Immunohistochemical Assessment of Mast Cells and Small Blood Vessels in Dentigerous Cyst, Odontogenic Keratocyst, and Periapical Cyst

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Abstract

Purpose: The aim of this study was to verify the density of mast cells (MCs) and microvessels in odontogenic cysts. Furthermore, the correlation between MCs and microvessels was evaluated to assess the contribution of MCs to angiogenesis and growth of odontogenic cysts. This approach may be a basis for the development of future pharmaceuticals addressed to MCs performance to manage odontogenic cysts. To our knowledge, no study investigating the correlation between MCs and microvessels has been performed to date.

Methods: 60 cases of odontogenic cysts consisting of 20 radicular cysts (RCs), 20 odontogenic keratocysts (OKCs) and 20 dentigerous cysts (DCs) were included in this study. Five high power fields in superficial connective tissue and five high power fields in deep connective tissue were counted for each sample. Moreover, a total mean of ten fields was calculated.

Results: RC showed the highest mean numbers of MCs and microvessels (p<0.05). The subepithelial zones of all cysts contained more MCs and microvessels compared to the deeper zones. A statistically significant correlation between the numbers of MCs and microvessels was not observed (r=0.00, p=0.49).

Conclusion: Although the number of MCs was not significantly associated with microvessels, these cells may be related to the growth of odontogenic lesions, particularly RCs. Further studies on the in vivo functions of MCs will make the concept more clear.

Introduction

Cysts are the most common destructive lesions of the jaw bones.1,2 Odontogenic cysts are derived from the epithelium of the dental apparatus and constitute more than 95% of the cysts of the jaws. The far most common odontogenic cysts are RCs, DCs, and OKCs. Odontogenic cysts are either inflammatory or developmental in origin. However, secondary inflammatory changes may be seen in the developmental cysts.3,4

MCs are granulocytes (with large cytoplasmic granules) that originate from the bone marrow.2,3 These cells are spread in the connective tissue and mucosal environments.5 MCs produce numerous proinflammatory cytokines e.g. IL-1α, 3,6 and TNF-α and play an important role in the initiation of the inflammatory responses. MC degranulation releases several factors including tryptase that play role in proteolytic cascades and affect endothelial cells by stimulating their proliferation and migration.5,8

It is not certain to date whether MCs are for or against the growth of the lesions. MCs play an important role in the angiogenesis by producing numerous factors such as heparin, VEGF, platelet-activating factor, tryptase, chemotactic mediators, and FGF.2,8,9 The role of MCs in vasoinductive events has been investigated by many researchers and these cells have been reported to promote angiogenesis in many lesions.10,12 On the other hand, MCs may suppress the growth of the lesions by synthesizing various factors e.g. TNF-α, IL-1 and IL-6.13,14 There has been much interest in assessing the contribution of MCs in pathology of different lesions. However, the definitive in vivo roles of MCs remain controversial.

Angiogenesis, the development of new blood vessels, is the hallmark of several diseases. Unlike the normal conditions, in pathological situations, new blood vessels are formed in a less organized fashion, serving to fuel the disease progression by aberrations in blood flow and oxygenation.15 A seminal work carried out about 70
years ago confirmed the importance of angiogenesis in the development of diseases.\textsuperscript{15} Angiogenesis has received great attention in malignant lesions, and more recently, similar investigations have been carried out in a number of non-malignant diseases.\textsuperscript{7,16} Few studies have been performed evaluating the presence of MCs in periapical granulomas and odontogenic cysts. These studies have reported the presence of MCs in these lesions, particularly in lesions with cystic morphology. However, it is not clear that MCs are present to promote the growth or suppress the development of odontogenic cysts. To our knowledge, no study investigating the correlation between MCs and microvessels has been performed to date.

The aim of this study was to determine the density of MCs and microvessels in odontogenic cysts. The relationship between MCs and microvessels was evaluated to assess the role of MCs in stimulating angiogenesis and growth of odontogenic cysts. This will make the concept of contribution of MCs to the growth and pathogenesis of the odontogenic cysts more clear and help for planning future nonsurgical treatment strategies targeting MCs.

Materials and Methods

In this work, the study samples included 60 paraffin blocks, retrieved from Department of Oral and Maxillofacial Pathology, Tabriz University of Medical Sciences, Tabriz, Iran. Three groups were considered in this study (OKC, DC and RC) and each category consisted of 20 cases. Only samples with adequate radiographic and clinical data were included and the histopathologic slides were assessed to confirm the diagnosis. Cases with improper paraffin blocks and insufficient microscopic fields for immunohistochemical analysis were excluded.

Four micrometer thick tissue sections were stained through standard immunohistochemical staining procedures according to the instructions of the manufacturer (DAKO, Glostrup, Denmark). The cut sections were mounted onto glass slides, deparaffinized in xylene, and rehydrated in alcohol. Endogenous peroxidase activity was blocked using hydrogen peroxide (1%). To retrieve antigen, citrate buffer solution (0.01 M, PH= 6.0) was used and the slides were rinsed with distilled water. Distribution of MCs and microvessels was determined using 1:20 diluted anti-CD31 and 1:100 diluted anti-MC tryptase primary mouse monoclonal antibodies. In the next step, the secondary antibody was applied and 3,3-diaminobenzidine (DAB) was used as chromagen. At last, the sections were counterstained with Harris hematoxylin.

For immunohistochemical counting, the sections were scanned at \times100 magnifications. Hot spot fields (the fields most populated by microvessels and MCs) were identified and ten representative high power fields (five field in superficial connective tissues and five fields in deep connective tissues) were analyzed in each section. The means of five superficial and five deep fields were calculated. Moreover, the mean of ten fields was considered as the total count for each sample. Evaluations were performed by two independent observers, with an agreement level of 92%.

Statistical analysis of the collected data was carried out by Statistical Package for Social Sciences version 20.0 (SPSS, Chicago, IL). Interobserver reproducibilities were established on six double assessments. One-way ANOVA test was carried out to compare the numbers of MCs and microvessels among odontogenic cysts. The numbers of MCs and microvessels between subepithelial and deep connective tissue regions were compared using paired t-test in each lesion. To compare the number of MCs and microvessels between inflamed and non-inflamed DCs and OKCs, independent t-test was used. Spearman’s rank correlation coefficient test was used to determine the correlation between the number of MCs and microvessels. A p-value of <0.05 was considered statistically significant.

Results

MC tryptase positive cells were mostly located beneath the epithelium of odontogenic cysts. However, MCs were spread throughout the connective tissue walls of odontogenic cysts. Likewise, microvessels were more commonly observed subjacent to the epithelium of odontogenic cysts (Figure 1).

Data from immunohistochemical assessments are illustrated in Figure 2 and Table 1. The mean ± SEM value for MCs was significantly higher in RC in comparison with DC and OKC (p<0.05). Likewise, the mean ± SEM value for microvessels was considerably higher in RC when compared to DC and OKC. However, statistically significant difference was only observed when RC was compared with non-inflamed DC and non-inflamed OKC (p<0.05).

Mean numbers of MCs and microvessels were higher in subepithelial regions. Statistically significant differences in the number of MCs, between superficial and deep areas,
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were observed in OKC and DC (p<0.05). Analysis of the microvessels, revealed statistically significant differences between superficial and deep zones in RC (p<0.05).

Discussion

In the present study, RCs showed the highest mean numbers of MCs and microvessels. The number of MCs was relatively higher in DC compared to OKC and relatively similar expression of MC tryptase and CD31 was observed in DC and OKC. These findings suggest that MCs may be somewhat associated with the angiogenesis and development of odontogenic cysts, particularly in RC. We used anti-MC tryptase antibody to stain MCs. In immunohistochemical assays, antibodies are used to detect antigens, hence have considerably higher sensitivity and specificity compared with special stains such as toluidine blue. Moreover, immunohistochemistry detects immature as well as mature MCs. On the other hand, toluidine blue is not very effective in detecting MCs due to the metachromatic staining of macrophages and fibroblasts, because of the phagocytosis of MC granules. Moreover, immature MCs might be failed to be stained with toluidine blue.\(^1,17\)

Few studies have investigated MCs in periapical granuloma and odontogenic cysts, demonstrating the presence of MCs in these lesions. Patidar et al.,\(^17\) using toluidine blue, found that MCs were observed more commonly in RC, followed by DC and OKC, respectively. Debta et al.\(^19\) showed that MCs were associated with RC, OKC and DC, in descending order. They used thionin and toluidine blue to stain MCs. de Oliveira Rodini et al.,\(^20\) using toluidine blue, found that MCs were more commonly observed in periapical cysts compared to periapical granulomas. However, it is an uncertain question that MCs are present to promote the growth or suppress the development of odontogenic cysts. Verifying the role of MCs in the pathogenesis and growth of odontogenic cysts will offer a basis for the future nonsurgical treatment strategies.

The mean numbers of MCs and microvessels were considerably higher in inflamed DC and OKC compared to non-inflamed counterparts (Figure 2). Regarding microvessel density, statistically significant difference was seen between inflamed and non-inflamed DCs. There was no correlation between the numbers of MCs and microvessels (r=0.00, p=0.49) (Figure 3).

### Table 1. Mast cell count and microvessel density in odontogenic cysts

<table>
<thead>
<tr>
<th>Studied groups*</th>
<th>Number of cases</th>
<th>Mast cells Mean±SEM</th>
<th>Microvessels Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC</td>
<td>20</td>
<td>15.28±1.62</td>
<td>14.14±0.88</td>
</tr>
<tr>
<td>NI-DC</td>
<td>10</td>
<td>8.34±1.53</td>
<td>9.1±0.78</td>
</tr>
<tr>
<td>I-DC</td>
<td>10</td>
<td>12.93±1.61</td>
<td>13.75±1.10</td>
</tr>
<tr>
<td>NI-OKC</td>
<td>10</td>
<td>8.67±0.90</td>
<td>9.39±1.44</td>
</tr>
<tr>
<td>I-OKC</td>
<td>10</td>
<td>10.24±2.30</td>
<td>13.07±1.55</td>
</tr>
</tbody>
</table>


The subepithelial regions of all studied cysts contained more MCs and microvessels compared to the deeper regions, which reflects the fact that MCs may be partially associated with boneresorption and spread of these lesions. Chatterjee et al., Chetterjee et al. and Patidar et al.\(^2,8,17\) found that MCs were more commonly observed beneath the epithelium compared to the deeper zones. However, Netto et al.\(^2\) showed higher numbers of MCs in the deep connective tissue compared to the subepithelial region.

We specifically considered two subdivisions for DC and OKC (inflamed and non-inflamed). In the case of non-inflamed cysts, MCs were more frequently observed in OKC compared to DC. The controversial results of previous studies could be due to the fact that inflamed and non-inflamed cysts were not separated appropriately in most of them. In our study, inflamed DCs and OKCs showed higher mean numbers of MCs and microvessels compared to non-inflamed counterparts, which indicate the involvement of MCs in the inflammatory reactions in odontogenic cysts.

In the immunohistochemical evaluation of the microvessels, relatively similar counts were observed in...
OKC and DC. Alaeddin et al. evaluated the microvessel density in 20 OKCs, 13 DCs, 14 solid ameloblastomas, and 6 unicystic ameloblastomas. They reported that the mean microvessel density was significantly higher in solid ameloblastomas compared to OKC and in OKC compared to DCs. Finally, our results did not reveal a statistically significant correlation between the numbers of MCs and microvessels. Many researchers have investigated the correlation between MCs and microvessels in numerous lesions of various sites in the body. For instance, in a study on renal cell carcinoma, Mohseni et al. did not observe a correlation between the MCs and microvessels. On the other hand, Mondal et al. in a study on cervical squamous cell carcinoma, and Sharma et al. in a study on oral squamous cell carcinoma reported a positive correlation between the MCs and microvessels. Collectively, the roles of MCs in the angiogenesis and development of the various lesions is an area of controversy. Further investigations are required to verify the role of MCs in odontogenic cysts.

Conclusion
MC count was not significantly associated with microvessel density in this study; however, mast cells may have a role in the growth of odontogenic lesions, particularly RC. Further investigations are desired to make the role of these cells in odontogenic cysts more clear.

Acknowledgments
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Ethical Issues
Not applicable.

Conflict of Interest
The authors declare that they have no competing interest.

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