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Metal ion release from fixed orthodontic appliances—an *in vivo* study

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SUMMARY The aim of this study was to test the hypothesis that there is no difference in salivary metal ion content between subjects with fixed orthodontic appliances and their same-gender sister or brother without any orthodontic appliance.

This retrospective study was carried out on 28 subjects (16 females and 12 males) who had undergone fixed orthodontic therapy for a duration of 12–18 months. In order to limit the effects of dietary and hygiene habits on salivary metal ion concentration, a same-gender brother or sister (total of 28 subjects) was selected as a control. Approximately 5 ml of saliva was collected from each subject, and the samples were analysed using an atomic absorption spectrophotometer. The detection limit of the method for sample solutions was 1 ng/ml. Since some variables were not normally distributed, non-parametric tests (Mann–Whitney U and Wilcoxon W) were used for statistical analysis.

The mean salivary nickel (Ni) content in subjects with and without a fixed orthodontic appliance was 18.5 ± 13.1 and 11.9 ± 11.4 ng/ml, respectively. A statistically significant difference ($P < 0.035$) was found between the two groups. The mean salivary chromium (Cr) ion level recorded was 2.6 ± 1.6 ng/ml in the study group and 2.2 ± 1.6 ng/ml in the control group. The difference, however, was statistically insignificant. Within the limits of this *in vivo* study, it can be concluded that the presence of fixed orthodontic appliances leads to an increased concentration of metal ions in salivary secretions.

Introduction

How safe are orthodontic appliances? Almost all fixed metallic orthodontic appliances comprise metals, such as nickel (Ni), chromium (Cr), and cobalt. Apart from withstanding physical, mechanical, and biological assaults, a fixed orthodontic set-up should also be biocompatible in the oral environment (Bourauel *et al.*, 1998). Degradation products released from brackets and/or archwires can have a toxic effect on the surrounding oral tissues (Toms, 1988; Eliades *et al.*, 2004; Eliades and Bourauel, 2005). Unsafe and hazardous materials, especially those that are used over a very long period of time and are in close contact with the oral mucosa, particularly in a wet environment, as in orthodontic therapy, should be avoided. Several studies have shown that orthodontic appliances release metal ions through emission of electro-galvanic currents, with saliva acting as the medium for continuous erosion over time (Gjerdet *et al.*, 1991; Kerosuo *et al.*, 1997; Kocadereli *et al.*, 2000; Ağaoğlu *et al.*, 2001; Fors and Persson 2006; Levrini *et al.*, 2006; Amini *et al.*, 2008; Matos de Souza and Macedo de Menezes, 2008; Singh *et al.*, 2008).

Ni has been systematically studied for its detrimental effects at the cell, tissue, organ, and organism levels (Costa *et al.*, 1994; Zhou *et al.*, 1998). It has been reported that Ni complexes, in the form of arsenides and sulphides, can be allergic, carcinogenic, and act as mutating substances (Costa *et al.*, 1994; Oller *et al.*, 1997; Zhou *et al.*, 1998; Novelli *et al.*, 1998; Wataha *et al.*, 1999; Dayan and Paine, 2001; Burgaz *et al.*, 2002; Zoroddu *et al.*, 2002; Genelhu *et al.*, 2005). Previous research has shown that the amounts of metal released from orthodontic appliances in saliva or serum are significantly below the average dietary intake (Kocadereli *et al.*, 2000; Ağaoğlu *et al.*, 2001). However, a low ion concentration can induce biological effects in cells. Ni can lead to DNA alterations mainly through base damage and DNA strand scission even at very low concentrations (Lee *et al.*, 1995; Faccioni *et al.*, 2003).

A number of studies have investigated the release of metal ions from orthodontic alloys, but the results were not consistent. For example, while some authors have shown an increase in metal ion concentration in the oral fluid of patients with orthodontic appliances (Barrett *et al.*, 1993; Ağaoğlu *et al.*, 2001; Faccioni *et al.*, 2003; Fors and Persson, 2006; Levrini *et al.*, 2006; Amini *et al.*, 2008; Matos de Souza and Macedo de Menezes, 2008; Singh *et al.*, 2008), Kerosuo *et al.* (1997), Gjerdet *et al.* (1991), Kocadereli *et al.* (2000), and Staffolani *et al.* (1999) reported no differences in salivary metal ion concentration between subjects with and without fixed orthodontic appliances after a period of 30–90 days. However, a significantly higher concentration of Ni was reported in the oral mucosa of patients wearing fixed orthodontic appliances by Amini *et al.* (2008).

Food, tobacco smoking, and Ni in air and water are substances that can affect salivary Ni level (International Programme on Chemical Safety, 1991). Gjerdet *et al.* (1991), Ağaoğlu *et al.* (2001), Kratzenstein *et al.* (1988), and Bishara *et al.* (1993) reported that the Ni salivary content of patients with orthodontic appliances varies during different period of orthodontic treatment, possibly due to dietary intake. The other factor that may influence the release of Ni ions into saliva is the pH level of the saliva itself (Huang *et al.*, 2001). Since the effect of dietary Ni and Cr intake on metal content of saliva cannot be calculated, it cannot be concluded that the salivary metal ion differences in subjects with and without orthodontic appliance may be partly attributed to dissimilarities in nutritional habits.

On the other hand, Eliades *et al.* (2003) and Huang *et al.* (2001) criticized previously published findings. They believed that a sampling period of such a short duration is unrealistic. The period of typical orthodontic treatment of 2–3 years is such that the effect of fatigue and corrosion, which are substances of time, should be considered in each study. However, in most previous investigations, this factor has not been taken into account. In addition, most reported corrosion studies were performed *in vitro*, thereby rendering the results and conclusions potentially irrelevant to the clinical situation.

The aim of this study was therefore to determine the metal ion concentrations in the saliva of subjects with and without fixed orthodontic appliances. The null hypothesis tested was that the concentration of metal ions in saliva does not change due to the presence of orthodontic appliances.

Subjects and methods

The objectives of the study were explained to the participants and informed consent was obtained before salivary collection. The research was approved by the ethics committee of the Dental College of Azad University.

A total of 56 subjects were included in this study. Twenty-eight (16 females and 12 males) were healthy orthodontic patients with fixed appliances in both arches for a period of 12–18 months (study group). The age range of the subjects in this group was from 16 to 19 years (mean 17.5 ± 2.5 years). To limit the effect of food and oral hygiene habits on salivary metal ion concentration, a same-gender sister or brother without any orthodontic appliance formed the control group. Their age range was from 14 to 22 years (mean 18.2 ± 3.9 years). The average age difference between the two groups was 1 ± 3 years.

All patients were from the clinic of one author (FA). The criteria for the selection were having a same-gender sister or brother; absence of any piercings or metal restorations; good health and medication-free; and absence of any systemic diseases. No palatal or lingual arches or devices soldered or welded to bands or extraoral auxiliary appliances were used. Since placement of nickel titanium (NiTi) archwires can temporarily cause an increase in Ni concentration (Petoumenou *et al.*, 2009), none of the patients had a NiTi archwire in their set-up for at least 1 month prior to sample collection. Similarly, no subjects in the control group had any piercings, metal restorations, systematic diseases, or were receiving any medication.

The fixed appliance consisted of bonded 0.018 inch slot pre-adjusted Roth prescription stainless steel brackets on all teeth except the molars (Discovery; Dentaurum, Pforzheim, Germany); an average of four to eight stainless steel orthodontic bands (Unitek/3M, Monrovia, California, USA); NiTi alloy (Nitinol; Ormco Corporation, Orange, California, USA); and stainless steel archwires (Remantium; Dentaurum).

The sampling was performed 16 ± 2 months after the start of treatment with fixed orthodontic appliances (range 12–18 months). Sample collection was carried out such that after rinsing with 15 ml of distilled and deionized water for 30 seconds, approximately 5 ml of saliva was collected from each subject and transferred to an assigned cold polypropylene tube. The samples were kept at −20°C until they were processed and diluted with Zolal deionized water (BahreZolal Tehran Co., Tehran, Iran) to eliminate interference and to reduce the effects of the biological matrix (protein, salt, etc.).

A volumetric flask was used to dilute 1 ml of saliva in 10 ml of deionized water and the samples were analysed using an atomic absorption spectrophotometer (Varian SpectrAA-220; Varian Australia Pty Ltd, Mulgrave, Australia). The results were considered as nanograms per millilitre (ppb). The detection limit of the method for sample solutions was 1 ng/ml. Determination of the metal content was performed at the Analytical Chemistry Department, Nuclear Research Centre, and Atomic Energy Organization of Iran. The same batch of glass tubes and diluting agent were used for both groups.

Statistical analysis

Normal distribution of data was examined using the non-parametric Kolmogorov–Smirnov test. Since some variables were not normally distributed, the non-parametric tests (Mann–Whitney *U* and Wilcoxon *W*) were used for statistical analysis. Statistical significance was set at $P < 0.05$.

Results

A large variation of Ni and Cr concentration was observed in both the study and control groups. The Ni concentration varied from 1 to 48.0 ng/ml in the controls and from 1 to 49.60 ng/ml in the study group. The mean salivary Ni content was 18.5 ± 13.1 and 11.9 ± 11.4 ng/ml in the subjects and controls, respectively. Statistically significant differences were found between the groups ($P < 0.035$; Table 1).

The salivary concentration of Cr varied from 1.2 to 6.10 ng/ml in the controls and from 0.30 to 5.10 ng/ml in

Table 1 Salivary nickel (Ni) and chromium (Cr) content [mean and standard deviation (SD), nanograms per millilitre] in subjects with and without orthodontic appliances. Min, minimum; Max, maximum.

Ions	With appliance (N = 28)					Without appliance (N = 28)					P
	Mean	SD	Min	Max	Variance	Mean	SD	Min	Max	Variance	
Ni	18.5	13.1	1.0	49.6	171.4	11.9	11.4	1.0	48.9	130.3	0.03
Cr	2.6	1.6	0.3	6.3	2.8	2.2	1.8	1.0	7.1	2.7	0.2

the subjects with an appliance. The mean level of Cr ion was 2.2 ± 1.6 ng/ml in the controls and 2.6 ± 1.6 ng/ml in the study group. However, the minimal increase in Cr concentration in the study group was not statistically significant (Table 1).

Discussion

Most orthodontic appliances are made of stainless steel and NiTi alloys (Staerkjaer and Menné, 1990; Bass *et al.*, 1993), which can release metal ions into the oral cavity (Munksgaard, 1992; Wataha *et al.*, 1999; Valko *et al.*, 2005). The corrosion of orthodontic appliances and their subsequent metal ion release in the oral environment is governed by two main factors. The first is the manufacturing process, which includes the type of alloy and the characteristics of the metals used (Matasa, 1995). The second is environmental factors, such as mechanical stress, diet, time of the day, salivary flow rate, and health and psychosomatic condition of the individual (Gjerdet *et al.*, 1991).

Water ionizes into hydrogen (H^+) and hydroxyl (OH^-) ions. When these ions are in equal proportions, the pH is a neutral 7. When there are more H^+ ions, then the water is said to be 'acidic'. If OH^- ions outnumber H^+ ions, then the water is said to be 'alkaline'. The pH scale ranges from 0 to 14 and is logarithmic, which means that each step is 10 times the previous one. In other words, a pH of 4.5 is 10 times more acidic than 5.5, 100 times more acidic than 6.5 and 1000 times more acidic than 7.5.

Minerals with a negative electrical charge that are attracted to the H^+ ions are called acid minerals. Acid minerals include chlorine, sulphur, and phosphoric acid. Minerals with a positive electrical charge that are attracted to negatively charged OH^- ions are called alkaline. Nutritionally important alkaline minerals include calcium, potassium, magnesium, and sodium.

Kuhta *et al.* (2009) reported that if the salivary pH is reduced from 6.75 to 3.5, it can increase the release of metal ions from orthodontic appliances up to 100-fold. Low pH values also reduce the resistance of dental alloys to corrosion (Huang *et al.*, 2003). On the other hand, Sandin and Chorot (1985), who investigated the effects of levels of anxiety and stress on salivary pH, found a statistically significant direct correlation between increasing levels of anxiety and stress and increases in salivary pH. Hypotheses of shared genetic aetiologies as a potential basis for stress and anxiety (Eley and Stevenson, 1999) have been tested and there is clear scientific evidence that anxiety runs in families (Silberg *et al.*, 2001; Mineka and Zinbarg, 2006).

In addition, a diet rich in sodium chloride and acidic carbonated drinks can provide a regular supply of corrosive agents (House *et al.*, 2008). Another contributor to an acidic oral condition is fluoride-containing products, such as mouth washes and tooth pastes (Toumelin-Chemla *et al.*, 1996; Schiff *et al.*, 2005). Vegetables, grains, and cereals are all foods rich in Ni (Barrett *et al.*, 1993). On the other hand, the Ni content in glandular saliva, like Ni in other body fluids, is largely influenced by dietary intake. In previous studies, the effects of diet on metal ion release from orthodontic appliances were not considered, as a lack of information on the Ni content in various food makes sampling difficult (International Programme on Chemical Safety, 1991; Livsmedelsverket, 1997).

To overcome or at least to reduce the effects of emotional behaviour and dietary intake on salivary ion concentration and to obtain a closer match between the study and control group, the same-gender sibling was selected as the control in the present study.

The corrosion of metal and release of ions such as Ni have an additive rather than a linear relationship with time (Eliades *et al.*, 2003). Except for a few reports (Ağaoğlu *et al.*, 2001; Eliades *et al.*, 2003; Fors and Persson, 2006), the majority of previous publications have calculated the amount of metal ions released inside the oral cavity over a short period of time (1–3 months). Such a short period is not sufficient to effectively evaluate the salivary metal ion content of orthodontic patients (Eliades *et al.*, 2003). Therefore, the present study was planned to cover an average period of 12–18 months, from appliance insertion until sample collection.

The use of an atomic absorption spectrophotometer permits analysis of metals in biological samples without separating the metal from its biological matrix. By utilizing this technique, there was no necessity for extraction procedures to analyse the elements. To eliminate interference and the effects of the biological matrix (protein, salt, etc.), a dilution of each salivary sample is adequate (Smith and Stewart, 1981).

In general, this study showed an increase in salivary Ni and Cr concentration in patients with fixed orthodontic appliances compared with their same-gender control sister or brother. This finding is in agreement with those of Ağaoğlu *et al.* (2001), who reported an increase in salivary Cr and Ni concentration 1 year after appliance insertion. Fors and Persson (2006) also showed that the amount of Ni in saliva debris retained on filters was significantly higher in orthodontic patients compared with controls when collecting the salivary sample after an average period of 16 months.

On the other hand, Eliades *et al.* (2003) and Gjerdet *et al.* (1991) failed to show increased levels of metal ions in the saliva of orthodontic patients. This difference may be due to diverse methods for analysing the levels of the metals or sample selection. Other studies, however, were either carried out over a short period of time of 1 week to 3 months (Staffolani *et al.*, 1999; Kocadereli *et al.*, 2000; Singh *et al.*, 2008; Petoumenou *et al.* 2009) or were *in vitro* investigations. Currently, the concentration of metal ions at a specific time point cannot be applied to full-term treatment, so the results could not be directly compared.

Conclusions

Using an appropriate sample collection method, a sufficiently long study period, a sibling of each patient as a control, and consideration of the same criteria for both groups and within the limits of an *in vivo* study, it can be concluded that:

1. Fixed orthodontic appliance therapy for an average period of 16 months can lead to increased levels of Ni and Cr ions in the saliva of patients.
2. While the low levels of these metal ions can be of concern to patients with allergies, they do not lead to problems in the majority of orthodontic patients as toxic levels are never attained.

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