Growth potential in localized reactive gingival lesions that affect alveolar bone

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ABSTRACT

Localized gingival reactive lesions usually develop in soft tissue, but they may sometimes involve the underlying bone. They are thought to arise from the periodontal ligament or the periosteum. However, there are many inconsistencies in terms of their etiology, growth potential and biological behavior – recurrence. The purpose of this study was to compare clinico-morphological features and Ki67 expression of localized reactive hyperplastic lesions of the gingiva. All cases used in this study were immunopositive for Ki67. The expression of Ki67 was not strictly associated with the clinical behavior of reactive lesions and as such, cannot be a prognostic factor of bone resorption.

Key words: Reactive gingival lesions, Ki67, bone resorption.

INTRODUCTION

Localized reactive hyperplastic lesions (LRHL) of the gingiva are the most common enlargements in the oral cavity. However, their classification, as well as etiology has been inconsistent (Agrawal, 2015). They are usually clinically similar but possess distinct histopathological features. On the basis of their histology, localized reactive gingival lesions can be classified into pyogenic granuloma, fibrous hyperplasia and peripheral giant cell granuloma (Anneroth and Sigurdson, 1983). Some authors classify fibrous hyperplasia with calcification as peripheral ossifying fibroma (POF) (Mergoni et al., 2015).

It is believed that localized reactive gingival lesions develop in response to chronic and recurring tissue injury such as calculus, iatrogenic injuries (overhanging dental restorations, carious teeth, orthodontic appliances). However, often the etiological factor cannot be diagnosed and the cause of development and progression of the lesion remains unknown (Kadeh et al., 2015; Buchner et al., 2010).

Localized reactive hyperplastic lesions of the gingiva are mostly present as small, pedunculated and painless mass of gingiva. Usually, the size of the localized gingival enlargements varies from a few millimeters to several centimeters; however, they can rarely attain a larger size and cause slight to severe alveolar bone loss (Niedzielska and Borgiel-Marek, 2009). Although a conservative surgical excision is considered to be the standard treatment of gingival LRHL, these lesions often demonstrate a high recurrence rate after insufficient surgery (Carvalho et al., 1995).

In view of the above, the etiology, clinical behavior and growth potential of reactive hyperplastic lesions of the gingiva are still a matter of discussion. Although there are numerous studies on the Ki-67 gene expression in benign and malignant neoplastic oral lesions, few studies have been conducted on the Ki-67 proliferative gene expression in reactive hyperplastic gingival lesions (Bodner et al., 1997; Bugala-Musiatowicz, 2004; Kranti et al., 2015; Mergoni et al., 2015).

This study aims to compare the proliferative activity of gingival reactive lesions with their clinical features with particular emphasis on alveolar bone resorption.

MATERIALS AND METHODS

In this study, a total number of 51 reactive lesions of the gingiva were collected from archives of the Oral Pathology Department, Medical University of Warsaw. Out of the total number of cases, 46 samples, including pyogenic granuloma (PG), focal fibrous hyperplasia (FFH) and peripheral giant cell granuloma (PGCG) that affect the alveolar bone were selected. The superficial erosion of the bone was confirmed...
on radiographs. Fifteen oral soft-tissue reactive lesions without bone resorption were considered as the control group. Demographic data, such as age, gender as well as clinical data including duration and location of the lesions, were obtained from medical records. The data were then analyzed to determine the correlation between the occurrence of osteolysis with demographic parameters and with the histopathological type, size and duration of LRHL.

Slices of 4-5-µ in thickness were prepared from paraffin embedded sections for morphological diagnostic on coloured sections of haematoxylin-eosin. Additional sections from each case were immunohistochemically coloured for the identification of Ki67. The slides were deparaffinized in xylene, and rehydrated in graded ethanol solutions. Slides were rinsed in water and incubated in Citra Plus Buffer (BioGenex) under the following conditions for antigen retrieval: microwave for 5 min, cool for 5 min, heat at 98°C for 5 min, and cool for 5 min. The slides were left at the room temperature for 30 min and then rinsed with distilled water. Endogenous peroxidase activity was blocked by incubating the slides in Peroxidase Block for 10 min.

Staining was done using mouse antibody diluted 1:15 and Bio Genex labeled Super Sensitive One Step Polymer – HRP (HC Detection System Large Volume DAB) reagents. Ultimately, the slides were rinsed with water for 5 min. The slides were then incubated with a specific antibody. Finally, the slides were mounted and examined under light microscope by 2 independent pathologists.

The histopathological preparations stained with HE were evaluated for the presence of inflammation based on the intensity of inflammatory cell infiltrates in connective tissue.

Cells were considered to be Ki-67 positive if there was brown staining of the nucleoplasm. Determination of the Ki-67 positive score was done by selecting areas containing the greatest number of positive cells from observation at 400x magnification. The percentage of Ki-67 positive scores was calculated by dividing the number of positive epithelial cells by the total number of epithelial cells in the respective areas counted at 100x magnification.

The data were analyzed statistically and P values <0.05 were considered as significant.

RESULTS

In the study group, out of 36 cases causing bone resorption, there were 19 peripheral giant-cell granulomas, 12 pyogenic granulomas and 5 tumors of focal fibrous hyperplasia. In the control group, there were 6 patients with FFH, 5 patients with PGCG and 4 patients with PG. The distribution of different types of epulides causing bone resorption is shown in Figure 1. PGCG was the most common histological sub-type among lesions with bone resorption in 53% of cases, followed by PG with 33%, while 14% of cases was FFH. Resorption of the alveolar crest in case of peripheral giant-cell granulomas occurred statistically more often in men than in women (p=0.0303). There was no such relationship in the case of other types of epulis (Figure 2).

LRHL affecting bone were equally distributed in the oral cavity. The relationship between site and LRHL was not statistically significant (Figure 3). However, in the anterior maxilla, the most frequently diagnosed lesion was pyogenic granuloma and in the remaining areas of oral cavity the most common was PGCG.

No significant differences in the clinical features in terms
Figure 2: Distribution of localized reactive lesions of gingiva according to sex and bone osteolysis.

Figure 3: Site distribution of localized reactive lesions of gingiva with bone osteolysis.

of patients’ age, tumors’ diameter and duration was observed between the studied sub-types of bone affecting epulides (Table 1).

**Ki67 immune reactivity**

All cases used in this study were immunopositive for Ki67 with a varying degree of expression (Figures 4 and 5). In peripheral giant cell granuloma, out of all stromal cells, only mononucleated cells showed positivity to Ki67, all multinucleated giant cells were negative. Immune-expression of Ki67 in stromal cells of peripheral giant-cell granulomas with bone osteolysis was significantly higher than in FFH and PG with bone osteolysis (p<0.005). No significant difference in Ki67 expression was found between FFH and PG with bone osteolysis (Figure 6).

**Peripheral giant cell granuloma analysis**

The difference in the expression of Ki67 was not significant between PGCG with bone osteolysis and the control group. However, there was a significant difference in terms of diameter and duration of focal fibrous hyperplasia with and without bone osteolysis. Tumors affecting bone assumed significantly larger sizes (p=0.01) than those of the control with significantly shorter time (p=0.02) (Table 2).

**Pyogenic granuloma analysis**

There was no significant difference in the expression of Ki67, age of patients, duration of epulides between PG affecting bone and the control group. We observed significantly longer duration of the PG affecting bone than
Table 1: Differences in the clinical features in terms of patients’ age, tumors’ diameter and duration in peripheral giant cell granuloma, pyogenic granuloma and focal fibrous hyperplasia causing bone resorption.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FFH</th>
<th></th>
<th></th>
<th>PG</th>
<th></th>
<th></th>
<th>PGCG</th>
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<th>Test ANOVA</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Average</td>
<td>Standard deviation</td>
<td>N</td>
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<tr>
<td>Age</td>
<td>5</td>
<td>54</td>
<td>20.8</td>
<td>12</td>
<td>55.6</td>
<td>15.8</td>
<td>19</td>
<td>58.1</td>
<td>23.2</td>
<td>0.9019</td>
</tr>
<tr>
<td>Diameter mm</td>
<td>5</td>
<td>20.4</td>
<td>7.5</td>
<td>12</td>
<td>22.9</td>
<td>11.8</td>
<td>19</td>
<td>28.9</td>
<td>11.6</td>
<td>0.1958</td>
</tr>
<tr>
<td>Duration</td>
<td>5</td>
<td>9.2</td>
<td>36</td>
<td>12</td>
<td>7.7</td>
<td>2.6</td>
<td>19</td>
<td>6.5</td>
<td>2.3</td>
<td>0.1028</td>
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</tbody>
</table>

Figure 4: Immunohistochemical staining showing high expression of Ki67 in pyogenic granuloma.

Figure 5: Immunohistochemical staining showing Ki-67 expression in peripheral giant cell granuloma affecting bone.
**Figure 6**: Levels of Ki67 in the stroma of localized reactive lesions of gingiva causing bone resorption.

**Table 2**: Peripheral giant cell tumors affecting bone were assuming significantly larger sizes from those of control group $p = 0.01$ in significantly shorter time $p = 0.02$.

<table>
<thead>
<tr>
<th>Group</th>
<th>Boneosteolysis</th>
<th>Control group</th>
<th>Test T</th>
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<tr>
<td></td>
<td>N</td>
<td>Average</td>
<td>Standard deviation</td>
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<tr>
<td>Age</td>
<td>19</td>
<td>58.1</td>
<td>23.2</td>
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<tr>
<td>Ki67 stroma</td>
<td>19</td>
<td>12.9</td>
<td>4.5</td>
</tr>
<tr>
<td>diameter mm</td>
<td>19</td>
<td>28.9</td>
<td>11.6</td>
</tr>
<tr>
<td>duration</td>
<td>19</td>
<td>6.5</td>
<td>2.3</td>
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</tbody>
</table>

**Table 3**: Observation of significantly longer duration of pyogenic granuloma affecting bone than pyogenic granuloma of control group ($p = 0.08$).

<table>
<thead>
<tr>
<th>Group</th>
<th>Boneosteolysis</th>
<th>Control group</th>
<th>Test T</th>
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<tr>
<td></td>
<td>N</td>
<td>Average</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Age</td>
<td>12</td>
<td>55.6</td>
<td>15.8</td>
</tr>
<tr>
<td>Ki67 stroma</td>
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<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Ki67 inflammatorycells</td>
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<td>17.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Diameter mm</td>
<td>12</td>
<td>22.9</td>
<td>11.8</td>
</tr>
<tr>
<td>Duration</td>
<td>12</td>
<td>7.7</td>
<td>2.6</td>
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There was no significant difference in the expression of Ki67 and parameters such as age of patients, tumors’ diameter between FFH with bone osteolysis and the control group. There was a significant difference in terms of the duration of tumors between the two groups (Table 4).

**DISCUSSION**

Ki-67 antigen is a large protein found in the nucleus. Its presence is found in many cells of normal tissues, which is
associated with cell proliferation. The nuclear antigen Ki-67 is a protein found only in dividing cells in the active phases of the cell cycle and is a good proliferation marker, as it stains strongly in paraffin sections. Therefore, it provides important information on the mitotic activity of the tumor. The results of the evaluation of the proliferation activity are expressed as a proliferative index (IP) which is the ratio of the number of positive cells (stained by immunohistochemistry) to all the cells counted under a microscope, which is expressed as a percentage. The proliferative index indicates the behavior of the tumor. Ki-67 also informs about the ability of the cells to undergo mitotic divisions within the specified time. Many normal tissues have a relatively high proliferation activity (Bodner et al., 1997).

Soft tissue reactive lesions are the most common lesions that occur in the oral cavity. Various studies have reported differences in the type of reactive lesions, age distribution, gender, location, and clinical behavior of these lesions in different populations (Anneroth and Sigurdson, 1983; Birajdar et al., 2014; Buchner et al., 2010; Niedzielska and Borgiel-Marek, 2009). Reactive lesions often present a great diagnostic challenge because of their histopathological resemblances and deceptive clinical presentation. In our study, we assessed the proliferative index in the stroma of gingival reactive hyperplastic lesions and compared it with the clinical features of these lesions. We found out that the expression of Ki67 in the stromal cells of peripheral giant-cell granulomas with bone osteolysis was significantly higher than in FFH and PG with bone osteolysis. However, there was no significant difference in the proliferative index between lesions with and without bone osteolysis. Our findings are in line with those reported by Souza et al. (2000). In their study, they concluded that although Ki67 indicates a proliferative stage of the cell in PGCL, it is not strictly associated with the clinical behavior of reactive lesions such as recurrence or cortex perforation.

Milner et al. (2014) assessed the proliferative activity of various proliferative lesions in the oral cavity. They evaluated the expression levels of Ki67 in the basal layer and the stroma of tumors. Based on the results, they concluded that the value of the proliferation index in the basal layer of epulis was moderate and did not exceed 50%, indicating their mild inflammatory character. In the present study, the highest stromal IP was found in PGCG and did not exceed 13%. This confirms the benign, reactive character of peripheral giant cell granulomas. On the other hand, Niedzielska et al. (2007) obtained different results by comparing epulis and malignant gingival hyperplasias (cancer) with regard to the activity of the genes associated with apoptosis, proliferation, and inflammation using RT-PCR. As regards the proliferation process, peripheral giant cell resembled neoplastic processes. Different H3 histone expression patterns were also determined for inflammatory and fibrous epulis. This was higher in inflammatory than in fibrous epulis, confirming the suggestion of a researcher that inflammation contributes to an increase in the expression of histone H3.

In conclusion, bone resorption in case of peripheral giant-cell granulomas occurred statistically more often in men than in women and was related to the size of tumors. However, Bodner et al. suggest that patients with large (>2 cm) peripheral giant cell granuloma lesions are more likely to be women (Bodner et al., 1997). In the present study, by comparing some clinical features of LRHL of the gingiva with bone osteolysis, it was found that bone resorption was related to the long duration and large size of the lesions. This is in line with some previous hypotheses that cupping bone resorption is due to the size of PGCG (Carvalho et al., 1995; Chaparro-Avendaño et al., 2005; Reddy et al., 2012; Sudiono and Hassan, 2012).

According to other investigators, the proliferation markers such as Ki67 cannot be a prognostic factor of their clinical behavior in localized reactive gingival lesions (Souza et al., 2000). Bone resorption in PGCG is connected to the diameter of the lesion and in PG and FFH depends on the duration of tumors. There is a need for further study of localized reactive gingival lesions to better predict their growth potential and their ability of bone osteolysis.

REFERENCES


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