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In vivo study of metal content of oral mucosa cells in patients with and without fixed orthodontic appliances

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Structured Abstract

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Objective – To compare the concentration of nickel, chromium and cobalt in oral mucosa cells of patients with and without fixed orthodontic appliances.

Materials and Methods – A total of 60 patients were included in this study. The control group consisted of 30 patients without any type of fixed orthodontic appliances or metal restoration in the mouth (20 females and 10 males from 16 to 20 years with a mean age of 18 years). The test group consisted of 30 patients who had fixed orthodontic appliance in their upper and lower arches (20 females and 10 male from 16 to 20 years with a mean age of 18.2 years). The metal content determinations were carried out using atomic absorption spectrophotometry with a graphite furnace.

Results – According to spectrophotometric analysis, no significant differences in chromium ($p = 0.09$) and cobalt ($p = 0.10$) content of oral mucosa cells were found between the test and control samples. The nickel content in mucosa samples was significantly higher ($p = 0.003$) in orthodontic patients compared with the controls. The mean levels of nickel in control and orthodontic patient group were 12.26 and 21.74 ng/ml, respectively.

Conclusion – Our findings indicate that there was no difference in the concentration of chromium and cobalt in oral mucosa cells of patients with or without fixed appliances. However, a significantly higher concentration of nickel can be found in oral mucosa cells of patients wearing fixed orthodontic appliances. Continued follow-up is needed to determine the long-term significance of nickel release.

Key words: chromium; cobalt; fixed orthodontic appliances; nickel; oral mucosa cell content

Introduction

The warm and moist condition in the mouth offers an ideal environment for the biodegradation of metals, consequently facilitating the release of metals ions that can cause adverse effects. Biocompatibility is strongly related to ionic release and therefore the public may express concern about possible leakage of metal ions from an orthodontic appliance.

Fixed orthodontic appliances usually include brackets, bands, arch wires, and springs. They are made of stainless steel, nickel–titanium, or nickel-cobalt alloys. The stainless steel currently used in orthodontic clinics is of type 302 or 304, both of which contain 8–10% nickel. Nickel is

added to maintain the steel's face-centered cubic structure, and is created when heated at 912°C or higher. Nickel also increases the strength, ductility, and resistance to general, crevice and erosion corrosion.

The major corrosion products are iron, chromium, and nickel for stainless steel, and Ti and Ni for nickel–titanium alloys. Among stainless steel and nickel–titanium corrosion products nickel and chromium have received the most attention because of their reported adverse effects. Nickel is a known allergen (1) with carcinogenic (2) and mutagenic effects (3). However, the cause and effect relationship between intra-oral use of nickel alloys and carcinogenicity has never been demonstrated (4). Nickel is a component of certain enzyme systems in humans and it is considered an essential trace element. Daily intake of nickel is estimated to be 100–600 μg/day (5).

Nickel is one of the most common causes of allergic contact dermatitis, and the incidence of such contact dermatitis is as high as approximately 20–30% (6–8). Adverse reactions related to nickel containing orthodontic devices such as arch wires, brackets, and soldered stainless steel face-bows have been reported (9–11). Surprisingly, nickel sensitivity has been reported to be lower in subjects who have received orthodontic treatment. It seems that treatment with nickel-containing metallic orthodontic appliances before sensitization to nickel (ear piercing) may have reduced the frequency of nickel hypersensitivity (12) and patients developed immunologic tolerance over a long period of treatment (13, 14). Allergic response to nickel-containing alloys is mainly type IV hypersensitivity reaction, cell mediated by T-lymphocytes (15). It has been suggested that long-term exposure to nickel-containing dental materials may adversely affect both human monocytes and oral mucosal cells (16–18).

Chromium ions provide an electrochemically formed passive film that offers protection against aggressive ions in the oral environment and prevents corrosion. This effect increases as the chromium content increases. As a result stainless steel and chromium-containing alloys do not corrode easily. The mechanism occurs by creating a thin oxide film, which delays corrosion. Integrity of chromium oxide on a metal surface is very important and chromium oxide must be maintained and kept stable throughout the entire material. However, when stainless steel is heat-treated, surface oxidation can occur, and an uneven oxide film may cause localized corrosion. Each heat treatment environment and cooling method can affect the thickness and form of the uneven oxide film on the wire surface, which can create various degrees of corrosion.

Aside from nickel, chromium and cobalt ions can also cause hypersensivity and dermatitis. These metals can induce cytotoxicity and genotoxicity (19–21). Previous *in vitro* (22–26) and *in vivo* (27–32) studies have investigated the release of metals, such as nickel and chromium from fixed orthodontic appliances. These studies reported a measurable level of metals in simulated medium (sodium chloride solution), saliva and blood. However, nickel released from all tested arch wires was considerably lower than the concentrations necessary to elicit cytotoxic reactions (25). Overall, differences in nickel content (in saliva) between individuals with or without orthodontic appliances, or between saliva collected before and after appliance insertion was not significant. The release of nickel seemed to be related to both the composition and the method of manufacture of the appliances (23, 25). Nickel release does not seem to be proportional to the nickel content of used fixed appliance (23). Heat treatments of the alloys under laboratory conditions have been shown to markedly increase the release of metal ions by 15–60 times (33). Exposure to acidic environment (34), simulated function conditions (dynamic) compared to static conditions (24), and the use of toothpaste (35) and some mouth washes (36) have been shown to increase the release of nickel ions from nickel alloys.

To our knowledge, most studies investigated the level of released ions in saliva or blood, overlooking the cells with prolonged contact with fixed appliances. The aim of this study was to compare the content of nickel, chromium and cobalt in oral mucosa cells in young patients with and without orthodontic appliances.

Materials and methods

Study cohort

Subjects were selected from the pool of patients who registered for a routine checkup at the Department of Orthodontics, Dental school of Azad Medical University within the past 3 years. A sample of 60 selected subjects was used comprising of a test group of 30 orthodontic patients who had fixed orthodontic appliances in both arches. The control group included 30 subjects without

any type of fixed orthodontic appliances or metal restoration in the mouth. The exclusion criteria in both groups were 1) smoking, pre-existing systemic diseases or medications associated with oral mucosa changes and 2) intraoral piercing/metal restorations. Informed consent was obtained after the objective of the study was fully explained.

In total, 20 females and 10 males, from 16 to 20 years (mean age 18.2 years) agreed to participate in the test group. For the patients in the test group, average period since appliance insertion was 16 months at the time of sample collection. All patients were bonded with new 0.018 in stainless steel brackets with standard edgewise slot in both arches (Discovery; Dentaurum, Pforzheim, Germany), and eight also had stainless steel orthodontic bands (Unitek/3M; Monrovia, CA, USA) on their upper and lower first molars. Six patients had a nickel–titanium alloy arch wire (Nitinol©; Ormco Corporation, Orange, CA, USA) at the time of sampling, and the remaining participants had stainless steel wires (Remantium; Dentaurum). Twenty females and 10 males aged 16–20 years (mean age 18 years) formed the control group.

Sample collection

The participants were asked to rinse their mouth for 1 min to remove exfoliated dead cells. Mucosa samples were collected by gentle brushing of the internal part of the right and left buccal mucosa with an interdental brush. The brushes were transferred to polyprophylene tubes and stirred in 5 ml of phosphate-buffered saline solution.

Metal content determination

Mucosa samples were diluted in water and acidified in nitric acid, kept at 60°C for 10 min to dissolve the metal content before analysis. The concentration of nickel, chromium, and cobalt ions was quantified using atomic absorption spectrophotometry with a graphite furnace (Varian SpectrAA-220; Mulgrave, Australia). Results were given as ng/ml. The detection limit of the method for samples solutions was as low as 1 ng/ml (p.p.b.). All metal content determinations were performed at the Analytical Chemistry Department, Nuclear Research Centre, Atomic Energy Organization of Iran.

Statistical analysis

Taking into account the approximately normal distributions of metal contents in samples, The Student's *t*-test was applied to assess differences in nickel, chromium, and cobalt mucosa cell contents between orthodontic patients (test group) and the control group. All analyses were carried out using SPSS 12 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL, USA). Statistical significance was determined at the 0.05 level throughout.

Results

The nickel contents in the buccal mucosa cells are given as mean and standard deviations and are shown in Table 1. The mean levels of nickel in control and test group were 12.26 and 21.74 ng/ml, respectively. Examining the content of nickel in the buccal mucosa cells of orthodontic patients (test group) and controls, the nickel content in mucosa samples of test group was significantly higher ($p < 0.01$) than that in the controls (Table 1).

There were slight increases in the content of chromium and cobalt in the buccal mucosa cells of the test group (Tables 2 and 3). In the control group, the mean levels of chromium and cobalt were 3.46 and 0.44 ng/ml, respectively, whereas in the test group the same metals were 4.24 and 0.84 ng/m, respectively. However the Student's *t*-test did not reveal a statistically significant difference between test and control groups for both chromium and cobalt.

Discussion

The present study investigated the presence of metal ions in oral mucosa cells in orthodontic patients wearing fixed appliances. Orthodontic appliances are

Table 1. **Mucosa cell nickel content in collected samples (mean ± SD, ng/ml) in controls and test groups**

Samples (ng/ml)	Controls (n = 30)	Patients (n = 30)	*p*-value
Nickel content (Mean ± SD)	12.26 ± 12.9	21.74 ± 11.41	0.003

Table 2. **Mucosa cell chromium content in collected samples (mean ± SD, ng/ml) in controls and test groups**

Samples (ng/ml)	Controls (n = 30)	Patients (n = 30)	*p*-value
Chromium content (Mean ± SD)	3.46 ± 1.65	4.24 ± 1.82	0.09

Table 3. **Mucosa cell cobalt content in collected samples (mean ± SD, ng/ml) in controls and test groups**

Samples (ng/ml)	Controls (n = 30)	Patients (n = 30)	*p*-value
Cobalt content (Mean ± SD)	0.44 ± 0.74	0.84 ± 1.06	0.10

mostly made of stainless steel and nickel–titanium alloys. The orthodontic alloy constituents are mostly iron, cobalt, chromium, and nickel. Because the corrosion products from orthodontic appliances can be harmful to the surrounding structure or body, we decided to evaluate the buccal mucosa cell content of three main possibly harmful constituents of orthodontic fixed appliances. Variety of factors can affect the amount of metal released from orthodontic appliances including the corrosion resistance of the material, the brazing or welding effects on the metal, galvanic corrosion of dissimilar metals, the surface of the appliance (37, 38).

Oral cavity provides an environment that makes aqueous corrosion in metals and alloys more favorable. Saliva as an electrolyte and a medium for chemical reactions between metals can cause corrosion. The organic acids and enzymes that microbes produce or the bacteria existing within the mouth can also cause corrosion. The present study used atomic absorption spectrophotometry with a graphite furnace for analysis of metal content in oral tissues. This is a common method used for trace element analysis in the literature (32, 39).

In our study, the nickel content in buccal mucosa cells of orthodontic patients (test group) was found to be significantly higher than in controls. This *in vivo* observation is in line with previous study by Faccioni et al. (16) in which the presence of nickel and cobalt has been shown in oral mucosa cells of orthodontic patients. Contrary to Faccioni's work (16), we did not find a significant difference in chromium and cobalt cell contents in patients with orthodontic appliances compared with their non-appliance controls. The failure to reach statistical significance was probably due to the wide variation in metal contents, and larger numbers may be required to demonstrate significant differences.

Nickel can be taken up into cells by diffusion via the $Mg2^{+}$ transport system (2), or via the calcium and iron channels (40). The most effective way of nickel uptake into cells is by phagocytosis of metallic nickel or nickel compound dust which has been seen in cultured cells, the efficiency of which depends on the size and surface charge of the nickel particles (41). Of the two environmentally available forms of chromium, hexavalent and trivalent, the hexavalent form has been demonstrated to be associated with the toxic parameters and classified as human carcinogen and mutagen (20). Several studies have shown that the cellular uptake of chromate is several fold greater than that of the trivalent ion, because trivalent chromium is predominantly octahedral and diffuses slowly (42). The tetrahedral hexavalent ion has been shown to enter the cell through general anion channels and bind to cellular components, causing disruptions in biochemical pathways.

Reductive metabolism of chromium within the cell by the cell's redox system leads to the formation of various intermediate forms, Cr (V), Cr (IV), and Cr (III) (43). While there is overwhelming evidence to show that Cr (VI) complexes are mutagenic in bacterial and mammalian cells, most of the Cr (III) complexes are shown to be non-mutagenic. Entry of Cr (III) into cells has also been shown to be diffusion controlled and macrophage mediated. Chromium (III) has been recognized as an essential trace element (44). Interpretation of the metal content of buccal mucosa cells is hampered by inherent limitations of the atomic absorption spectrophotometry to differentiate between oxidation levels of the metal contents. However, the valence of a metal affects its biologic activity, e.g., being mutagenic, hexavalent Cr crossed the cell membrane in contrast to trivalent Cr during *in vitro* studies.

There have been many studies on the amount of metal released from orthodontic appliances under various physical and chemical conditions (22–32). These studies demonstrated that these metals were released and absorbed by patients during the early stages of orthodontic therapy. They concluded that nickel ions, released from orthodontic appliances in

saliva or blood samples was significantly below the average dietary intake and did not reach toxic concentrations.

However, a review of the literature reveals that prolonged *in vitro* exposure to low levels of nickel ions can alter cellular metabolic activity (17, 18). Furthermore studies taking oral mucosal cell brushings in orthodontic patients compared with control subjects concluded that nickel release from fixed orthodontic appliances could induce DNA damage in oral mucosal cells (16). Therefore, to insure the safety of patients, further research and continued follow-up would be needed to determine the long-term significance of nickel release and other corrosion products.

Conclusion

Our findings indicate that there was no difference in the concentration of chromium and cobalt in oral mucosa cells of patients with or without fixed appliances. However, a significantly higher concentration of nickel can be found in buccal mucosa cells of patients wearing fixed orthodontic appliances. Continued follow-up is needed to determine the long-term significance of nickel release.

References

1. Staerkjaer L, Menne T. Nickel allergy and orthodontic treatment. *Eur J Orthod* 1990;12:284–9.
2. Oller AR, Costa M, Oberdorster G. Carcinogenicity assessment of selected nickel compounds. *Toxicol Appl Pharmacol* 1997;143:152–66.
3. Lee YW, Broday L, Costa M. Effects of nickel on DNA methyltransferase activity and genomic DNA methylation levels. *Mutat Res* 1998;415:213–8.
4. Setcos JC, Babaei-Mahani A, Silvio LD, Mjor IA, Wilson NH. The safety of nickel containing dental alloys. *Dent Mater* 2006;22:1163–8.
5. Uthus EO, Seaborn CD. Deliberations and evaluations of the approaches, endpoints and paradigms for dietary recommendations of the other trace elements. *J Nutr* 1996;126:2452S–9S.
6. Dalmau LB, Alberty HC, Parra JS. A study of nickel allergy. *J Prosthet Dent* 1984;52:116–9.
7. Espana A, Alonso ML, Soria C, Guimaraens D, Ledo A. Chronic urticaria after implantation of 2 nickel-containing dental prostheses in a nickel-allergic patient. *Contact Dermatitis* 1989;21:204–6.
8. Romaguera C, Vilaplana J, Grimalt F. Contact stomatitis from a dental prosthesis. *Contact Dermatitis* 1989;21:204.
9. Greig DGM. Contact dermatitis reaction to a metal buckle on a cervical headgear. *Br Dent J* 1983;155:61–2.
10. Dunlap CL, Vincent SK, Barker BF. Allergic reaction to orthodontic wire: report of case. *J Am Dent Assoc* 1989;118:449–50.
11. Greppi AL, Smith DC, Woodside DG. Nickel hypersensitivity reactions in orthodontic patients. *Univ Toronto Dent J* 1989;3:11–4.
12. Kerosuo H, Kullaa A, Kerosuo E, Kanerva L, Hensten-Pettersen A. Nickel allergy in adolescents in relation to orthodontic treatment and piercing of ears. *Am J Orthod Dentofac Orthop* 1996;109: 148–54.
13. Vreeburg KJJ, von Blomberg M, Scheper RJ. Induction of immunological tolerance by oral administration of nickel and chromium. *J Dent Res* 1984;63:124–8.
14. Van Hoogstraten IMW, Andersen KE, Von Blomberg BME, Boden D, Bruynzeel DP, Burrows D. Reduced frequency of nickel allergy upon oral nickel contact at an early age. *Clin Exp Immunol* 1991;85:441–5.
15. Schmalz G, Garhammer P. Biological interactions of dental alloys with oral tissues. *Dent Mater* 2002;18:396–406.
16. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosal cells. *Am J Orthod Dentofac Orthop* 2003;124:687–93.
17. Wataha JC, Lockwood PE, Schedle A, Noda M, Bouillaguet S. Ag, Cu, Hg and Ni ions alter the metabolism of human monocytes during extended low-dose exposures. *J Oral Rehabil* 2002;29: 133–9.
18. Noda M, Wataha JC, Lockwood PE, Volkmann KR, Kaga M, Sano H. Low-dose, long-term exposures of dental material components alter human monocyte metabolism. *J Biomed Mater Res* 2002;62:237–43.
19. Burrows D. Hypersensivity to mercury, nickel and chromium in relation to dental materials. *Int Dent J* 1986;36:30–4.
20. International Agency for Research on Cancer (IARC). *Monographs on Evaluation of the Carcinogenic Risk of Chemicals to Humans: Cr, Ni and Molding*. Lyon, France: IARC; 1990.
21. International Agency for Research on Cancer (IARC). *Monographs on the Evaluation of Carcinogenic Risk to Humans: Chlorinated Drinking Water, Chlorination By-products, some other Halogenated Compounds, Cobalt and Cobalt Compounds*, Vol. 52. Lyon, France: IARC; 1991.
22. Park HY, Shearer TR. In vitro release of nickel and chromium from simulated orthodontic appliances. *Am J Orthod* 1983;84:156–9.
23. Grimsdottir MR, Gjerdet NR, Hensten-Pettersen A. Composition and in vitro corrosion of orthodontic appliances. *Am J Orthod Dentofacial Orthop* 1992;101:525–32.
24. Kerosuo H, Moe G, Kleven E. In vitro release of nickel and chromium from different types of simulated orthodontic appliances. *Angle Orthod* 1995;65:111–6.
25. Jia W, Beatty MW, Reinhardt RA, Petro TM, Cohen DM, Maze CR et al. Nickel release from orthodontic arch wires and cellular immune response to various nickel concentrations. *J Biomed Mater Res* 1999;48:488–95.
26. Hwang CJ, Shin JS, Cha JY. Metal release from simulated fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop* 2001;120:383–91.
27. Gjerdet NR, Erichsen ES, Remlo HE, Evjen G. Nickel and iron in saliva of patients with fixed orthodontic appliances. *Acta Odontol Scand* 1991;49:73–8.
28. Bishara SE, Barrett RD, Selim MI. Biodegradation of orthodontic appliances, Part II. Changes in the blood level of nickel. *Am J Orthod Dentofacial Orthop* 1993;103:115–9.

29. Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop* 1997;111:595–8.
30. Agaoglu G, Arun T, Izgi B, Yarat A. Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. *Angle Orthod* 2001;71:375–9.
31. Eliades T, Zinelis S, Eliades G, Athanasiou AE. Nickel content of as-received, retrieved, and recycled stainless steel brackets. *Am J Orthod Dentofacial Orthop* 2002;122:217–20.
32. Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. *Eur J Orthod* 2006;28:292–7.
33. Gjerdet NR, Hero H. Metal release from heat-treated orthodontic archwires. *Acta Odontol Scan* 1987;45:409–14.
34. Geis-Gerstorfer J, Passler K. Studies on the influence of Be content on the corrosion behavior and mechanical properties of Ni–25 Cr–10 Mo alloys. *Dent Mater* 1993;9:177–81.
35. Wataha JC, Lockwood PE, Mettenburg D, Bouillaguet S. Toothbrushing causes elemental release from dental casting alloys over extended intervals. *J Biomed Mater Res B Appl Biomater* 2003;65:180–5.
36. Mueller H. Some considerations regarding the degradational interactions between mouth rinses and silver-soldered joints. *Am J Orthod Dentafac Orthop* 1982;81:140–6.
37. Maijer R, Smith DC. Corrosion of orthodontic bracket bases. *Am J Orthod* 1982;81:43–8.
38. Magnusson B, Bergman M, Bergman B, Soremark R. Nickel allergy and nickel and nickel-containing dental alloys. *Scand J Dent Res* 1982;90:163–7.
39. Garhammer P, Schmalz G, Hiller KA, Reitinger T. Metal content of biopsies adjacent to dental cast alloys. *Clin Oral Invest* 2003;7: 92–7.
40. Refvik T, Andreassen T. Surface binding and uptake of nickel (II) in human epithelial kidney cells: modulation by ionomycin. *Carcinogenesis* 1995;16:1107–12.
41. Costa M, Simmons-Hansen J, Bedrossian CWM, Bonura J, Caprioli RM. Phagocytosis, cellular distribution, and carcinogenic activity of particulate nickel compounds in tissue culture. *Cancer Res* 1981;41:2868–76.
42. Biedermann KA, Landolph JR. Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis and anchorage independence in diploid human fibroblasts. *Cancer Res* 1990;50:7835–42.
43. Connett P, Wetterhahn KE. Metabolism of the carcinogen chromate by cellular constituents. *Struct Bonding* 1983;54:93–124.
44. Mertz W. Chromium in human nutrition: a review. *J Nutr* 1993;123:626–33.