Preliminary evaluation of morphological parameters of the saliva in patients undergoing orthodontic treatment

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Abstract

Purpose: In recent years, many reports have focused on clinical changes in the oral cavity of orthodontic patients, manifested in general inflammation of the mucosa. In order to better understand histopathological alterations in the mouth and the use of easily available diagnostic material, we decided to assess the morphology of salivary cells at different time points of treatment with orthodontic appliances.

Material and methods: The study material included non-stimulated saliva obtained from 21 orthodontic patients and 11 healthy secondary school students (controls). After fixation in 96% ethanol the smears were stained with PAS + hematoxylin or H+E, and using the methods of May-Grünwald-Giemsa and Feulgen.

Results: As revealed by the histopathological examinations of saliva smears, patients treated with intra-oral fixed orthodontic appliances showed morphological changes in oral epithelial cells and in the number of leukocytes as compared to the control group. The changes were most pronounced in the first months of treatment.

Conclusions: The preliminary data indicate that orthodontic patients develop changes in the composition and morphology of salivary cells, the intensity of which depends on the time of exposure to the appliance.

Key words: morphology, salivary cells, orthodontic patients.

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Introduction

Clinical changes in the oral cavity manifested in general gingivitis are frequently observed in orthodontic patients. Changes described in literature usually refer to single cases and are mainly detected by clinical evaluation or sometimes confirmed by basic histopathological investigations of oral tissues [1,2]. The increase noted in the number of patients with masticatory abnormalities treated with fixed orthodontic appliances and their clinical picture seem to justify the necessity for the use of accessory diagnostic methods. The saliva, an easily available material, has not been investigated so far. The composition of the saliva, which constitutes a damp environment in the mouth, depends on a number of factors, including plasma composition. In the saliva, desquamating oral epithelial cells, leukocytes and bacteria are suspended. Besides, it contains various biologically active substances, among which some can act as markers not only of oral but also systemic abnormalities. Taking the above into consideration, as well as to better understand histopathological changes in the mouth, we decided to perform a morphological analysis of cells in the saliva of patients at different time points of exposure to fixed orthodontic appliances.

The aim of the current study was to assess the morphological changes in orthodontic patients and their juxtaposition with different time of exposure to the orthodontic appliance.

Material and methods

Investigations were conducted on the non-stimulated saliva obtained from 32 subjects. The control group consisted of 11 generally healthy and non-orthodontic students of the Catholic Secondary School, aged 15-20 years. The study group included 21 patients with food allergy, aged 15-30 years, treated with intra-oral fixed appliances in the Department of Orthodontics in Białystok. The patients were divided into three groups, according to the orthodontic treatment duration: R1 – up to 10 months

Figure 1. **Mucosal epithelial cells and leukocytes in the saliva of control subjects. Mag. x 200 and x 400**

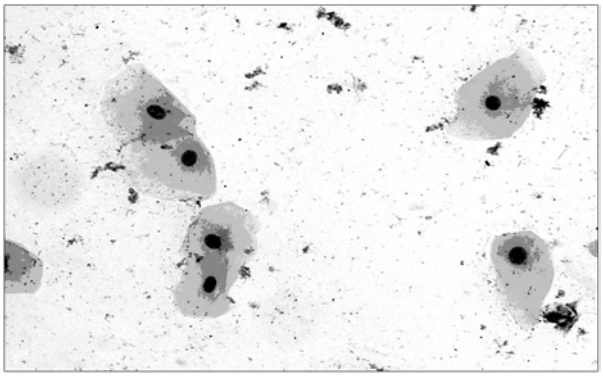


Figure 2. **Mucosal epithelial cells and leukocytes in the saliva of control subjects. Mag. x 200 and x 400**

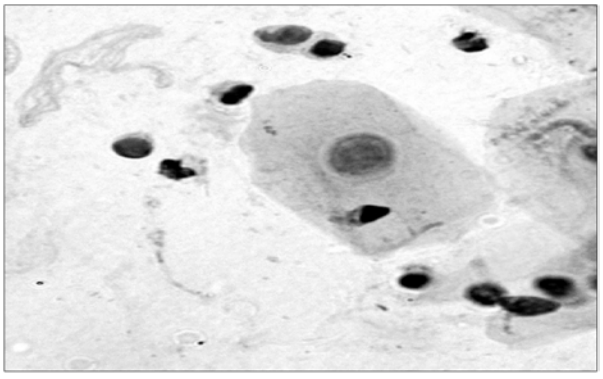


Figure 3. **Varied morphological picture of the epithelial cells and only single leukocytes in the saliva of a patient after a 7-month--exposure to an intra-oral appliance. Mag. x 250**

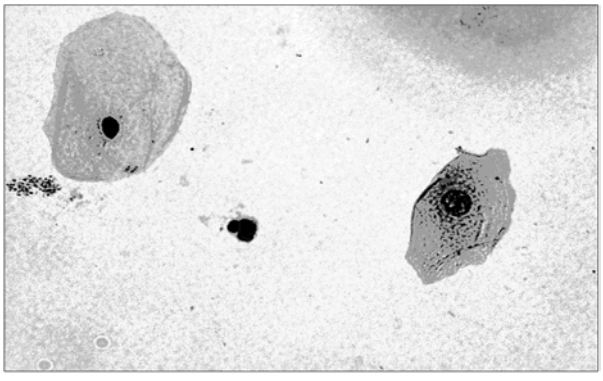
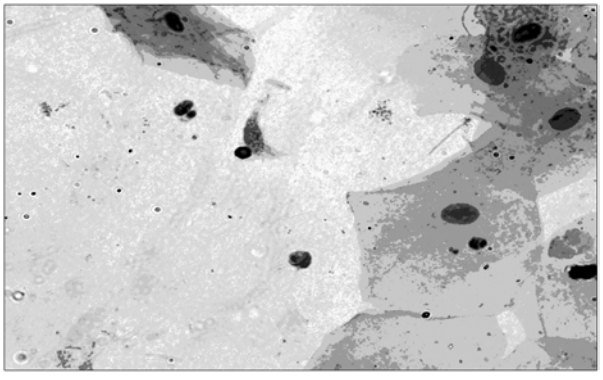


Figure 4. **Morphological picture of the patient's saliva after 24-month-treatment with a fixed orthodontic appliance. Mag. x 250**



(8 patients), R2 – up to 20 months (7 patients), R3 – over 20 months (6 patients).

The study obtained the approval of the Local Bioethics Committee for Research on Humans, Medical University of Białystok.

Each patient gave a detailed history of allergy and other ailments, used medications and accompanying systemic diseases. Then, the status of the oral cavity was assessed by a standard method using artificial light, a mirror and a periodontally calibrated probe.

Saliva smears were done on cleansed and defatted glass slides. After fixation in 96% ethanol the smears were stained with PAS + hematoxylin or H+E, and the methods of May-Grünwald-Giemsa and Feulgen.

Oral epithelial cells and inflow elements underwent microscopic analysis, with regard to shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results

In control salivary samples, epithelial cells were numerous, equal in shape and size, and their nucleus-to-cytoplasm ratio was normal and levelled (*Fig. 1*).

Leukocytes, mainly neutrophilic granulocytes and single lymphocytes were observed among epithelial cells (*Fig. 2*).

In orthodontic patients, oral epithelial cells showed morphological alterations. Some of them exhibited changes in the nucleus-to-cytoplasm ratio; others were less regular in shape. Frequently, the cells had a bigger in size, dark pycnotic or vesicular nucleus with loose chromatin. Worth special noting is the presence of characteristic chromatin condensations in the form of rods or serpentine structures throughout the nucleus in some preparations. The morphological changes in epithelial cells were most pronounced in patients in the first (up to 10) months of wearing the appliance (*Fig. 3*). In patients with over 20 months of exposure to the orthodontic appliance, changes in oral epithelial cells were observed only in single preparations (*Fig. 4*).

Smears of the saliva collected after a few months of the exposure to the orthodontic appliance showed a considerably smaller number of inflow cells, as compared to patients after longer treatment duration or control subjects. Only single cells, mainly neutrophilic granulocytes, were sporadically observed (*Fig. 3*).

Discussion

Metals and alloys have a wide range of applications as prosthetic materials for dental tissue reconstruction. The most common metals used in orthodontics are of a composition simi-

lar to 18/8 (18% chromium and 8% nickel) stainless steel and nickel-titanium alloys are widely used in orthodontic treatment [3]. The biocompatibility of dental casting alloys is a critical issue because these alloys are in long-term intimate contact with oral tissues. The release of elements from commonly used dental casting alloys directly into the oral cavity may cause harmful reactions to the host body both locally and systemically [4-6].

The aim of the present study was to investigate the cytotoxicity of materials used in orthodontic appliances by evaluating their effects on changes of cell morphology in the saliva. The results of our study indicate that metal ions released from the alloys can cause adverse effects on cellular metabolism manifested by morphological alterations in salivary cells. In clinical investigations, generalized gingivitis is observed during the first few months of treatment with fixed orthodontic appliances. The mechanisms of these adverse effects have not yet been fully clarified. Some authors have suggested that trace amounts of metal ions can be localized in blood or serum [7], urine [8], and other organs [4,9]. The biological consequences of these released ions on the tissues or cells are still investigated. In order to evaluate the toxicity of the alloys used in orthodontics, an essential approach is to study specific cell populations *in vitro*. Results of the researches have shown that metal ions can reduce cell activity and inhibit specific cellular functions, such as alkaline phosphatase activity [6]. Human gingival fibroblasts exposed to the alloys exhibit alterations in proliferation, glucose-6-phosphate-dehydrogenase activity and in intracellular ATP levels [10-13]. Alterations in cell morphology described in this study can be caused by metal ions binding to endogenous macromolecules, leading to inhibition of various enzymes and having ongoing effects on cell metabolism. In this study, we observed most pronounced alterations in cell morphology in patients who had been undergoing orthodontic treatment for less than 10 months. In this group of patients, we also observed a significantly smaller number of mononuclear cells, perhaps due to cell death. This suggestion is in agreement with those reported by Silvennoinen-Kassinen, who showed that higher concentrations of nickel can induce lymphocyte death *in vitro* [14].

In our study, patients from R2 and R3 groups exhibited slighter metal ions-induced alterations in cell morphology than those with less than 10 months of orthodontic treatment. These data suggest that the longer the treatment continues, the slighter the metal-induced histopathological changes; this in turn suggests that mechanisms of oral tolerance might develop. *In vitro* cytotoxicity tests [1,15] and clinical evidence have been presented to show that small doses of nickel from dental appliances may induce tolerance to this allergen [10,16]. On the other hand, it is likely that metal ions exert a toxic effect only after some time of low-dose exposure. These results indicate that only when the effects of ion release from dental alloys are assessed, extended exposures should be considered.

Further studies are needed to clarify the effects of these metal ions on cellular functions.

Concluding, the current study has demonstrated that release of elements from dental alloys may affect cellular metabolism, which can be manifested by alterations in the number and morphology of salivary cells.

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