Quantification of Mast Cells in Periodontal Diseases: A Comparative Study

ABSTRACT

Objective The present study was undertaken to identify and quantify the presence of mast cells in human periodontal disease using histochemical (toluidine blue [TB]) technique.

Materials and Methods A total of 45 tissue samples were obtained for the study: GROUP 1, 15 cases of clinically healthy gingival tissues, GROUP 2, 15 cases of pericoronitis and GROUP 3, 15 cases of chronic periodontitis were selected. Periodontally healthy tissue samples were obtained from premolar teeth extracted for orthodontic reasons. Samples of chronic periodontitis were obtained from teeth extracted due to poor periodontal prognosis. In pericoronitis group impacted third molar and operculum with inflammatory signs were selected. Sample fixed in 10% buffered formalin and stained with TB stain and observed under binocular microscope.

Result Mast cell densities (cells per mm²) were significantly increased in chronic periodontitis, and pericoronitis group compared to clinically healthy gingival tissues by histochemical technique.

Conclusion In human periodontal disease there is an increase in the number of mast cells that may be contributing either in the destructive events or in the defense mechanism of periodontal disease via secretion of cytokines, cellular migration and healing processes.

KEYWORDS chronic periodontitis, pericoronitis, mast cells, toluidine blue

INTRODUCTION

Periodontitis is considered the most common inflammatory oral disease triggered by bacteria in the dental plaque. It is elucidated by the presence of dense infiltrate of inflammatory cells, loss of connective tissue (CT), formation of periodontal pockets, breakdown of the alveolar bone which subsequently leads to tooth mobility and tooth loss¹.

Bacterial plaque has been implicated as the primary etiological factor in the development of inflammatory periodontal disease, but recently several studies have focused on the role of the immune system, indicating that bacterial antigens trigger an immunopathological reaction and the ultimate outcome of the disease process is dependent on the individual host response².

Among the cells found in the periodontal tissues, mast cells have been detected in varying quantities at both healthy and inflamed gingival sites. Mediators derived from mast cells are stored within the secretory granules and are released by degranulation when these cells get stimulated or activated³.

Mast cells are implicated in various activities ranging from control of vasculature to tissue injury repair, allergic inflammation and host defense. Their significant contribution to tissue damage and propagation of inflammatory responses makes the control of mast cell activity vital in the management of many inflammatory diseases⁴.

Nowadays, there is an increased awareness of the potential interactions between mast cells and other components of the immune response, contributing to the modulation of humoral and cellular events in host defense mechanisms against bacterial infections, and probably participating in the pathogenesis of inflammatory conditions such as periodontal disease⁵,⁶.

The aim of this study was quantification of mast cells in health and disease, whether they correlate with degree of inflammation.
**MATERIALS AND METHODS**

Patients who reported to the Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore, were enrolled in the study. A total of 45 tissue samples were obtained for the study. All individuals with age range of 16.47 ± 49.73 years, M = 57.8%, F = 42.2% (mean 16.47 ± 49.73, M = 57.8%, F = 42.2%) were included after performing the power analysis.

The study was conducted in accordance with the Helsinki declaration of 1973, revised in 2000. Prior to the execution of the treatment, written informed consent was obtained and the treatment procedure was explained to the patient. Ethical clearance was obtained from Institutional Ethical Committee Review Board. Fifteen periodontally healthy tissue samples were obtained from tooth extractions for orthodontic treatment, generally premolars. Fifteen gingival tissue samples of inflamed pericoronal flap with clinical parameters such as gingival inflammation, bleeding on probing (BOP) with no clinical attachment loss (CAL) and 15 moderate-to-advanced chronic periodontitis (probing depth [PD] and CAL more than 4 mm with BOP), tissue samples were obtained from patients undergoing extraction of involved tooth which had poor periodontal prognosis.

The patients had no systemic diseases and had not used any medications with probable effects on periodontal tissues for the previous 2 months; they were non-smokers with no special hormonal conditions, such as pregnancy, menopause, menstruation or puberty.

The clinical examination, including Turesky-Glimore-Glickman plaque index, modified Loe and Silness gingival index, probing depth (PD), CAL and BOP were recorded using a Williams periodontal probe.

### Biopsies

Biopsies were obtained with a scalpel blade (no. 15) by incisional biopsy method from suitable sites immediately after extraction of diagnosed periodontitis tooth, from the deepest sites of interproximal pocket. The specimens were immediately fixed in 10% formalin for further processing and then dehydrated, cleared and embedded in paraffin.

### Histological technique

Two 5 μ 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% ethyl alcohol and distilled water. Toluidine blue (TB) staining was performed with a 1% TB for 30 min diluted in phosphate buffer (pH = 4–6) for 45 s. Then the tissue samples were placed at 37°C in incubator. After rinsing in phosphate buffer for 1 min. sections were blotted carefully, quickly dehydrated through, 96% ethanol and absolute alcohol to xylene and mounted in synthetic resin and observed under binocular microscope. Mast cells were identified by deep blue purple staining.2

### Quantitative and statistical analysis

Number of positively stained mast cells in the periodontal tissues was determined in four consecutive microscopic high-power (×800; objective ×64; eyepiece ×12.5; tube factor ×1) fields. Mast cell counts in inflammatory cell infiltrate of diseased tissue and periodontally healthy tissue areas have been performed. The results of TB-stained mast cells were expressed as mean ± SD of on observations per mm². Also the comparative analysis of the number of mast cells/mm² between periodontally healthy and diseased group was performed. ANOVA was used to test the difference between the groups. To find out which of the two groups means is significantly different post hoc test of Scheffe test was used. p value less than 0.05 was taken to be statistically significant. The data were analysed using SPSS package (Version 11.5).

### RESULTS

Table 1/Fig. 1 show mean values of age in quantification of mast cells in periodontally healthy, pericoronitis

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min.</th>
<th>Max.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (N = 15)</td>
<td>15</td>
<td>16.47</td>
<td>1.959</td>
<td>16</td>
<td>14</td>
<td>20</td>
<td>274.902</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periodontitis (N = 15)</td>
<td>15</td>
<td>22.6</td>
<td>2.586</td>
<td>22</td>
<td>19</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic periodontitis (N = 15)</td>
<td>15</td>
<td>49.73</td>
<td>6.386</td>
<td>50</td>
<td>41</td>
<td>61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Quantification of mast cells in periodontal diseases

Table 2: Total no of mast cells.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (N = 15)</td>
<td>15</td>
<td>2.8</td>
<td>1.014</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>83.186</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periodontitis (N = 15)</td>
<td>15</td>
<td>6.47</td>
<td>1.885</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic periodontitis (N = 15)</td>
<td>15</td>
<td>12.4</td>
<td>2.849</td>
<td>12</td>
<td>8</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2: Comparison of mean total number of mast cells among the study groups.

Table 3: Degree of inflammation.

<table>
<thead>
<tr>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference (I-J)</th>
<th>’p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>Periodontitis</td>
<td>-3.667</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Chronic periodontitis</td>
<td>-9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Chronic periodontitis</td>
<td>5.933</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Fig. 3: Degree of inflammation.

differentiation in this site, then enter the circulation to complete their differentiation in the peripheral mucosal tissue or CT micro-environment rich in fibroblasts and other mesenchymal elements2.

The inflammatory cell infiltrate in chronic periodontitis has been observed7–10, and interestingly, found high numbers of mast cells equal to and often mounting the numbers of macrophages in the inflamed periodontal lesion7. Many reports focused on the mast cell as pivotal cell in both innate and acquired immunity11–13 and in wound-healing processes8,14.

The results of the present study signify higher mast cell counts in chronic periodontitis compared to healthy/gingivitis cases, which is consistent with the studies carried out by Kennett15, Myint7, Ka-bashima16, Jeffcoat17 and Næsse et al.18. Mast cells are seen distributed throughout gingival CT, often in association with endothelial cells, but are also found sub- and intraepithelially. These findings point the role of mast cells in chronic periodontal tissue breakdown8,19,20.

DISCUSSION

Mast cells taking origin from the pluripotent hematopoietic cells in the bone marrow, undergo part of their
Table 4  Degree of inflammation.

<table>
<thead>
<tr>
<th></th>
<th>No inflammation</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Mean difference (I–J)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (I)</td>
<td>60.0%</td>
<td>40.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>−3.667</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peri­cor­onitis (J)</td>
<td>0.0%</td>
<td>53.3%</td>
<td>33.3%</td>
<td>13.3%</td>
<td>−9.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic periodontitis (J)</td>
<td>0.0%</td>
<td>46.7%</td>
<td>33.3%</td>
<td>20.0%</td>
<td>5.933</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>20.00%</td>
<td>46.70%</td>
<td>22.20%</td>
<td>11.10%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Walsh et al. have showed that mast cell numbers were dramatically increased in inflamed sites of periapical granulomas and lichen planus when compared with non-lesion sites representing higher activity of that cells in that area. In 1991, he describes various key mediators in mast cells have a role in tissue breakdown. Degranulation induced by MC activation releases pro-inflammatory substances, like proteases, histamine, proteoglycans, arachidonic acid metabolites, chemokines and growth factors. These cytokines secreted by the mast cell, tumor necrosis factor alpha is of particular interest, being related to inflammation of the oral cavity (Walsh et al. 1995). Furthermore, mast cells can also synthesize a range of mediators, including the interleukins (IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13 and IL-16), together with granulocyte-macrophage colony-stimulating factor, platelet-activating factor, RANTES, macrophage inflammatory protein, convincing their presence and activity in inflammatory sites.

Mast cells are believed to contain most of the body’s histamine which breaks down the tissue barrier, causes edema and helps cellular infiltration. Expression of matrix metalloproteinases 1, 2 and 8, are strongest in mast cells, which are crucial in the degradation of the main components in extracellular matrices. Furthermore, tryptase can cleave another component of collagen and activate latent collagenase that can participate in tissue destruction in periodontitis; even tryptase activity is confined to mast cell granules. Kennett assessed the activity of mast cell tryptase by histochemical technique and indicated that the number, distribution and morphology of the cells stained with TB were similar to those stained with methoxy-2-naphthylamine.

In the present study, chronic periodontitis cases had higher mast cell counts compared to healthy tissues. Næsse et al. showed mast cell counts were significantly higher in chronic periodontitis as compared to healthy/gingivitis group in both HIV-positive and HIV-negative patients. Günhan et al. 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.

In this study, mast cell counts were statistically significant difference between healthy group, periocorontitis and chronic periodontitis, which might be attributed to immunological and microbiological differences between different disease entities. In the present study, TB was used because of its simplicity and we had some limitations in selecting the method.

CONCLUSION
In light of these results, it may be concluded that there is an increased number of mast cells in human periodontal disease compared with healthy tissues, considering inflammatory cell-rich areas of diseased tissues. Chronic periodontitis sites had higher mast cell counts compared to pericorontitis sites or healthy tissues.

FUTURE PERCEPTION
Though bacteria are thought to initiate the pathogenesis of the periodontal disease the host response is the one which plays a crucial role in the disease manifestation. Even though varied expression of aggressive form of periodontal disease progression has elucidated researchers till date. Analysis of role of mast cells in the disease progression in aggressive periodontitis may be required for the clear understanding of the pathogenesis of the disease.

REFERENCES