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Relationship between Mast Cell Counts and Different Types of Periodontitis

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Abstract

Background and aims. Mast cells play an important role in allergic reactions, host defense, local homeostasis, inflammation and angiogenesis. The aim of this study was to evaluate the relationship between mast cell counts and different types of periodontitis.

Materials and methods. Gingival specimens were taken from 20 moderate-to-advanced chronic and 19 moderate-to-advanced aggressive periodontal sites as case groups and 18 healthy/gingivitis sites as control group in routine periodontal flap and crown lengthening procedures. All the specimens were stained by toluidine blue for mast cell counting and hematoxylin/eosin to assess inflammation. Inflammatory and mast cells were assessed in 5-micron sections by two observers 3 times, utilizing light microscopy at ×100 and ×400 magnification. ANOVA and t-test were used to analyze data. Statistical significance was defined at α=0.05.

Results. Mast cell counts were higher in chronic versus aggressive periodontitis and healthy/gingivitis cases (P = 0.000). The aggressive periodontitis cases did not demonstrate higher counts of mast cells as compared to healthy/gingivitis cases (P >0.05). There was no relationship between mast cell counts and degree of inflammation in the three groups.

Conclusion. The present study indicated higher mast cell counts in the chronic periodontitis sites compared to other sites. The results of this study suggest that further studies are necessary to simultaneously evaluate dynamic aspects of host defense and other aspects of immune system.

Key words: **Aggressive, chronic, inflammation, mast cells, periodontal disease.**

Introduction

Periodontitis is the most common inflammatory oral disease triggered by bacteria in the dental plaque.[1] It is characterized by a dense infiltrate of inflammatory cells, loss of connective tissue, formation of periodontal pockets and breakdown of the alveolar bone, finally leading to tooth mobility and tooth loss.[1,2]

At present, common periodontal treatments are mechanical debridement, including oral hygiene measures, scaling and root planing (SRP) and surgical techniques and chemical methods, such as use of antibiotics and mouthwashes.[1]

Biologic information of the disease mechanisms is the first step to prevent and treat periodontal disease. The role of the host in the pathogenesis of periodontal disease has been studied by many investigators.[3,5,22-25] Substantial evidence has implicated certain immune and inflammatory responses as destructive mechanisms in the periodontal disease process.[3,4] One possible host reaction against periodontal breakdown may be mediated by mast cell release. Some investigators have proposed a role for mast cell constituents in periodontal destruction.[4,22,24,25] These cells play a key role in gingival homeostasis and express matrix metalloproteinases (MMPs) that may be important in the progression of periodontitis.[3,11] However, the contribution of mast cell mediators to periodontal disease progression is not clearly known.[4]

Increased mast cell counts have been reported in the gingiva as compared to other healthy tissues.[7] Alternatively, Carranza et al[9] in 1955 and Dummett et al[19] in 1963 observed that mast cell population decreases in inflamed gingival tissue. Gemmell et al[5] compared chronic periodontitis lesions with healthy/gingivitis ones and indicated lower mast cell counts in periodontitis lesions. Günhan et al[8] showed significant increase in mast cell counts in infected tissues compared to healthy samples. Some studies have evaluated the effect of lodoxamide ethyl and disodium cromoglicate, the releasing inhibitors of mast cells; for example Jeffcoat et al[4] reported a decrease in the rate of alveolar bone loss and Nukin et al[22] showed failure to alter the development of experimental gingivitis by these factors.

The present study was undertaken to examine the relationship between mast cell counts and periodontal diseases because of the discrepancies in the results of various studies, limited attention and insufficient knowledge about the precise role of mast cells in periodontal diseases.

Materials and methods

Patients

Patients referred to a specialized dental center were enrolled in the study. The patients had no systemic diseases[1,18] and had not used any medications[15] with probable effects on periodontal tissues for the previous 2 months;[1,16,17] they were non-smokers with no special hormonal conditions, such as pregnancy, menopause, menstruation or puberty. All the subjects signed a consent form and their oral cavities were examined by two observers. The clinical examination, including Turesky-Glimore-Glickman plaque index (PI),[20] Modified Loe and Silness gingival index (GI),[21] probing depth (PD), clinical attachment loss (CAL) and bleeding on probing (BOP) were recorded using a Williams probe by two dental interns under supervision of an experienced periodontist. Three sample groups were included:

1. 20 samples with healthy tissues or gingivitis (PD less than 3 mm and CAL less than 1 mm)
2. 20 samples with moderate-to-advanced chronic periodontitis (PD and CAL more than 4 mm with BOP)
3. 20 samples with moderated-to-advanced aggressive periodontitis (PD and CAL more than 4 mm with BOP)

All the patients in the chronic and aggressive periodontitis groups had previous oral hygiene instructions, including flossing and Bass brushing technique and SRP at least one month before surgery.[12] The sites with endodontic lesions[6] or suppuration were excluded.

Biopsies

Informed consents were obtained from all the adult participants, including the name of the appropriate institutional review board that approved the project. Ethics Committee of Qazvin University of Medical Sciences approved the protocol. Each patient underwent periodontal surgery, independently of this study, as a part of their routine periodontal treatment (crown lenghtening and full-thickness mucoperiosteal flap/debridement) by one surgeon in an identical manner and technique. Informed consent was obtained from the patients to collect, preserve and analyze the gingival tissues for this study. Biopsies were obtained from suitable sites immediately after diagnosis, from the deepest sites of interproximal pocket at the time of surgeries.

Histological technique

The specimens were immediately fixed in 10% formalin for further processing and then dehydrated, cleared and embedded in paraffin. Two 5-micron sections were obtained from each sample. The sections were placed on slides, dried and subsequently deparaffinized in 3 changes of xylol and rehydrated in 3 changes of 95 percent ethyl alcohol and distilled water. Adjacent sections were stained with hematoxylin/eosin (H&E) for inflammation assessment and toluidine blue for mast cell counting. Each section was examined twice utilizing the Olympus light microscope by 2 dental

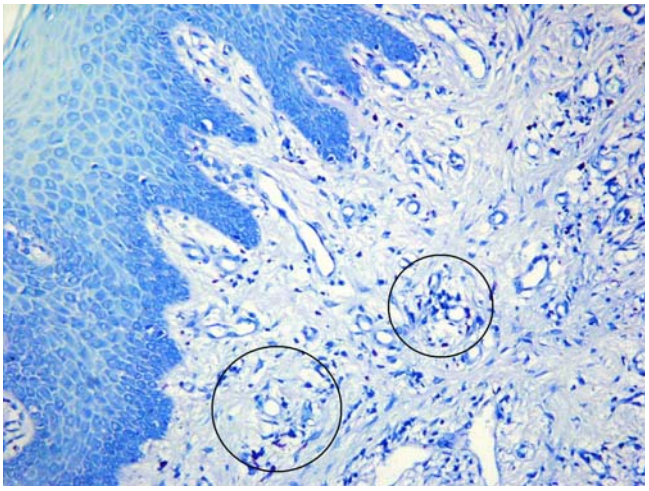

Figure 1. Microscopic view of mast cells (Magnification, ×400).

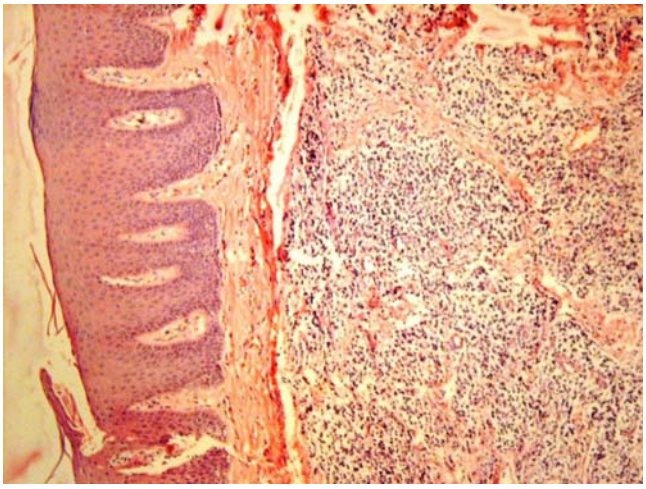

Figure 2. Microscopic view of inflammatory cells (Magnification, ×100).

students as observers. All the mast cells were counted in areas directly below the epithelium in 5 high-power fields (×400) (Figure 1). The density of the inflammatory infiltrate was assessed at ×40 and ×100 (Figure 2) based on the slight and severe criteria.[10] A pathologist checked the 15 sites with triple counting to evaluate the counting errors of the two observers. ANOVA and *t*-test were used to analyze data. Statistical significance was defined at α=0.05.

Results

The correlation coefficient between the two observers and the pathologist was 95.6 percent. On the whole 20 chronic periodontitis, 19 aggressive periodontitis and 18 healthy/gingivitis biopsies were obtained from 26 subjects. Three biopsies were excluded because of laboratory problems.

Table 1. Means of patient ages and oral characteristics in the three study groups

Variable	Healthy/ Gingivitis	Aggressive periodontitis	Chronic periodontitis
Age (years)	36.2	34.1	45.2
PD (millimeters)	2.22	7.02	5.85
CAL (millimeters)	0.8	5.83	4.85

The mean of the clinical parameters (PD and CAL) of the three groups are presented in Table 1.

BOP was positive in all the sites of chronic and aggressive periodontitis (case groups) and 11 sites of healthy/gingivitis (control group).

PI was under 2 in all the sites and GI was between the grades 1 and 3.

The results of the analyses of mast cell counts between the three groups are shown in Table 2.

There were significantly more mast cells in the chronic periodontitis cases as compared to aggressive periodontitis cases and the healthy/gingivitis ones (P = 0.000); however, the aggressive periodontitis cases did not show higher counts as compared to the healthy/gingivitis cases (P>0.05) (Table 2).

In addition, there was no relationship between mast cell counts and degree of inflammation in the three groups.

Discussion

The results of this study suggest that mast cell counts may be associated with periodontitis. The results of the present study seem to indicate that mast cells have higher counts in chronic periodontitis compared to healthy/gingivitis cases, which is consistent with the results of studies carried out by Kennett,[14] Myint,[6] Kabashima,[15] Jeffcoat,[4] and Næsse et al.[11] This finding indicates the role of mast cells in chronic periodontal tissue breakdown. One of the biological and biochemical factors is histamine, which breaks down the tissue barrier, causes edema and helps cellular infiltration. In addition, mast cells are believed to contain most of the body's histamine.[7] Another reason is that the expression of Matrix Metalloproteinases (MMPs) 1, 2, and 8 are strongest in mast cells. MMPs are crucial in the degradation of the main components in extracellular

Table 2. Means of mast cell counts in the three study groups

	Mean ± SD			
	Healthy/Gingivitis	**Aggressive periodontitis**	**Chronic periodontitis**	**P value**
Mast cell count	13±9.8		34.5±23.1	0.000
	13±9.8	10.8±7.6		>0.05
		10.8±7.6	34.5±23.1	0.000

matrices.[11] Furthermore, tryptase can cleave the third component of collagen and activate latent collagenase that can participate in tissue destruction in periodontitis. Furthermore, it has been indicated that tryptase activity is confined to mast cell granules. Kennett[14] assessed the activity of mast cell tryptase by histochemical technique and indicated that the number, distribution and morphology of the cells stained with toluidine blue were similar to those stained with methoxy-2-naphthylamine.

In the present study chronic periodontitis cases had higher mast cell counts compared to gingivitis sites or healthy tissues. In a study carried out by Næsse et al[11] mast cell counts were significantly higher in chronic periodontitis as compared to healthy/gingivitis group in both HIV-positive and HIV-negative patients. Zappa et al[13] evaluated cell populations in progressing and non-progressing sites in chronic periodontitis patients. Increased mast cell counts in the progressing sites of periodontal diseases may indicate the importance of these cells in the progression of chronic periodontitis. Since previous studies have not elaborated on this finding, active lesions were identified by bleeding on probing (BOP) in the present study.

The results of the present study are different from the results of studies carried out by Gemmell[5] and Aeschlimann et al.[7] Gemmell et al showed decreased counts of tryptase-positive mast cells in chronic periodontal sites as compared to healthy/gingivitis samples. Different techniques for evaluating mast cells and use of PD for defining the type of the disease may account for this difference.

In most previous studies, biopsies were taken from chronic periodontitis sites; however, the comparison between aggressive periodontitis and other groups cannot be directly compared to other studies.

In this study, mast cell counts were not significantly different in chronic versus aggressive periodontitis, which might be attributed to immunological and microbiological differences between different disease entities. As far as our available literature is concerned, these data are the first assessment of mast cell population to discriminate between these groups of diseases.

In the present study toluidine blue was used because of its simplicity and we had some limitations in selecting the method. However, similar results have been achieved by immunohistochemical and immunofluorescense assays for counting mast cells because almost 75% of mast cells are formalin-sensitive.[23]

The time for taking biopsies following SRP is another explanation for different results in different studies; however, none of the previous studies have taken this into account. Since dental treatments, such as SRP, usually result in hormonal responses to some of the subgingival bacteria, including *Actinobacillus actinomycetemcomitance* and *Porphyromonas gingivalis*, and peak of the response of serum antibodies occurs at about 2 to 4 months following SRP, in this study we used the period of 1 month to decrease the effect of these responses on mast cell degranulation.

The biopsies were obtained during the first session of the surgeries in all the patients to avoid the effects of previous surgeries on bacterial load and not to change inflammation and immunological process; moreover, in this study biopsies were taken from the bottom of the gingival pockets in the interproximal areas that show the greatest intensity of disease. However, except for Myint et al,[6] in the other studies, biopsies were taken from papilla that does not seem to be a suitable site to demonstrate the intensity of inflammation and progression of the disease.

We selected moderate-to-advanced areas of periodontitis to minimize the overlapping of different degrees of periodontal diseases; except for Gemmell[5] and Zappa et al,[13] others have not categorized the severity of periodontal diseases in this manner.

It seems that the evaluation of consumption rate is as important as the production rate while evaluating mast cell counts. Therefore, endocrine and immunologic factors such as interleukin-3, and 4 (IL3, 4), the complement system,[3] mediators such as transforming growth factor β-1 (TGF-β-1), SCF[3] and cellular communicators such as fibroblasts[7] and TH-1, and 2,[5] that affect mast cell turnover, should also be noted.

By using techniques such as immunohistochemical assays, evaluation of cellular contents and tracing the receptors, mast cell counting and cellular turnover assessment can be carried out simultaneously; however, we did not find any information in this regard in previous studies.

The life style of patients with long-term periodontal and heart diseases, in which the immune system would be exposed to continuous and slight insults, may also be important.

Furthermore, there were no relationships between the degree of inflammation and mast cell counts in this study, consistent with the results of a study by Cobb et al.[26]

Although numerous investigations have tried to correlate mast cell counts with the severity of inflammation,[5,7,8] the conflicting results among these studies is in part due to differences in histological techniques and a lack of adequate controls. The improved techniques and precise controls used in recent investigations have yielded more consistent results. In this study no significant relationships could be explained due to

the following factors:

a) In severe inflammation, mast cells are possibly undetectable by conventional histologic staining because of their degranulation.

b) Inflammation was assessed only in 2 grades (slight, severe) and the moderate grades were not evaluated separately. It decreased the error of eyes in grading because of wide differences between the two grades.

Finally, we suggest longitudinal studies on animal samples due to ethical and time limitations in taking standard samples from human biopsies and evaluation of cellular changes in different types of periodontal diseases.

Conclusion

Because of the importance of periodontal diseases, inadequate studies and possible relationships between mast cells and pathogenesis of periodontal diseases, further research is needed to elucidate the cellular interactions and immunologic and dynamic aspects of the disease so that the pathogenesis of periodontitis might be elucidated more clearly and effective treatment approaches can be suggested.

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