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# Correlation of Mast Cell Numbers and Different Periodontal Diseases

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**Abstract:** Mast cells play an important role in allergic reactions, host defense, local homeostasis, inflammation and angiogenesis. The objective of this study was to evaluate the relationship between mast cell numbers and different types of periodontal diseases. Gingival specimens were taken from 20 moderate to advanced chronic periodontal and 19 moderate to advanced aggressive periodontal sites as case groups and 18 healthy/gingivitis sites as control group in routine periodontal flaps and crown lengthening procedures. All the specimens were stained by toluidine-blue for mast cells counting and hematoxylin-eosin to assess inflammation. Inflammatory and mast cells in 5 micron sections were assessed by two observers 3 times, utilizing light microscope at 100 and 400× magnification. Anova and T tests with an alpha error level <5% were used to analyze data. Mast cells numbers were higher in chronic versus aggressive periodontitis and healthy/gingivitis ($p = 0.000$). The aggressive periodontitis did not have more numbers of mast cells as compared to healthy/gingivitis ($p>0.05$). There were no relationship between mast cell numbers and degree of inflammation in 3 groups. The present study indicates more mast cell numbers presence in the chronic periodontitis sites than other sites. The results of this study suggest more studies to evaluate dynamic aspects of host defense in conjunct with other aspects of immune system, simultaneously.

**Key words:** Mast cell, periodontal disease, inflammation, chronic, aggressive, Iran

## INTRODUCTION

Periodontitis is an inflammatory disease triggered by bacteria in dental plaque that is one of the most common oral diseases (Newman *et al*., 2002). It is characterized by a dense infiltrate of inflammatory cells, loss of connective tissues, formation of periodontal pockets and breakdown of the alveolar bone which finally leads to tooth mobility and tooth loss (Newman *et al*., 2002; Lindhe *et al*., 2003). In present, the common periodontal treatments are mechanical debridements including oral hygiene measures, Scaling, Root Planning (SRP) and surgical techniques and chemical methods such as use of antibiotics and mouth washes (Newman *et al*., 2002).

Biologic information of the disease mechanisms is the first step to prevent and treat the periodontal disease. The role of the host in the pathogenesis of periodontal disease has been studied by many investigators (Steinsvoll *et al*., 2004; Nuki and Farnoush, 1975; Myint *et al*., 2002; Mirbod *et al*., 2001; Kennett *et al*., 1993; Zappa *et al*., 1992). Substantial evidences have implicated certain immune and inflammatory responses as destructive mechanisms in the periodontal disease process (Steinsvoll *et al*., 2004; Gemmell *et al*., 2004). One possible host reaction against periodontal breakdown may be mediated by mast cell releasing. Some investigators have proposed a role for mast cell constituents in periodontal destruction (Gemmell *et al*., 2004; Myint *et al*., 2002; Kennett *et al*., 1993; Zappa *et al*., 1992).

These cells play a key role in gingival homeostasis and express Matrix Metallo Proteinases (MMP) that may be important in the progression of periodontitis (Steinsvoll *et al*., 2004; Aeschlimann *et al*., 1980) However, the contribution of mast cell mediators in periodontal disease progression is not obviously known (Gemmell *et al*., 2004).

Increased numbers of mast cells have been reported in gingivae as compared to healthy tissues (Neiders *et al*., 1979). Alternatively, Carranza and Cabrini (1955), Jeffcoat *et al*. (1985) and Turesky *et al*. (1970) observed that the mast cell population decreased in inflamed gingival tissue. Gemmell *et al*. (2004) compared chronic periodontitis lesions with healthy/gingivitis ones and indicated lower numbers of mast cells in periodontitis

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lesions. Gunhan *et al.* (1991) has showed significant increase in numbers of these cells within infected tissues compared to healthy samples.

Some reports studied effects of Lodoxamide ethyl and disodium cromoglicate, the releasing inhibitors of mast cells, for example, Jeffcoat *et al.* (1985) indicated reduction of the rate of alveolar bone loss and Nuki and Farnaush (1975) showed failure to alter the development of experimental gingivitis by these factors.

Hence, because of the different results, limited attention and insufficient knowledge about the precise role of mast cells in periodontal diseases, this study has been undertaken to examine the relationship between mast cell numbers and periodontal diseases.

## MATERIALS AND METHODS

**Patients:** Persons who have been referred to a specialized dental center were entered the study. Patients had no systemic diseases (Newman *et al*., 2002; Roitt *et al*., 2001) and had not used any medication (Movahedi, 1996) with probable effects on periodontal tissues since last 2 months (Newman *et al*., 2002; Kabashima *et al*., 2002; McDonald *et al*., 2004) they were nonsmokers and with no especial hurmonal conditions such as pregnancy, menopause, menstrual or puberty.

All the persons signed a consent form and their oral cavities were examined by two observers. The clinical examination including Turesky-Glimore-Glickman Plaque Index (PI) (Lobene *et al*., 1986), Modified Loe and Silness Gingival Index (GI) (Carranza *et al*., 1996), 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:

- Twenty samples with healthy tissues or gingivitis (PD <3 mm and CAL <1 mm)
- Twenty samples with moderate to advanced chronic periodontitis (PD and CAL >4 mm with BOP)
- Twenty samples with moderated to advanced aggressive periodontitis (PD and CAL >4 mm with BOP)

All patients in chronic and aggressive periodontitis groups had previous oral hygiene instruction including flossing and Bass brushing technique ® and SRP at least one month before surgery (Carranza and Cabrini, 1955). The sites with endodontic lesions (Roberts and Brenchley, 2000) or suppuration were excluded.

**Biopsies:** Informed consents were obtained from all human adult participants include the name of the appropriate institutional review board that approved the project. Ethical committee of Qazvin Medical University verified the protocol. Each patient was being undergone periodontal surgery, independently of this study, as a part of their routine periodontal treatment (crown lenghtening and full thickness mucoperiostal flap/debridements) by one surgeon in the same condition. Inflamed consent was obtained from the patients to collect, preserve and analyze of the gingival tissues for this study. Biopsies were obtained immediately after their diagnosis of suitable sites, 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. About 2 sections obtained from each sample at 5 micron. The sections were placed on slides, dried and subsequently deparaffinized in 3 changes of xylol and rehydrated in 3 changes of 95% ethyl alcohol and distilled water. Adjacent sections were stained with Hematoxylin-Eosin (H and E) for inflammation assessment and toluidine-blue for mast cell counting. Each section examined twice utilizing the Olympus light microscope by 2 dental students as observers.

All the mast cells were counted in areas directly below the epithelium in 5 high-power fields (400X) (Fig. 1). The density of the inflammatory infiltrate was assessed at 40 and 100X (Fig. 2). According to the slight

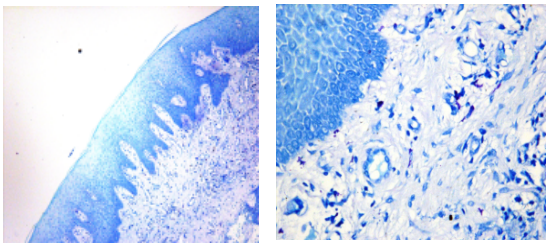

Fig. 1: Pathologic view of mast cells

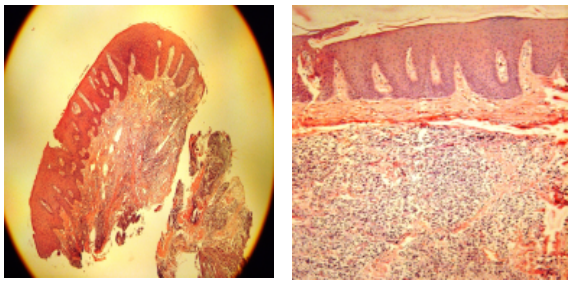

Fig. 2: Pathologic view of inflammatory cells

and severe criteria (Naesse *et al*., 2003). A pathologist checked the 15 sites with triple counting to evaluate the counting errors of the two observers. Anova and t-tests with an alpha error level <0.05% were used to analyze data.

## RESULTS AND DISCUSSION

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

The mean of the clinical parameters (PD and CAL) of 3 groups are shown in Table 1. BOP in all sites of chronic and aggressive periodontitis (Case groups) and 11 sites of healthy/gingivitis (control group) were positive. PI in all sites was under 2 and GI was between the grades 1 and 3.

The results of the analyses of mast cell counts between 3 groups have been shown in Table 2. There were more mast cells in the chronic periodontitis as compared to aggressive periodontitis and the healthy/gingivitis significantly ($p = 0.000$) however, the aggressive periodontitis did not show more numbers as compared to the healthy/gingivitis ($p>0.05$) (Table 2). Also, there were no relationship between mast cell numbers and degrees of inflammation in 3 groups.

The results of this study suggest that the number of mast cells may be associated with periodontitis. It appeared that the mast cells had higher numbers in chronic periodontitis than the healthy/gingivitis. This agrees with the findings of Kennett *et al*. (1993), Myint *et al*. (2002), Kabashima *et al*. (2002), Jeffcoat *et al*. (1985) and Naesse *et al*. (2003) can indicate the mast cell involvement in the chronic periodontal tissue breakdown. One of the biological and biochemical explanations is histamine. The function of histamine is to break the tissue barrier down, cause edema and help cellular infiltration. Also mast cells are believed to contain most of the human's body histamine (Neiders *et al*., 1979).

Table 1: Means of patient ages and oral characteristics in the 3 study groups

| Parameters | Healthy/ gingivitis | Aggressive periodontitis | Chronic periodontitis |
|---|---|---|---|
| Age ( years ) | 36.20 | 34.10 | 45.20 |
| PD (mm) | 2.22 | 7.02 | 5.85 |
| CAL (mm) | 0.80 | 5.83 | 4.85 |

Table 2: Means of mast cell numbers in the 3 study groups

| Mast cell count | | | |
|---|---|---|---|
| Healthy/ gingivitis | Aggressive periodontitis | Chronic periodontitis | p-value |
| 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 |

Another reason is that the expression of Matrix Metallo Proteinases (MMPs) 1, 2, 8 were strongest in the mast cells. MMPs are crucial in the degradation of main components in extra cellular matrices (Aeschlimann *et al*., 1980). Also , tryptase can cleave the third component of collagen and activate latent collagenase then could participate in tissue destruction in periodontitis.

Further more, it has been indicated that tryptase activity was localized to progress mast cell granules, Kennett *et al*. (1993) 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.

Chronic periodontitis had a higher numbers of mast cells than gingivitis sites or healthy tissues. In Naesse *et al*. (2003)'s study numbers of mast cells were significantly increased in chronic periodontitis as compared to healthy/gingivitis group in both HIV positive and HIV negative patients. Zappa *et al*. (1992) evaluated cell populations in progressing and non-progressing sites in chronic periodontitis patients.

In result, increased numbers of mast cells in the progressing sites of periodontal diseases may indicate the importance of these cells in the progression of chronic periodontitis. Since previous studies haven't notice to this point in present study, active lesions were identified by Bleedings on Probing (BoP).

Results of the present study are different from the results of Gemmell *et al*. (2004) and Aeschlimann *et al*. (1980). Gemmell showed decreased tryptase positive mast cells in chronic periodontal sites as compared to healthy/gingivitis samples. Different techniques for evaluating mast cell and use of PD for defining of the type of the diseases may be the reason of this difference. In most of previous studies, biopsies were taken from chronic periodontitis sites; however the comparison between aggressive periodontitis and other groups can not be directly compared to other studies.

In this study, the number of mast cells have not had any significant difference in chronic versus aggressive periodontitis, may be due to immunological and microbiological differences between different diseases. As far as the available literature, these data are the first assessment of mast cell population to discriminate between these groups of disease.

In the present study, toluidine blue was used because of its simplicity and the researchers have some limitations in selecting the method. However, similar

results have been taken by immunohistochemical and immunofluorescense assays for counting mast cells, because around of 75% total mast cells are formalin sensitive (Mirbod *et al*., 2001).

Special time for taking biopsies following SRP is another explanation for different results among the studies, however in none the former studies have been noticed to. Since dental treatments such as SRP, usually leads hurmonal responses to some of the subgingival bacteria including actinobacillus actinomycetem commitance and porphyromonas gingivalis and peak of the response of serum antibodies is about 2-4 months following SRP; in this study we used the period of 1 month to decrease the effects of these responses on mast cells degranulation.

The biopsies were obtained in first session of the surgeries in all patients to inhibit the effects of previous surgeries on load of bacteria and not to change of inflammation and immunological process; moreover in this study, biopsies were taken from the bottom of the gingival pockets in the interproximal areas that shows most intensity of disease, however except Myint *et al*. (2002) and Roberts and Brenchley (2000), in the other studies, biopsies were taken from papilla that does not seem to be a suitable site to show the intensity of the inflammation and progression of the disease.

The researchers select moderate to advanced areas of periodontitis to minimize the overlapping of different degrees of periodontal diseases that except Gemmell *et al*. (2004) and Zappa *et al*. (1992), others have not categorized the severity of the diseases in this way.

It seems that the evaluation of consumption rate is as important as the production rate while evaluating mast cell numbers. Hence, endocrine and immunologic factors such as interleukin-3, 4 (IL3, 4), complement system (Steinsvoll *et al*., 2004), mediators such as transforming growth factor B1 (TGF B1), SCF (3) and cellular communications such as fibroblasts and TH 1 and 2 that affected mast cell's turn over should also be noted.

By using developed techniques such as immunohistochemical assays with evaluating cellular contents or tracing the receptors can specially count the mast cells and assess cellular turnover simultaneously, however we did not find any information in the former studies.

The life style of patients with continuous long term mode such as periodontal and heart diseases in which immune system would expose to continuous and slight insults may also be important. Furthermore, there were no relationships between the degree of inflammation and mast cell numbers in this study that agreed with results of Cobb *et al*. (1976).

Although, numerous investigations have tried to correlate mast cell numbers with the degree of inflammatory involvement (Nuki and Farnoush, 1975; Neiders *et al*., 1979; Nisengard, 1977) the conflicting results that is seen among these studies is in part due to differences in histological technique 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 may be explained by these following factors:

- In severe inflammation, mast cells are possibly undetectable by the ordinary histologic staining because of their degranulation
- 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 high differences existed between 2 grades

Ultimately, 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 the progression of different types of periodontal diseases.

## CONCLUSION

Because of the importance of periodontal diseases, inadequate studies and possible relationships between mast cells and pathogenesis of this disease; further researches is needed to conceive the cellular interactions and immunologic and dynamic aspects of the disease then the pathogenesis of periodontitis will be elucidated more clearly and effective treatment approaches can be suggested.

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