Histological evaluation of oral maintenance programs upon gingival condition in orthodontic patients

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Abstract
The aim of the study was to conduct a histological evaluation of gingival condition in patients under orthodontic treatment with fixed appliances, according to different oral hygiene maintenance programs. We performed a randomized prospective study on 36 patients with fixed orthodontic appliances (17–25 years of age) divided in three study groups. The investigations were represented by measurements of plaque index and sulcular bleeding index, followed by pathological examination of specimens from gingival tissue. Treatment of orthodontic patients must follow an interdisciplinary approach. All modalities of oral hygiene procedures and their effect on the periodontal tissues must be explained to the patient prior to fixed orthodontic treatment. Fixed orthodontics do not induce periodontal disease if basic principles of oral hygiene are followed in compliant patients, which are correctly instructed to deal with real challenge, represented by complete elimination of debris and bacterial accumulation.

Keywords: fixed orthodontic appliances, gingivitis, histopathology, oral hygiene methods.

Introduction
Numerous clinical studies have demonstrated the presence of gingivitis in patients subjected to fixed orthodontic appliances. These have evaluated hard and soft tissues alterations to orthodontic treatment, with emphasis on bone metabolism and periodontal ligament reactions [1–6]. Clinical recordings involve inherent limitations in that they are subjective and susceptible to examiner variability, therefore a histological evaluation is more accurate and offer more objective information upon periodontal status. Long-term successful outcomes of orthodontic treatment are influenced by the patient periodontal status before, during and after active orthodontic therapy, which also includes post-treatment maintenance of the patient [7, 8].

The purpose of our study was the histological evaluation of the severity of gingival inflammation during orthodontic treatment in correlation with different oral hygiene protocols.

Materials and Methods
We performed a prospective study on 18 patients between 17–25 years of age (seven males and 11 females) scheduled for fixed orthodontic treatment in the Clinic of Orthodontics, Tirgu Mureș, Romania, between October 2012–February 2014. The study had the approval of the Scientific Research Ethical Committee, University of Medicine and Pharmacy of Tirgu Mureș.

We used the following including criteria: patients with good general health, over 16 years of age, non-smokers, without signs of periodontal disease and reduced or moderate accumulation of dental plaque prior to orthodontic treatment. The exclusion criteria were: teeth with extensive restorations, significant alterations of oral mucosa (others than gingivitis), adverse reactions to oral hygiene products, administration of anti-inflammatory or antibiotic drugs one month prior to this investigation, regular use of antibacterial oral products two weeks before this study.

The patients were randomly divided in three groups: Group A – patients examined prior to fixed orthodontic treatment with Straight-Wire technique, which were considered control; Groups B and C included patients with passive fixed appliances for at least three months (average period 3±1.5 months), which had different oral hygiene programs. The former used electric brush, water flosser and interdental brush (Group B) and the latter manual brushing and fluoride (Group C).

The histological examination was carried out after fragments of gingival tissue was obtained from molar interdental papillae according to a specific protocol: fixation in Lille neutral formaldehyde for five days, dehydration in consecutive solutions of alcohol, immersion in xylene, inclusion in paraffin, sectioning, staining with Hematoxylin–Eosin (HE) and examination under optic microscope at different magnifications. In order to evaluate the degree of inflammation we used the following criteria:
- 0 – reduced: width of inflammatory zone similar to control group, absence of or only a few inflammatory cells;
- 1 – moderate: mild inflammatory reaction, macrophages and plasma cells;
- 2 – severe: very strong inflammatory reaction, macrophages, plasma cells, with foci of granulocytes and lymphocytes.
For the immunohistochemical study, the histological sections were collected on poly-L-Lysine covered blades, and dried in a thermostat at 37°C for 24 hours. Then, the sections followed the classical protocol: deparaffinization and hydration. For antigen demasking, the blades were boiled in a sodium citrate solution, pH 6, for 21 minutes (seven cycles of three minutes each) in a microwave oven. After blade boiling and cooling, they were washed in tap water and distilled water for 15 minutes. The endogenous peroxidase blocking was performed by blade incubation in 3% oxygenated water for 30 minutes, at room temperature. After another washing step, the Biotin was amplified with a Streptavidin-peroxidase conjugate (GBI Labs, diluted as 1:200), and detected with 3,3’-diaminobenzidine (Liquid DAB, Dako). The slides were counterstained with Hematoxylin and coverslipped with a xylene-based mounting medium (Dako).

In our study, we used the following immunohistochemical markers for T-lymphocytes. In our study, we noted the presence of increased numbers of T-lymphocytes in the inflammatory infiltrate of the gingival mucosa chorion of patients in Group B (Figure 4), B-cells were identified by CD20 immunostaining. Phosphorylated CD20 is a glycoprotein antigen present on the surface of all B-lymphocytes. In our study, we identified a small number of B-cells in the gingival mucosal chorion (Figure 5). Macrophages were highlighted by using the CD68 antibody. The CD68 antigen is a glycoprotein that is found in the lysosomes of macrophages. We identified numerous diffuse scattered macrophages in the gingival mucosa chorion of Group B patients (Figure 6). Overall, we can say that in the gingival inflammatory infiltrate of patients in Group B, most cells were represented by T-lymphocytes, then macrophages, while B-lymphocytes were the least numerous. Using the anti-CD34 antibody allowed us to identify a number of angiogenesis vessels within the gingival chorion (Figure 7). We recorded a degree of gingival inflammation of 1.

Gingival biopsies taken from subjects in Group C – subject with fixed appliance and oral hygiene program consisting of manual brushing and fluoride – presented flattening of gingival papillae, increased mitosis in basal epithelial layer, connective tissue degradation due to the rich inflammatory infiltrate, numerous dilated blood vessels and thrombosis (Figure 8). Gingival epithelium showed uneven thickness, with surface erosion, and the inflammatory infiltrate was much more abundant than in patients from Group B. Immunohistochemical reactions showed in this group that most cells of the inflammatory infiltrate were T-lymphocytes, which often had a tendency to organize in nodes, followed by macrophages and then B-lymphocytes (Figures 9–11). This makes us believe that inflammation occurring in patients with passive fixed appliances in the gingival chorion is predominantly cellular, dominated by T-lymphocytes and macrophages. We have identified numerous angiogenesis vessels in patients of this group, preponderant of capillary type (Figure 12). The degree of gingival inflammation was 2.

Table 1 – Characteristics of the antibodies used in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Code</th>
<th>Clone</th>
<th>Antigen retrieval</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
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<tr>
<td>CD3</td>
<td>A0452</td>
<td>F7.2.38</td>
<td>Sodium citrate buffer, pH 6</td>
<td>T-lymphocytes</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>CD20</td>
<td>M0755</td>
<td>L26</td>
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<td>B-lymphocytes</td>
<td>1:100</td>
<td>Dako</td>
</tr>
<tr>
<td>CD68</td>
<td>M0814</td>
<td>KP1</td>
<td>Sodium citrate buffer, pH 6</td>
<td>Macrophages</td>
<td>1:200</td>
<td>Dako</td>
</tr>
<tr>
<td>CD34</td>
<td>ab81289</td>
<td>EP373Y</td>
<td>Sodium citrate buffer, pH 6</td>
<td>Endothelial cells</td>
<td>1:100</td>
<td>Abcam</td>
</tr>
</tbody>
</table>
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Figure 1 – Gingival biopsy taken from a 23-year-old healthy subject without orthodontic appliance. Normal aspect of epithelium layers, dermal papilla and connective tissue. HE staining, ×200.

Figure 2 – Gingival biopsy taken from a 19-year-old subject with fixed appliance and oral hygiene program consisting of electric brushing, water flossing and interdental brushing. We could observe a relatively higher number of inflammatory cells in the gingival chorion, while the epithelium shows a slight thickening. HE staining, ×40.

Figure 3 – Inflammatory infiltrate from the gingival chorion, preponderantly composed of lymphocytes and plasmocytes, in a patient from Group B. HE staining, ×400.

Figure 4 – T-type lymphocytes present in large number in the inflammatory infiltrate from the gingival mucosal chorion in a patient from Group B. Immunostaining with anti-CD3 antibody, ×400.

Figure 5 – Inflammatory infiltrate which was abundant in the gingival mucosa, rich in lymphocytes and plasmocytes; immunostaining with anti-CD20 antibody revealed the presence of a lower number of lymphocytes. Immunostaining with anti-CD20 antibody, ×100.

Figure 6 – Microscopic image of gingival chorion in a patient from Group B, in which we found an increased number of macrophages. Immunostaining with anti-CD68 antibody, ×100.
Figure 7 – Gingival chorion with a high number of angiogenesis vessels in a patient from Group B. Immunostaining with anti-CD34 antibody, ×200.

Figure 8 – Gingival biopsy taken from a 17-year-old subject with fixed appliance and oral hygiene program consisting of manual brushing and fluoride (Group C). The inflammatory infiltrate is much more abundant than those in Group B, blood vessels appearing more numerous and congested. HE staining, ×100.

Figure 9 – Microscopic image from a patient in Group C, in which we could observe the presence of an increased number of T-lymphocytes, with a tendency to organize. Immunostaining with anti-CD3 antibody, ×200.

Figure 10 – B-lymphocytes showing in small number within the inflammatory infiltrate of the gingival mucosal chorion, in a patient from Group C. Immunostaining with anti-CD20 antibody, ×400.

Figure 11 – Large number of macrophages in a patient from Group C. Immunostaining with anti-CD68 antibody, ×200.

Figure 12 – Angiogenesis vessels in large number in the gingival mucosa of a patient from Group C. Immunostaining with anti-CD34 antibody, ×100.
Discussion

Orthodontic treatment using fixed appliances represents a potential risk for periodontal health, as they increase the accumulation of oral biofilms and enhance inflammation of periodontal tissues. In these patients, periodontal maintenance programs must be carried out in conjunction to orthodontic treatment and also after completion of therapy [9–11]. Fixed appliances make brushing procedures more difficult and the patients are unable to completely remove dental plaque, which favors the development of gingival inflammation [12, 13]. This is accompanied by the presence of gingival pockets and the subsequent bacterial colonization of subgingival areas, which may cause periodontal breakdown. After initiation of orthodontic treatment with fixed appliances, the tooth movement determines remodeling reactions in the periodontal tissue represented in the early stages by enzymatic changes, vasodilatation and increased inflammatory cellular infiltration [14].

An important part of our study was directed toward gingivitis associated to fixed orthodontic therapy; biopsies were collected from the buccal aspects of upper molars which did not move during the experiment in order to exclude the effect of orthodontic forces upon gingival health. No forces were applied on the teeth in order to evaluate only the effect of fixed appliance in dental plaque accumulation and the influence of oral hygiene measures in development of gingival inflammation. Metallic bands fixed passively around teeth induced more clinical signs of gingival inflammation (Groups B and C) than those of untreated individuals (Group A). The presence of subgingival band margins accompanied by an oral hygiene protocol with manual brushing and fluoride showed the worse capacity of plaque removal. In these specimens, we noted the highest degree of gingival inflammation.

According to data from scientific literature, removable appliances do not cause alterations of periodontal tissues, due to their easy maintenance measures, but the presence of fixed orthodontic appliances makes the use of oral hygiene methods more difficult and prevents the complete mechanical removal of dental plaque, which increases the risk of detrimental consequences on soft dental tissues [15–17].

Based on the results of our histological investigation, the best oral health maintenance program is represented by the use of an electric brush, associated with water flosser and interdentally brush – Group B. The specimens from these patients showed only moderate inflammation, compared to Group C where the oral hygiene protocol included only manual brushing and use of fluoride solutions.

Treatment of orthodontic patients must follow an interdisciplinary approach. All modalities of oral hygiene procedures and their effect on the periodontal tissues must be explained to the patient prior to fixed orthodontic treatment [14, 15, 17]. A predictable treatment outcome needs coordination between the two disciplines, along with appropriate risk assessment. Successful therapy and its long time maintenance can be obtained through close collaboration between orthodontist and periodontist [18].

Our histological examination demonstrated the presence of gingival inflammation due to the presence of fixed orthodontic appliances even when there are no active forces. Tissue reactions varied from mild in the control Group A to severe in Group C, strongly correlated to the type of oral hygiene programs used by the patients. In the control group only minimal tissue alterations were noted due probably to the malposition of teeth, which favors dental plaque accumulation.

Conclusions

Within the limitations of this methodology and based on the results obtained after our investigation we can conclude that the accumulation of dental biofilms induces a pro-inflammatory state that can be followed by destructive periodontal reactions. The presence of fixed orthodontic appliances makes the use of oral hygiene methods more difficult and prevents the complete mechanical removal of dental plaque, which increases the risk of detrimental consequences on soft dental tissues. Our histological examination demonstrated the presence of gingival inflammation due to the presence of fixed orthodontic appliances, even when there are no active forces. Tissue reactions varied from mild to severe in close relation to the type of oral hygiene measures used. The best oral hygiene method implies the use of powered dental brushes associated with interdentally brushes and water devices.

Conflict of interests

The authors declare that they have no conflict of interests.

References


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