ORIGINAL PAPER

A histological analysis of gingival condition associated with orthodontic treatment

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Abstract
The aim of this histological study was to analyze the gingival reaction to fixed orthodontic appliances. Gingival specimens were obtained with minimal trauma from 11 patients treated with fixed appliances in different intervals during the orthodontic treatment, including post-treatment periods. Serial sections were stained with Hematoxylin–Eosin. T- and B-cells were identified by specific antibodies, using a double staining technique with Avidin–Biotin method. Histological observations demonstrated and confirmed the presence of gingivitis during orthodontic treatment. According to the usual histological evaluation, the biopsies revealed the presence of hyperplastic chronic inflammatory changes from mild to moderate severity. The lack of rapid increase of CD20+ cells demonstrated that the gingival inflammation did not cause overall tissue destruction.

Keywords: orthodontic, gingivitis, immunohistochemistry, T-cell, B-cell.

Introduction
Clinical investigations have demonstrated the presence of gingival inflammations associated with fixed orthodontic therapy. Bonding brackets directly to tooth surfaces within a certain distance to the gingival margin has become the most widely used method of securing fixed orthodontic appliances [1]. Evidence suggested that the accumulation of microbial plaque on teeth is a direct cause of gingivitis and that gingivitis may precede periodontitis [2]. Several studies have evaluated the hard and soft tissue responses as biological features during orthodontic treatment in animal [3–6] as well in human [7, 8] models. However, most of these have focused their attention on bone metabolism [5–7] or periodontal ligament changes that occur during tooth movement. Gingival modifications incidental to fixed orthodontic appliances have been reported as occurring in both histological and ultrastructural analyses and at a clinical level in certain cases [10]. This comprehensive histological and immunohistochemical study on the type and extension of gingival inflammation at different intervals during orthodontic treatment, including debonding and retention period allowed us to obtain more objective information regarding the degree of inflammation. It was also possible to study the density and distribution of cellular infiltration, the density and distribution of T- and B-cells in different levels of gingival inflammation.

Materials and Methods
Two millimeters wide gingival specimens were obtained with minimal trauma from 11 patients. Patients were informed and a parental, written consent was obtained. The study has the approval from the Scientific Research Ethics Committee, University of Medicine and Pharmacy of Tîrgu Mureș, Romania. All individuals (aged 13–17 years) were treated with Straight-Wire technique for an average of 14.3±1.2 months. The specimens were taken in different intervals during the orthodontic treatment, including post-treatment periods.

Prior to excision, a clinical assessment of gingival condition was made; the sites were the interdental papilla between second premolar and first molar and the two premolars in one case. All the selected patients followed a non-extraction treatment in order to observe the local irritations due to the presence of bands and labial tubes. In all cases, healing occurred within a few days after excision.

The excised specimens were fixed in Lillie’s neutral formaldehyde for five days, dehydrated in ascending alcohol, cleared in xylene and then embedded in paraffin, cut to 4–5 μm thickness. Serial sections were stained with Hematoxylin–Eosin. The immunophenotype of the inflammatory infiltrate of the lamina propria was studied using HIER antigen retrieval method and antibodies against T- and B-lymphocytes, primary antibodies originated from Thermo Scientific, Lab Vision, Freemont, CA: CD20 mouse monoclonal antibody RM-9107, clone L26, and CD3 rabbit monoclonal antibody RM-9107, clone SP7. We applied the immunodetection system Dako EnVision™ Flex, High pH, K8010, for all antibodies. For positive controls, sections from reactive lymph nodes were tested parallel. For negative controls, the primary antibodies were omitted. Membrane immunoreactivity was visualized...
using DAB chromogen, followed by Hematoxylin counterstaining.

**Results**

The biopsies obtained at three months after bracket placement showed a slight hyperplasia of pocket epithelium, with the small areas of keratinized mucosal tissue. Inflammatory cells are mostly lymphocytes; plasma cells were also detected. The subchronic type of slight infiltration of inflammatory cells in the connective tissue is mostly adjacent to the pocket epithelium and around blood vessels (Figure 1).

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**Figure 1** – HE stained histological section of the interdental papillae three months after bracket placement (a, ob. ×4) with lymphoplasmacytic infiltration adjacent to the pocket epithelium (b, ob. ×10) and around blood vessels (c, ob. ×20). EO – Oral epithelium; EG – Gingival epithelium; ch – Chorion; PC – Plasma cells; LC – Lymphocytes; HC – Histiocytes; FB – Fibroblasts; V – Blood vessels.

Epithelial projections are irregular; they protrude from the pocket epithelium. The cellular population is predominantly composed of lymphocytes and plasma cells, mainly in the central region of the specimen. We observed a slight hyperemia of the chorion; fibroblasts are not affected. By the visualization of T- and B-cells immunophenotype (Figure 2), we observed a slight increase of both types of cellular infiltrate, their concentration increased with the increase of inflammatory cellular infiltration.

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**Figure 2** – Three months after bracket placement: immunostains for CD20 (a) and CD3 (b) show a perivascular concentration of T- and B-cells. DAB chromogen, ob. ×10.
In time, the cellular picture shifted toward chronic inflammation with a dominancy of plasma cells, more accentuated hyperplasia and proliferation of pocket epithelium, the pegs are irregular. We also observed in certain cases acantholysis and marked edema of pocket epithelium, appearance of leukocytes inside vessels. Cellular infiltration was dominant in the pocket area as well as in the central region of the specimen. Lymphocyte infiltration increased considerably, a domination of T-cell infiltration in the bottom of the gingival pocket was observed (Figures 3 and 4).

Such changes persisted throughout the whole treatment period in some patients with good oral hygiene. In patients with poor oral hygiene, the cellular infiltration was anarchic. T-cells were dominating the cellular infiltrate while an increase in the number of B-cells was observed (Figure 5).

One month after removal of orthodontic appliance, the gingiva returned to its normal condition, although a few signs of former chronic inflammation remained. The hyperplasia of the epithelium might persist, acanthosis of pocket epithelium and slight cell infiltration might be observed.

**Discussion**

The biopsies were taken from the buccal part of the interdental papilla, whom inflammation is a very common symptom during orthodontic treatment. Banded molars during orthodontic treatment showed more clinical signs of gingival inflammation than those of untreated individuals. Moreover, major risk factors identified included the presence of plaque and the presence of subgingival band margins [11, 12].

The cases we included in this study were treated without extraction, so the interdental papilla moved little if at all, and all the reactive inflammatory infiltrate observed during this study were the result of the local irritation produced by buccal attachments. Tooth movement induced by orthodontic forces is consequent to remodeling of the periodontal tissue. The enzymatic gingival changes occur during the early phases of orthodontic treatment of human, vasodilatation and increased leukocyte infiltration may appear due to tooth movement [3] that was the reason we included in our study cases without extraction.

Histological observations demonstrated and confirmed the presence of gingivitis during orthodontic treatment.
According to the usual histological evaluation, the biopsies revealed the presence of hyperplastic chronic inflammatory changes from mild to moderate severity. The localization and the distribution of the inflammatory cell infiltrates, as well as the different cell types within the exudates, confirm the chronic gingivitis.

Figure 5 – The aspect of gingival specimen before debonding: hyperplasia, acantholysis (a) and inflammatory cells (lymphocytes, plasmocytes, fibroblasts) (b) can be seen. HE staining, ob. ×4 and ob. ×20. Slightly increase CD3 positive T-cell number can be observed (c). DAB chromogen, ob. ×10.

We obtained our specimens arbitrary and a part of epithelial lining remained on tooth surface, we could not assess the exact placement of epithelial attachment and the depth of pockets.

Histochemical staining revealed an almost overall presence of T-cell infiltration, which increased gradually with the advancement of the inflammation. The presence of B-cells was randomly at the beginning of the treatment and slightly increased with the advancement of gingival inflammation. CD20+ cells did not show a rapid increase, we consider that the gingival inflammation did not cause overall tissue destruction.

Very few histological studies have evaluated the immunohistological aspect of gingival and periodontal inflammation during orthodontic treatment. The presence of a chronic inflammation within the pulp, more or less intense, as well as pulp tissue necrosis, indicating the propagation of the periodontal inflammatory process towards the pulp tissues was also observed [13]. The most common aim of immunohistochemical studies was to evaluate the level of certain enzymes and the type of immunological reactions. Most of the studies insisted on evaluating the nitric oxide level in these inflammations [14–16]. Most of the studies we followed suggested the role of these radicals in tissue response to mechanical stress [10, 17, 18]. Interleukin 1β and prostaglandin E were also considered involved in the response of periodontal cells to mechanical stress [9, 11, 19].

Matsuo T et al. immunohistologically examined the cell densities and distribution of T- and B-cells in periodontitis and analyzed their relationship in terms of periodontal tissue destruction. Specimens were labeled with monoclonal antibodies for T-cells (CD3) and B-cells (CD20) and developed using the double staining technique. CD3+ cells appeared in low-density infiltrates and increased gradually with the number of total infiltrates; in contrast, CD20+ cells did not appear in small infiltrates but increased rapidly with the number of infiltrates. The number of fibroblastic cells negatively correlated with that of the total infiltrates and CD20+ cells, but it did not correlate with the number of CD3+ cells. These findings suggest that the T-cells infiltrated into the inflamed sites at the onset and increased gradually with the development of inflammation, B-cells infiltrated later than T-cells and their number increased rapidly with inflammation [20].

Further immunohistochemical studies are needed in order to assess the proportion of T-/B-cells, the eventually tissue destructions in order to describe the dynamic of the inflammatory process.

Conclusions

Histological and immunohistochemical examinations showed the existence of gingival inflammation during orthodontic treatment, hyperplastic chronic inflammatory
changes from mild to moderate severity was found. Minimal tissue destruction can be observed, the absence of fibrosis was constant. After the removal of orthodontic appliance, the gingiva returned to its normal condition, very few signs of former chronic inflammation remained.

References


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Received: February 22, 2013

Accepted: November 16, 2013