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Nickel and Chromium Ion Concentrations and Buccal Cell Cytotoxicity in Patients with Fixed Orthodontic Appliances

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Abstract

orthodontic appliances usually include brackets, bands, and archwires made of stainless steel, nickel-titanium, or nickel-cobalt alloys, and these can release metal ions. The purpose of this study was to determine nickel and chromium concentrations in oral mucosa cells and investigating their cytotoxicity. Epithelial cells of buccal mucosa from each patient were collected, by gentle brushing of buccal mucosa with an interdental brush of 25 orthodontic patients and 25 control subjects who were not receiving orthodontic treatment. Nickel and chromium cellular content was quantified by atomic absorption spectrometer with a graphite furnace. The results indicate that nickel and chromium concentrations were 2.2-fold and 2.4-fold higher, respectively, in the patients than in the controls. The biologic compatibility of fixed orthodontic appliances in buccal cells was evaluated by micronucleus assay. Results showed a remarkable increase of MN in the oral mucosa cells of the patients compared with the controls. MN assay demonstrated that metallic ions such as nickel and chromium released from orthodontic appliances could induce DNA damage in oral mucosa cells. This study indicates the potential toxicologic effects of nickel and chromium and shows that these metals can damage oral mucosa cells.

Keywords: chemical components, elements, metals, mucosa

Full-length article **Corresponding Author, e-mail: drshimaminoo29@gmail.com*

1. Introduction

Intra-oral fixed orthodontic appliances include brackets, bands, and archwires that are made of alloys containing nickel, cobalt, and chromium in different percentages [1, 2, 3]. Orthodontic appliances are usually made of stainless steel alloy, which contains metals such as chromium, nickel and iron. Several studies have investigated orthodontic appliances release metal ions through emission of electro-galvanic currents, with saliva as the medium [3,4-12]. Due to its thermal, microbiological, and enzymatic properties, the physiological environment of the oral cavity stimulates the release of compounds from dental alloys that are placed in to contact with the gingival and oral mucosa for prolonged periods [13, 3, 12].

Nickel is one of the most common allergens and the most potent sensitizer of all metals and it has toxic, immunological and carcinogenic effects [13]. Nickel ions have been demonstrated to enter the cell in various ways, to bind with several biological components and to alter cellular functions, morphology, and ultrastructure in several cell lines [13].

Chromium is known to be highly toxic to biological systems [14] And it also causes hypersensitivity, dermatitis, and asthma [3]. Many metal compounds are carcinogenic to animals or humans 'but their mechanisms are not overall known [13, 3]. Previous studies have investigated, both in vitro and invivo, the release of these metals from orthodontic appliances, but less information exists about adverse biologic effects of these metals invivo [3]. The purpose of this study was to measure the metal levels in the buccal mucosa cells and to evaluate their possible adverse biological effects.

2. Materials and Methods

Fifty subjects were included in this study with an average age of 18 to 30 years. The first group comprised 25 orthodontic patients with fixed appliances in both arches for 6 to 8 months. The fixed appliances consisted of an average of 4 to 8 bands (ortho technology) and 20 bonded brackets (ortho organizer). The second group comprised 25 subjects with no orthodontic treatment and had no dental restorations as the control group. Informed consent was obtained after the objective of the study was fully explained.

Epithelial cells of buccal mucosa from each patient were collected, by gentle brushing of buccal mucosa with an interdental brush, after washing out the mouth many times with tepid water to remove exfoliated dead cells. The brushes

were stirred in 5 ml of phosphate-buffer saline solution (PBS.PH :7.4) and cell suspensions were centrifuged [3]. Before the centrifuging the cells were counted. One milliliter of cell suspension (100 cells) from buccal mucosa cells of each patient was treated with nitric acid (5ml,0.5 %), and then diluted with deionized distilled water. To measure the amount of nickel and chromium release, a Perkin Elmer Model 4100 atomic absorption spectrometer, equipped with a GTA Graphite furnace was used. Results are given as n/ml that, in this study, is equivalent to ng/100 cells.

To determine the genotoxicity induced by metals from orthodontic appliances, we used micronucleus assay for healthy patients undergoing orthodontic treatment [12]. Slides of buccal cells were prepared by dropping the washed cell suspension on to slides [12]. After dropping, the cells were allowed to air-dry and fixed in ethanol. Staining was performed with May-Grunwald-Gimsa according to a standard protocol. Only cells that were not smeared, clumped or overlapping and that contained intact nuclei were included in the analysis. Micronucleus were identified according to the following characteristics:

1. less than 1/3 diameter of the main nucleus;
2. the same plane of focus, the same color, texture and refraction as the main nucleus;
3. smooth oval or round shape and
4. clearly separated from the main nucleus

Cells were observed in oil immersion at 1000 magnification with a light microscope to determine the presence of MN cells. Fisher exact test was used to compare the number of patients with MN in control and case group.

3. Results

The control group consisted of 25 subjects with no orthodontic treatment. The 25 orthodontic patients had been wearing different types of metallic alloy fixed appliances in both arches for 6 to 8 months. Nickel and chromium release from the fixed orthodontic appliances was analyzed by atomic absorption spectrometer (GTA Graphite furnace). When we examined the amount of nickel and chromium ions in the buccal mucosa cells, we observed a remarkable increase of both metals in the subjects with fixed orthodontic appliances: the nickel and chromium concentrations were about 2.2-fold and 2.4-fold higher, respectively than those in the control subjects (Table I). In the control group, the mean levels of nickel and chromium were 0.71 ng/ml and 0.24 ng/ml, respectively, whereas the same metals were 1.54 ng/ml and 0.59 ng/ml, respectively, in the study group, and between groups, these differences were significantly higher in the statistical analysis with Student t test (P< 0.000).

The biologic compatibility of fixed orthodontic appliances in buccal cells was evaluated by micronucleus assay. Results showed a remarkable increase of MN in the oral mucosa cells of the patients compared with the controls (Table II). There was a significant increase in MN frequency in study group in the statistical analysis with Fisher exact test (P<0/05). (Figure 1).

Table 1. Mean values of nickel and chromium levels (ng/ml) evaluated by atomic absorption spectrometer (GTA Graphite furnace) in buccal cells of controls and patients

Subjects	Nickel	Chromium
Controls	0.62-0.81	0.19-0.31
Patients	1.29-1.81	0.48-0.69
	P<.000	P<.000

Table 2. Comparison of micronucleus frequency evaluated by MN assay in buccal cells of controls and patients

Group		Micronucleus		Total
		+	-	
Control	(number of cases)	0	25	25
	%	0%	100%	100%
Patient	(number of cases)	23	2	25
	%	92%	8%	100%

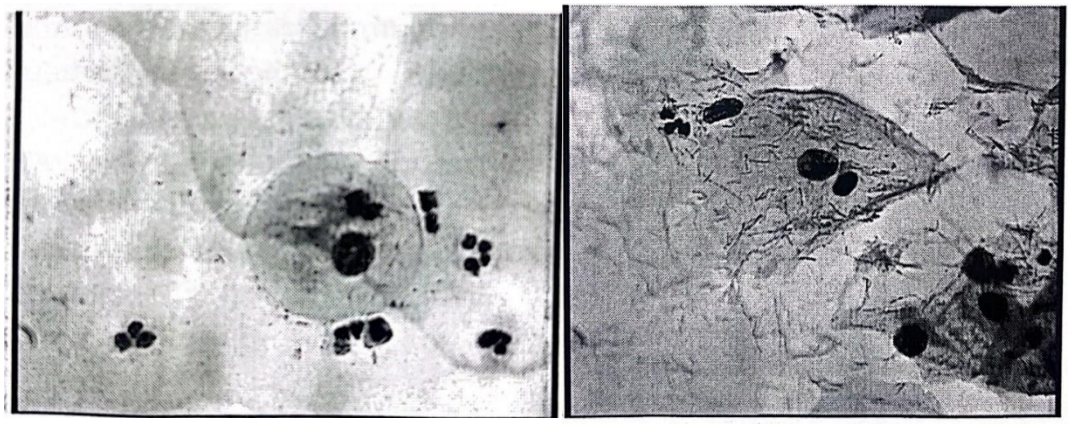

Figure 1. The micronucleus test. Cells of the oral epithelium. Micronucleus is indicated

4. Discussion

We investigated the presence of metal ions in oral mucosa cells in orthodontic patients wearing fixed appliances; we chose to evaluate nickel and chromium cellular levels as biomarkers of cytotoxicity and genotoxicity, because these metals were the higher constituents with harmful characteristics of the appliances used during orthodontic therapy. A standard orthodontic appliance consists of bands, brackets, and both stainless steel and nickel-titanium arch wire [15]. The most common alloy constituents are cobalt, chromium, and nickel, followed by other metals in different amounts. Corrosion events are very frequent in the oral cavity. The alloys used in dentistry are exposed to several aggressive physical-chemical events, such as high concentrations of oxygen and chloride mixtures in saliva, tartar, and plaque, and acid product deposit from microbiologic metabolism [3].

Several in vitro and in vivo methods have been used to study the release of metals and their content in biologic fluids, including saliva, blood, and urine. The studies have shown that these metals were released during the first 4 or 5 months of orthodontic therapy, and the metals were actually absorbed by patients with systemic distribution. When orthodontic appliances are present in the oral cavity, they are usually subject to corrosion processes, which lead to the release of metals. Some metallic elements present in orthodontic appliances, such as nickel and chromium, are known to be potential carcinogenic and mutagenic agent.

Other studies demonstrated that the amount of metals released from orthodontic appliances in saliva or blood samples was significantly below the average dietary intake and did not reach toxic concentrations. However, it cannot be excluded that even nontoxic concentrations could be sufficient to induce biological effects in cells from the oral mucosa. It is the least invasive method available for measuring DNA damage, and these cells could represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. Genotoxicity effects from orthodontic appliances were assessed by MN assay. MN may arise from either DNA breakage leading to centric chromosome fragments or from chromosome/ chromatin lagging in anaphase.

Analysis of exfoliated cells of buccal mucosa also provides evidence of other nuclear abnormalities such as binucleates (presence of two nuclei within a cell), karyorrhexis (nuclear fragmentation) and karyolysis (nuclear dissolution). Binucleus formation is considered as an indicator of cytotoxicity, while karyorrhexis and karyolysis are considered as indicators of apoptosis. In general, cells can adapt with mild lesions and can repair these lesions but loss of repair capacity due to a reduction in damage detection or to an enzymatic deficiency in repair processes might be. an initiating event of adverse biologic effects so MN formation is a type of cellular adaptation to nickel and chromium exposure that show cytotoxicity.

5. Conclusions

This invivo study demonstrated that metallic ions such as nickel and chromium released from orhodontic applinces could induce DNA damage in oral mucosa cells. The MN assay has been applied in biological or physical agents. The MN assay showed an increase in MN cells after the placement of the orthodontic appliances. Finally, because of possible adverse biologic effects, scientific research should be directed toward changing the alloys containing as a suitable recommendation in future. Also increasing the mechanical, tear and wear resistance maybe is a short time suggestion.

Also we suggest measurement the amount of Ni and Cr blood concentrations in patients who had higher cell concentrations than other to determine possible adverse effects specially in pregnant patients.

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