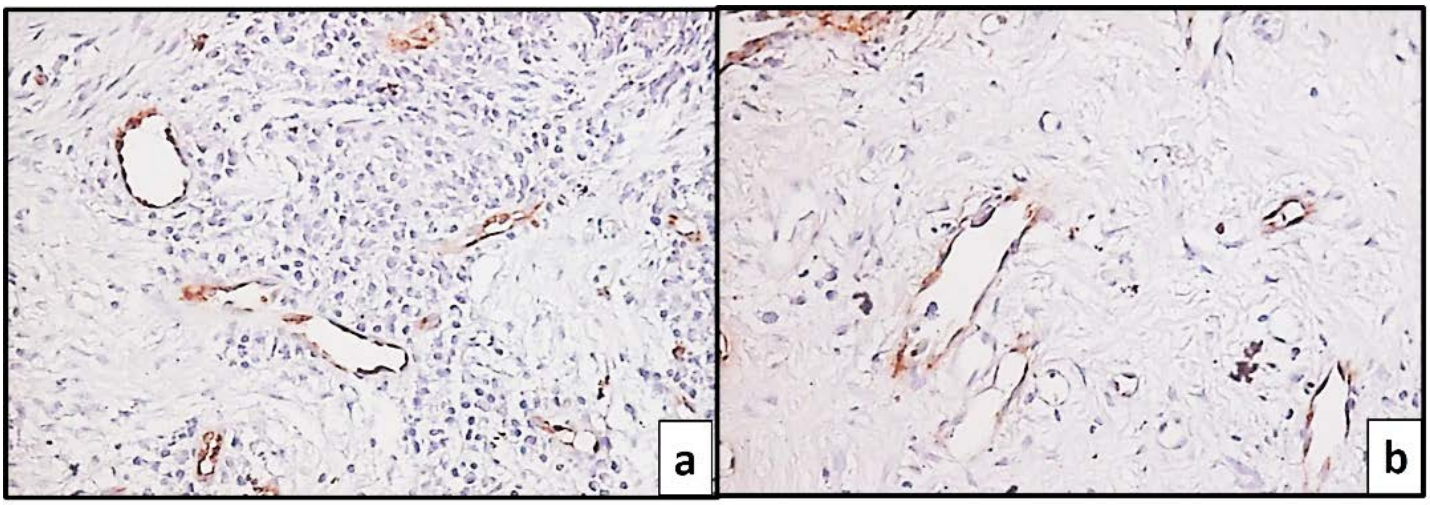
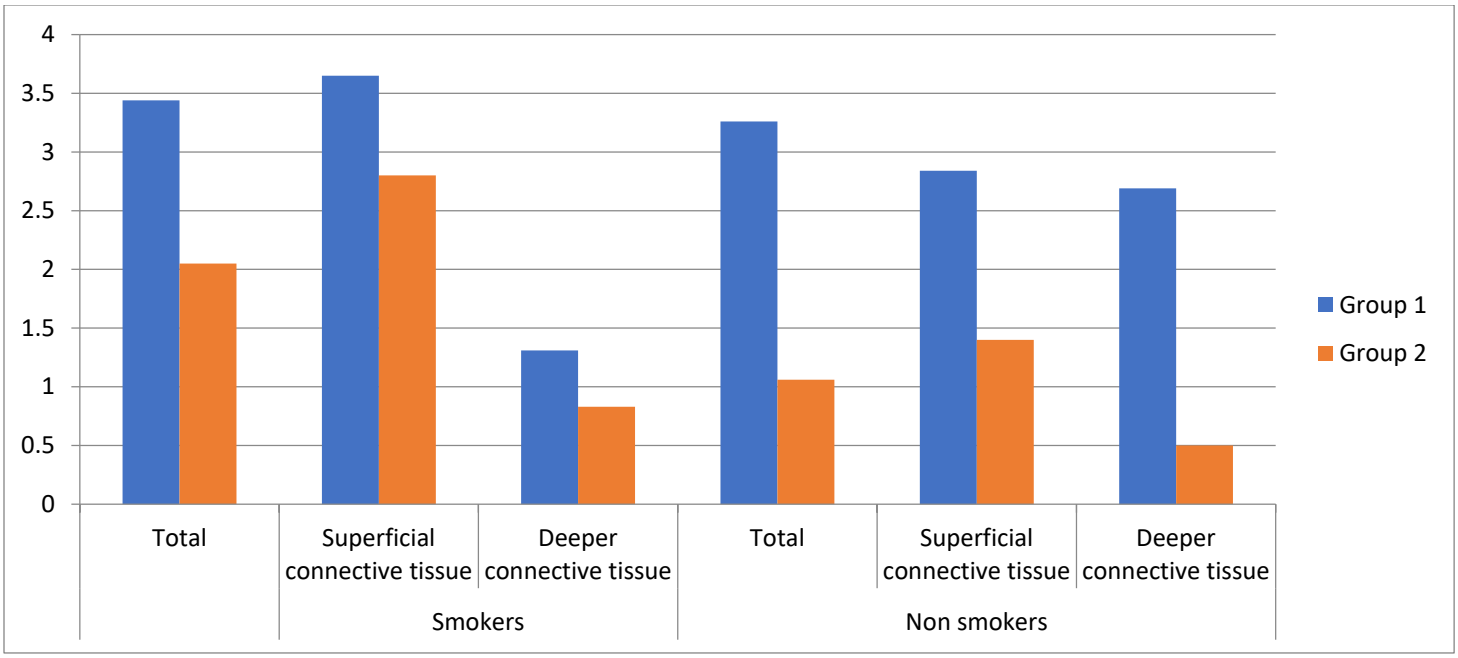
Figure 3**:** (a) Immunohistochemical analysis of Mean vessel density using Factor VIII IHC in patients before giving mast cell stabilizers (group 1),

(b) Immunohistochemical analysis of Mean vessel density using Factor VIII IHC in patients after giving mast cell stabilizers (group 2).



Correlation of Mean mast density (total, superficial connective tissue, deeper connective tissue) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers). The mean mast cell density (total, superficial and deep) was higher in group 1 compared to group 2 in smokers as well as non-smokers [Graph 1].

Graph 1: Correlation of Mean mast density with habit in study groups.



The above graph depicts correlation of Mean mast density (total, superficial connective tissue, deeper connective tissue) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers). The mean mast cell

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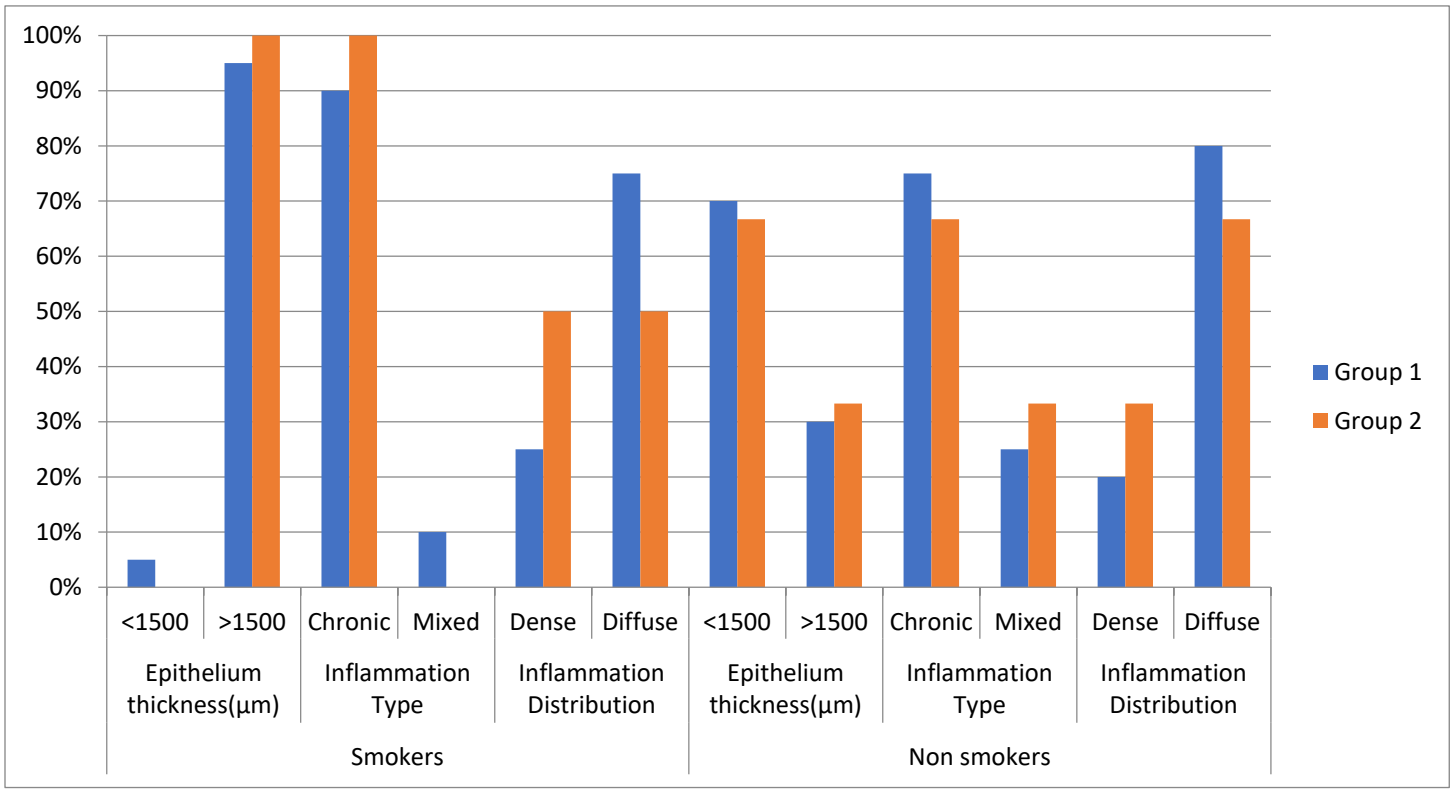
density (total, superficial and deep) was higher in group 1 compared to group 2 in smokers as well as non-smokers.

Correlation of histomorphological parameters (epithelium thickness and inflammation type and distribution) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers). In smokers group, the epithelium thickness of <1500 µm is shown by 5% cases whereas of >1500 µm is shown by 95% cases, whereas in non-smokers group, 70% cases show the epithelium thickness of <1500 µm while 30% cases show thickness of >1500 µm. Similarly, in smokers, chronic inflammation is seen in 90% of cases whereas mixed inflammation is seen in 10% cases; while in non-smokers, 75% cases show chronic inflammation and 25% show mixed inflammation. Distribution of inflammation is dense in 25% cases and diffuse in 75% cases in smokers group, whereas in non-smokers group, dense inflammation is seen in 20% cases and diffuse inflammation is seen in 80% cases. In smokers group, the epithelium thickness of >1500 µm is shown by 100% cases, whereas in non-smokers group, 66.70% cases show the epithelium thickness of <1500 µm while 33.30% cases show thickness of >1500 µm. Similarly, in smokers, chronic inflammation is seen in 100% of cases whereas mixed inflammation is seen in 0% cases; while in non-smokers, 66.7% cases show chronic inflammation and 33.30% show mixed inflammation. Distribution of inflammation is dense in 50% cases and diffuse in 50% cases in smokers group, whereas in non-smokers group, dense inflammation is seen in 33.3% cases and diffuse inflammation is seen in 66.7% cases. The results were statistically non-significant (p >0.05) [Graph 2].

Graph 2: Correlation of Histo-morphological parameters with habit in study groups.



The above graph depicts correlation of histomorphological parameters (epithelium thickness and inflammation type and distribution) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell

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stabilizers) and 5 cases of group 2 (after mast cell stabilizers).

Discussion

The term 'periodontal diseases' encompasses a wide variety of chronic inflammatory conditions of the gingiva (or gums, the soft tissue surrounding the teeth), bone and ligament (the connective tissue collagen fibres that anchor a tooth to alveolar bone) supporting the teeth [7].

Periodontitis is a complex interrelationship between infectious agents and host factors. Environmental and genetic risk factors may modify the expression of disease. Among the environmental risk factors, tobacco smoking has been found to be associated with an increased prevalence and severity of periodontal disease. Smokers have demonstrated a decreased inflammatory response and reduced gingival bleeding to plaque accumulation [3].

Smokers demonstrate reduced inflammatory clinical signs that might be attributed to changes in gingival microvasculature and increased gingival epithelial thickness. The by-products from tobacco oxidation produce peripheral vasoconstriction or damage the vasculature thereby reducing gingival bleeding, redness and oedema and affect the progression of periodontal diseases [8].

Mast cells originate from pluripotent hematopoietic cells in the bone marrow after partial differentiation they enter the peripheral mucosal or connective tissue microenvironments and complete their differentiation. Mast cells reside close to T-cells phagocytose and process antigens and initiate acquired immune responses by presenting antigens to T-cells. Since T lymphocytes have been shown to predominate in the stable periodontal lesion with an increase in the numbers of B-cells and plasma cells in the progressive lesion, there has been the suggestion that T-cells with a Th1 cytokine profile may be the major mediator in periodontitis while Th2 cells play a role in periodontitis [9].

Correlation of the mean mast cell density (total, superficial, deep) was elucidated with mean epithelium thickness in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers). The mean epithelium thickness (1675.32) in group 1 with mean mast cell density (total, superficial connective tissue, deeper connective tissue) were 3.34±3.2, 3.24±3, 2.00±3.3 compared to the mean epithelium thickness (1682.22) in group 2 with mean mast cell density (total, superficial connective tissue, deeper connective tissue) were 1.46±1.2, 1.96±1.9, 0.62±0.1. The results were statistically non-significant ($p > 0.05$). Ironically, the results of our study are inconsistent with Villar [10], Seyedmajidi [11], Prakash [12], Paul [2] who observed that the increase in mast cell density the epithelium thickness also increased. The results of our study could be attributed to the fact that the inflammation can interfere in the epithelial maturation process and in the original conformation of tonofilaments, compromising the complete keratinization [Table 1].

On correlation of Mean mast density (total, superficial connective tissue, deeper connective tissue) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers). In group 1, the smokers group depicts the mean mast cell density in the superficial connective tissue as 3.65±3.4 whereas in the deeper connective tissue the mean mast cell density is 1.31±2.9; while in the non-smoker group, the mean mast cell density in the superficial connective tissue is 2.84±2.6, whereas in the deeper connective tissue, the mean mast cell density is 2.69±3.5. In group 2, the

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smokers group depicts the mean mast cell density in the superficial connective tissue as 2.80±3.3 whereas in the deeper connective tissue the mean mast cell density is 0.80±0.2; while in the non-smoker group, the mean mast cell density in the superficial connective tissue is 1.40±0.8, whereas in the deeper connective tissue, the mean mast cell density is 0.50±0.1. In the present study, the total mean mast cell density was higher in smokers (3.44±3.5) compared to non-smokers (3.26±3.0) in both the groups [Figure 2] [Graph 1].

On correlation of histomorphological parameters (epithelium thickness and inflammation type and distribution) with habit status (smoker and non-smoker) in 40 cases of group 1 (before mast cell stabilizers) and 5 cases of group 2 (after mast cell stabilizers), it was observed that in smokers group, the epithelium thickness of <1500 µm was seen in 5% cases whereas of >1500 µm was seen in 95% cases, whereas in non-smokers group, 70% cases show the epithelium thickness of <1500 µm while 30% cases show thickness of >1500 µm [Figure 1]. Similarly, in smokers, chronic inflammation is seen in 90% of cases whereas mixed inflammation is seen in 10% cases; while in non-smokers, 75% cases show chronic inflammation and 25% show mixed inflammation. Distribution of inflammation is dense in 25% cases and diffuse in 75% cases in smokers group, whereas in non-smokers group, dense inflammation is seen in 20% cases and diffuse inflammation is seen in 80% cases.

In smokers group, the epithelium thickness of >1500 µm is shown by 100% cases, whereas in non-smokers group, 66.70% cases show the epithelium thickness of <1500 µm while 33.30% cases show thickness of >1500 µm. Similarly, in smokers, chronic inflammation is seen in 100% of cases whereas mixed inflammation is seen in 0% cases; while in non-smokers, 66.7% cases show chronic inflammation and 33.30% show mixed inflammation. Distribution of inflammation is dense in 50% cases and diffuse in 50% cases in smokers group, whereas in non-smokers group, dense inflammation is seen in 33.3% cases and diffuse inflammation is seen in 66.7% cases. The results were statistically non-significant ($p > 0.05$) **[**Graph 2].

So, the results of this study can be inferred as mast cells play an important role in progression and sustenance of the periodontal disease. Mast cell stabilizer can help in taming the progress of periodontal destruction.

The relationship of mean vessel density was seen in the study groups. It was observed that the mean vessel density (small, medium and large) was higher in group 1 (before mast cell stabilizers) 22.37±8.45, 47.96±11.73, 141.36±22.09 when compared to group 2 (after mast cell stabilizer) 17.6±2.34, 46.39±2.11, 110.09±17.33 [Figure 3]. The reasoning behind this could be because mast cells secrete pro angiogenic factors such as VEGF, TGF-beta, TNF-alpha and IL-8. Also, the proteases released by mast cell breakdown the ECM and help in contributing to release of angiogenesis modulating molecule [13]. Thereby, in group 2 after giving mast cell stabilizers the mean vessel density (small, medium and large) decreased as mast cell density also reduced [Table 3].

Conclusion

Mast Cells are one of the key effectors of early innate immunity and play a central role not only in host defense against invading pathogens and other environmental threats, but also in the underlying mechanisms of implementation, perpetuation, and regulation of the inflammatory response. Thus, normal mature MCs are involved in many physiological and pathological processes such as inflammation, angiogenesis, wound healing, allergic diseases, and carcinogenesis. Moreover,

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increasing knowledge accumulated over the years on the biology of MCs has led to the development of drugs that target specific molecules involved in activation of MCs such as omalizumab. Considerable progress has been made in our understanding of these immune cells in recent years. Additional efforts to define the complex interactions of mast cells will potentially lead to novel clinical approaches from therapeutic significance in progression of pathological conditions involving the periodontal tissues.

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