Preliminary evaluation of saliva composition in allergic patients subjected to orthodontic treatment; morphological examination

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Abstract

Purpose: Intra-oral fixed orthodontic appliances, so frequently used in the treatment of malocclusions, may cause pathomorphological changes in the mouth and can be a potential source of antigen stimulation. Therefore, the aim of the current study was to assess the changes in salivary cells of orthodontically treated allergic patients.

Material and methods: The study material was the non-stimulated saliva samples collected from 28 allergic patients subjected to orthodontic treatment with intra-oral fixed appliances and from 11 healthy secondary school students (controls).

After fixation in 96% ethanol, saliva smears were stained with PAS + hematoxylin or H+E, and using the methods of May-Grünwald-Giemsa and Feulgen.

The microscopic analysis was made of oral epithelial cells and inflow elements, with regard to their shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results: The results of preliminary investigations indicate that allergic patients with fixed orthodontic appliances exhibit changes in the morphology and composition of salivary cells as compared to control patients. Differences in the morphological picture were most pronounced in the first months of orthodontic treatment.

Conclusions: It was shown that the number and morphology of salivary cells in allergic patients altered in response to ions released from dental alloys. Thus, saliva can be used as diagnostic material.

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Introduction

In the last few years, the number of patients subjected to orthodontic treatment has shown a considerable increase and so has the number of scientific reports demonstrating the presence of generalized gingivitis and skin and mucosal reactions leading to tissue inflammation when fixed orthodontic appliances are used [1-6]. Orthodontic appliances are a potential source of ions of various metals released directly to the mouth. The most common metals used in orthodontics are chromium and nickel (stainless steel components) [7]. Because nickel can sensitize certain people and cause severe allergic reactions, the safe use of this alloy is still under study. The knowledge of side-effects, such as irritation, allergies and other toxic effects of long-term contact of the appliance with the adjacent tissues is still incomplete. Most researches investigating toxic effects of orthodontic materials have been conducted *in vitro* on a chosen cell population [7-9]. *In vitro* cytotoxicity tests have been common because they are faster, less expensive, and pose fewer ethical concerns than animal or clinical usage tests. However, considerable controversy remains about the relevance of *in vitro* cytotoxicity tests in the light of studies which have compared them with animal and usage tests [10]. In fact, *in vitro* the cells are free of the general influence of the organism and adapt to the particular conditions of the microenvironment in which they are maintained. Furthermore, in orthodontic therapy, different materials are used and subjected to a damp oral environment, which can modify their properties. The liquid environment of the oral cavity is the saliva, which apart from various biologically active substances contains mucous epithelial cells and numerous blood morphotic elements. Saliva as a diagnostic material is easily available and frequently underestimated. Detection of certain substances in the saliva can be a marker of pathological changes, not only in the mouth but also in the whole body.

Since the mechanisms behind many of the adverse effects have not been fully clarified, the knowledge of the composition of the materials, their irritative and allergenic properties, and other possible toxic effects is essential for their proper and safe use.

Therefore, the purpose of the current study was to assess the changes in salivary cells of allergic patients treated with fixed orthodontic appliances.

Any potential changes in the composition of the morphological activity of salivary cells can confirm the usefulness of saliva as a diagnostic material.

Material and methods

Investigations were conducted on the non-stimulated saliva obtained from 39 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 28 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 treatment duration: A1 – up to 5 months (8 patients), A2 – up to 20 months (14 patients), A3 – over 2 years (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 periodontically 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 using the methods of May-Grünwald-Giemsa and Feulgen.

Oral epithelial cells and inflow elements were analysed microscopically, with regard to their shape, size, the nucleus-to-cytoplasm ratio and nuclear chromatin condensation.

Results

The saliva smears of control subjects showed a relatively uniform picture of cell morphology. Epithelial cells were even, usually oval in shape and equal in size. The nucleus-to-cytoplasm ratio in these cells was normal. Among the epithelial cells, leukocytes, mostly neutrophilic granulocytes and single lymphocytes, were present (*Fig. 1*).

In allergic patients treated with fixed orthodontic appliances, the microscopic picture of epithelial cell morphology was less homogenous than in controls. Cells varied in shape, size, in the nucleus-to-cytoplasm ratio and in colour intensity of cell structures. These changes were most pronounced in subgroups A2 and A3. Differences were also observed in the number of mononuclear phagocytes and lymphocytes. Group A1 saliva smears showed numerous neutrophilic granulocytes, monocytes and lymphocytes (*Fig. 2*). The number of inflow cells in the saliva of group A2 patients was markedly smaller as compared to controls (*Fig. 3*). Even fewer inflow cells were found in group A3 – these were mainly "old" neutrophilic granulocytes with nuclei defragmented into numerous lobes, and rare lymphocytes (*Fig. 4*).

Figure 1. **Control salivary smear. The morphological picture of oral mucous epithelial cells is quite uniform. Mag. x 400**

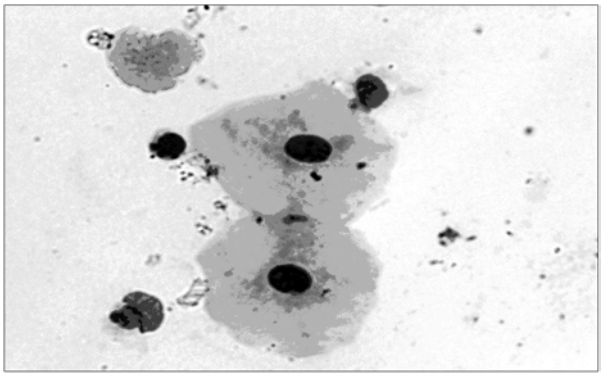


Figure 2. **Numerous neutrophilic granulocytes, monocytes and lymphocytes in the saliva of an allergic patient after 4 months of treatment with a fixed orthodontic appliance. Mag. x 250**

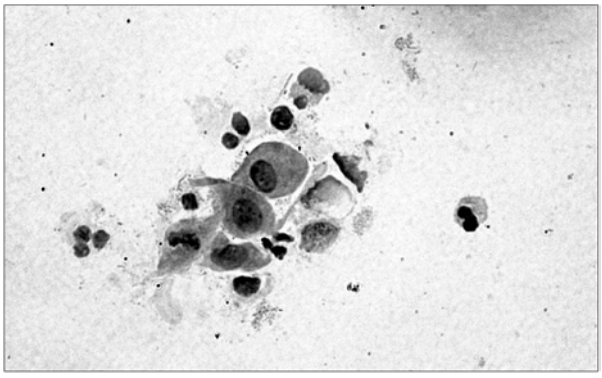
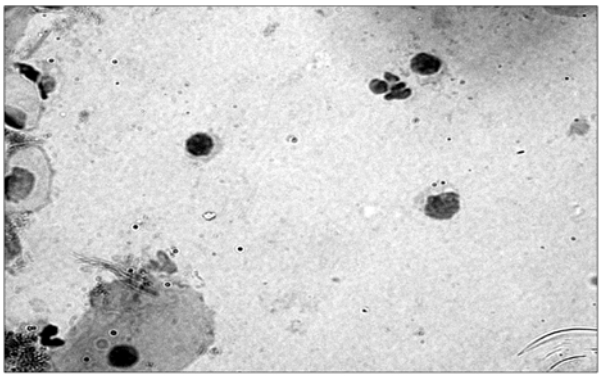


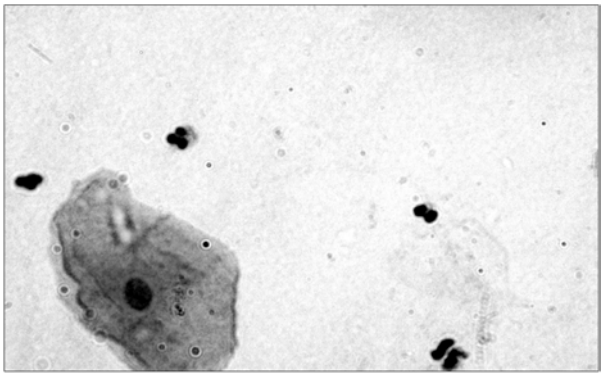
Figure 3. **Saliva cell picture of an allergic patient after 17 months of orthodontic treatment. Mag. x 250**



Discussion

Nickel is a constituent of many dental alloys, which in many people are in long-term intimate contact with oral tissues and

Figure 4. **Saliva smear of an allergic patient after 26 months of treatment with a fixed orthodontic appliance. Mag. x 250**



can induce nickel sensitivity. Clinically, nickel released from these alloys has been shown to cause adverse tissue reactions [11-14]. Reactions of the mucous membranes, such as stomatitis, gum hyperplasias, cheilitis, labial desquamation, and multiform erythemas are frequently noted [15]. Although several studies have reported normal morphology, ultrastructure, viability, and DNA synthesis [16-21], others have demonstrated decreases in DNA, RNA, and protein synthesis, intracellular ATP levels, and inhibition of various enzymes of cultured cells when exposed to nickel-based alloys [8,22,23].

The biologic consequences of released metal ions on tissues or cells have been extensively studied *in vitro*. However, a major problem is associated with relevance of *in vitro* cytotoxicity tests in the light of studies which have compared them with animal and usage tests. The lack of correlation between *in vitro* tests and clinical experience is probably related to many factors [24]. Therefore, in the diagnosis of dental alloy sensitivity it is a great challenge to discover and develop alternative *in vitro* assays to improve and minimize false-positive results.

In our study, patients who had been undergoing orthodontic treatment for less than 5 months showed more evident metal ions-induced alterations in epithelial cell morphology and in the number of mononuclear cells than those with extended exposure. These data indicate that intra-oral exposure to metal ions from orthodontic appliances may have ongoing effects on cellular metabolic functions manifested by morphological alterations. Our data suggest that the longer the treatment continues, the slighter the ions-induced alterations in cells; this in turn suggests that mechanisms of oral tolerance might develop in this context. The mechanisms by which metal ions act in cells are unknown. However, the current results indicate that the concentrations of metal ions which are known to be released from dental materials are potentially capable of altering cell metabolism as well as cellular proliferation [6,15,25,26]. Several investigators have reported that the cytotoxicity of dental alloys may be substantially different after a several months' exposure to a biological medium than after a short time (72-168 h) [6,26]. Studies with nickel-chromium alloys over 35 days [27] showed that the rate of release decreased with time. Clinical evidence has been presented to show that small doses of nickel from dental appliances may induce tolerance to this allergen [28]. Immunologic tolerance to nickel was described by Vreebur, when oral administration of nickel induced partial tolerance in guinea pigs with a splint fixed to their teeth or receiving nickel in their food [29].

In conclusion, it was shown that the number and morphology of salivary cells in allergic patients altered in response to ions released from dental alloys. Thus, saliva can be used as diagnostic material.

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