Dental Research Journal

Original Article

In vivo study on the release of nickel, chromium, and zinc in saliva and serum from patients treated with fixed orthodontic appliances

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ABSTRACT

Background: Fixed orthodontic appliances can release metal ions such as nickel, chromium, and zinc into saliva and blood, which can cause contact dermatitis, hypersensitivity, and cytotoxicity. This study was undertaken to assess the release of nickel, chromium, and zinc in saliva and serum of patients undergoing fixed orthodontic treatment.

Materials and Methods: This *in vivo* study was conducted on 80 participants with an age range of 15–40 years. Thirty were included as controls and 50 participants were treated with fixed orthodontic appliances. Saliva and blood samples were collected at five different periods, before insertion of fixed orthodontic appliance and at 1 week, 3 months, 1 year, and 1.5 years after insertion of appliance, respectively. The metal ion content in the samples were analyzed by atomic absorption spectrophotometry. Mean levels of nickel, chromium, and zinc in saliva and serum were compared between groups using independent sample *t*-test and before and after results using paired *t*-test. $P < 0.05$ was considered as statistically significant.

Results: At the end of 1.5 years, the mean salivary levels of nickel, chromium, and zinc in controls were 5.02 ppb, 1.27 ppb, and 10.24 ppb, respectively, as compared to 67 ppb, 30.8 ppb, and 164.7 ppb at the end of 1.5 years. This was statistically significant with $P < 0.001$. A significant increase in the metal ion levels were seen in participants with before and after insertion of appliance ($P < 0.001$).

Conclusion: Orthodontic appliances do release considerable amounts of metal ions such as nickel, chromium, and zinc in saliva and serum. However, it was within permissible levels and did not reach toxic levels.

Key Words: Chromium, nickel, orthodontic appliances, saliva, zinc

Received: December 2017
Accepted: October 2018

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INTRODUCTION

Nickel-titanium alloys are commonly used in orthodontics, in manufacturing various components of fixed orthodontic appliances.[1] Metal ions such as nickel, chromium, and zinc can be released from the orthodontic appliances as part of the dissolution and the biomechanical process of alloys.[2] Nickel is a common allergen and powerful sensitizer. With amounts as low as 2.5 ng/mL, nickel has been found to impair the chemotaxis of leukocytes and

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www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Quadras DD, Nayak US, Kumari NS, Priyadarshini HR, Gowda S, Fernandes B. *In vivo* study on the release of nickel, chromium, and zinc in saliva and serum from patients treated with fixed orthodontic appliances. Dent Res J 2019;16:209-15.

stimulate neutrophils to become aspherical and move slowly.[3] Ion release can also cause discoloration of the adjacent soft tissues, allergic reactions, or pain.[4]

The patients undergoing orthodontic treatment are exposed to metal alloys in the mouth that can corrode over a period during orthodontic treatment, resulting in the release of ions into oral mucosa and biologic fluids.[5,6] Corroded orthodontic appliances have shown to cause biologic health hazards including contact dermatitis, hypersensitivity, and cytotoxicity in the past studies.[7-10]

Orthodontic appliances are made up of austenitic stainless steel, which includes about 8%–12% nickel, 17%–22% chromium, and other trace metals. Thus, metal ions are released from fixed appliances through discharge of electro-galvanic currents, where saliva acts as medium for continuous erosion over a period during orthodontic treatment.[11,12] Such released metals from fixed orthodontic appliances can result in metal-mediated generation of free radicals which may cause various chemical alterations in DNA bases.[13]

In addition, metals such as chromium and nickel can produce free radical species from molecular oxygen, which in turn produce superoxide anion and highly toxic hydroxyl radical.[14] The generated free radicals also prevent antioxidant enzymes and consume the intracellular glutathione levels, thus intensifying the effects of prooxidants.[15] Fixed orthodontic appliances increase the risk of plaque deposition, periodontal trauma, dental caries, and alteration in endogenously synthesized antioxidants, namely, glutathione-S-transferase and superoxide dismutase in saliva.[16]

The release of metal ions can cause DNA damage in human cells due to oxidative DNA damage (direct interaction) or interference with DNA replication (indirect interaction).[17-19] The mutative action of nickel may derive from its action on inhibiting several enzymes known to restore DNA breaks, promoting mutations, thereby contributing to genetic instability.[20] It seems that multidisciplinary approach toward the problem of the assessment of exposure of the human organism to trace elements may reveal different aspects associated with the application of metals in dentistry.[21]

Although some studies in the past have shown that the level of metals released from orthodontic appliances in saliva or serum was significantly below the average dietary intake and did not reach toxic concentrations,[19] those were short-term studies conducted over few weeks to months. Hence, the present study was carried out to assess nickel, chromium, and zinc levels in saliva and serum samples of patients treated with fixed orthodontic appliances and to determine any significant changes in these concentrations, throughout the treatment.

MATERIALS AND METHODS

In this *in vivo* study The patients were selected from the Department of Orthodontics and Dentofacial Orthopedics, ABSMIDS, Deralakatte, Mangalore. Ethical clearance was obtained from the Institutional Ethical Committee before the commencement of the study (Ref: NU/CEC/PhD-30/2011).

Eighty participants who visited the department for seeking orthodontic treatment and consented to be a part of the study were included for the study after applying the inclusion and exclusion criteria. Healthy participants with permanent dentition within the age range of 15–40 years were included for the study. Participants with genetic syndromes, history of previous orthodontic treatment, allergies to artificial jewelry, under medication, amalgam and metal restorations, expansion and extraoral orthopedic appliance, smokers, and alcoholics were excluded from the study.

The study group included 50 patients who required orthodontic treatment. A detailed case history was obtained from these 50 participants followed by treatment with stainless steel fixed orthodontic appliances (preadjusted edgewise appliance) which consisted of 4–8 bands and 20 bonded brackets (3M Unitek, Monrovia, California). The material was American Iron and Steel Institute type 304 for the orthodontic bands and type 316 for the metal brackets. The archwires used in this study were nickel-titanium alloy and stainless steel (3M Unitek, Monrovia, California). In addition, 30 participants who did not require any orthodontic treatment were included to serve as controls resulting in a total sample size of 80.

Saliva and serum samples were collected from all 80 participants at five intervals, before insertion of the appliance, at 1 week, 3 months, 1 year, and 1.5 years after the insertion of the appliance. Saliva and serum samples were collected in the morning before breakfast. Saliva was collected by the spit method. The patients were asked to rinse their mouth thoroughly with distilled water before the sample collection. The saliva was sampled for 5 min with the mouth closed without stimulation and then transferred to plastic test tubes.

Five milliliters of blood was obtained from the antecubital fossa of the arm and centrifuged at 3000 rpm for 10 min to prepare the serum. Acid-washed plastic containers were used for storage of saliva and serum samples. The samples were kept at −20°C until they were processed. The estimation was carried out by Ağaoğlu *et al*.[22] method. The stored saliva and serum samples were subjected for analysis of nickel, chromium, and zinc in levels.

The spectrophotometric determinations were carried out using an electrothermal atomic absorption spectrophotometer in Nitte University Center for science education and research using Perkin Elmer AAnalyst 800 with graphite surface. Each test was analyzed three times, and the average was used as a result. Before each test distilled water sample was processed to prevent possible contamination. The insoluble precipitate was not included in the analysis because of the problem of particles causing variation in the results.

Data was ere analyzed using SPSS version 22 (IBM Corp, Armonk, NY, USA). The difference in mean levels between study and control groups was assessed using independent sample *t*-test. The comparisons before and after treatment in the test group were done using paired *t*-test. $P < 0.05$ was considered as statistically significant.

RESULTS

The mean age of the participants was 24.5 ± 1.2 years. Of 50 patients in the study group, 31 were female and 19 were male. Comparison of mean levels of nickel, chromium, and zinc in saliva and serum among study and control groups before the insertion of appliance did not show any significant difference [Table 1].

Table 2 shows the mean levels of nickel, chromium, and zinc in saliva and serum of patients with stainless steel appliances at the end of 1.5 years. A significant difference was found in metal ion levels at the end of study between control and study groups. While the nickel, chromium, and zinc levels in saliva of controls was 5.02 ppb, 1.27 ppb, and 10.24 ppb, respectively, and study group was 67 ppb ($P = 0.021$), 30.8 ppb ($P = 0.013$), and 164.7 ppb ($P < 0.001$), respectively. The same was true with the serum levels as well, with the test group having significantly higher levels of metal ions in serum as compared to controls ($P < 0.001$).

The mean levels of metal ions also showed a steady increase throughout the study in both saliva and serum in patients undergoing orthodontic treatment. In saliva, a slow increase of only 0.10 ppb of nickel, 0.49 ppb of chromium, and 26 ppb of zinc was found during the 1st week which reached a maximum of 67 ppb of nickel, 30.8 ppb of chromium, and 164.5 ppb of zinc at the end of 1.5 years [Table 3].

In serum, a slow increase of only 0.38 ppb of nickel, 0.36 ppb of chromium, and 27 ppb of zinc was found during the 1st week which reached a maximum of 81.65 ppb of nickel, 35.6 ppb of chromium, and 597.16 ppb of zinc at the end of 1.5 years. This result was statistically significant with $P < 0.001$ [Table 4].

Table 1: Comparison of mean metal ion levels in saliva and serum among control and study groups before insertion of appliance

Mean metal ions (ppb) before insertion of appliance	Control group (*n*=30)	Study group (*n*=50)	*P*
Salivary nickel level	4.33±0.002	4.24±0.009	0.98
Salivary chromium level	1.13±0.03	1.18±0.01	0.84
Salivary zinc level	10.73±1.9	11.8±2.4	0.53
Serum nickel level	8.31±0.004	8.46±0.008	0.91
Serum chromium level	6.18±0.006	6.46±0.008	0.42
Serum zinc level	29.1±1.9	28.3±2.3	0.26

Table 2: Comparison of mean levels of nickel, chromium, and zinc in saliva and serum among control and study groups at the end of 1.5 years

Mean metal ions (ppb) at the end of 1.5 years	Control group (*n*=30)	Study group (*n*=50)	*P*
Salivary nickel level	5.02±0.001	67±10.8	0.021
Salivary chromium level	1.27±0.09	30.8±4.3	0.013
Salivary zinc level	10.24±1.3	164.7±10.36	<0.001
Serum nickel level	8.47±0.001	81.65±3.78	<0.001
Serum chromium level	6.02±0.004	35.6±2.3	0.014
Serum zinc level	30.1±1.7	597.16±68.5	<0.001

Table 3: Mean nickel, chromium, and zinc levels in saliva at different periods in the study group

Periods	Nickel (ppb)	Chromium (ppb)	Zinc (ppb)
BA	4.24±0.009	1.18±0.01	11.8±2.4
After 1 week	4.34±0.006	0.59±0.026	37±3.6
After 3 months	11.1±0.009	1.57±0.08	61.6±3.4
After 1 year	6.84±0.005	0.94±0.069	84.75±6.85
After 1.5 year	67±10.8	30.8±4.3	164.7±10.36
P	0.015	0.021	<0.001

BA: Before insertion of appliance

DISCUSSION

Orthodontic patients are exposed to metal alloys that can corrode in the oral cavity and cause biologic health hazards.[20] The corrosion of metals and ion release has been shown to have additive rather than a linear relationship with time.[21-23] Dental materials within the mouth interact continually with physiologic fluids.[24,25] The presence of soldered joints increases corrosion susceptibility since they tend to emit electrogalvanic currents with saliva and release metal ions. The use of nickel is of particular concern since it is the most allergenic of all metallic materials. Galvanic current or release of ions could account for many types of dyscrasias such as lichenoid lesions, ulcers, leukoplakia, cancer, and kidney disorder.[26]

Urinary excretion levels of nickel in orthodontic patients have shown significant increase 2 months after treatment.[27] Mobile phone usage has a time-dependent influence on the concentration of nickel in the saliva of patients with orthodontic appliances.[28] Nickel and chromium ions in the scalp hair show slightly elevated levels in patients treated with fixed orthodontic appliances.[2] Listerine causes the highest release of nickel and chromium ions and Listerine Advanced White, Oral B 3D White Luxe, and distilled water were similar in terms of ion release. Oral B caused the lowest release of ions in patients undergoing fixed orthodontic treatment.[4]

Many studies in the past have measured the release of metals *in vivo* over a short period of 1–3 months. Such a small period is not enough to assess the release of salivary metal ion content of orthodontic appliances.[21] Hence, the present study was designed to cover the entire treatment course of 12–18 months, from appliance placement to the end of treatment. The mean age of patients in the present study was 24.5 ± 1.2 years. Patients were older compared to participants of a study by Ağaoğlu *et al.*[22] in which the mean age of the participants was 19.5 years and Kocadereli *et al.*[29] in which the mean age was 13 ± 1.2 years.

Table 4: Mean nickel, chromium, and zinc levels in serum at different periods in the study group

Periods	Nickel (ppb)	Chromium (ppb)	Zinc (ppb)
BA	8.46±0.008	6.46±0.008	28.3±2.3
After 1 week	9.08±0.008	6.08±0.008	55.25±6.5
After 3 months	6.4±0.006	8.4±0.006	224±12.6
After 1 year	7.88±0.005	5.88±0.005	390.2±32.5
After 1.5 year	81.65±3.78	35.6±2.3	597.16±68.5
P	0.023	0.041	<0.001

BA: Before insertion of appliance

The most significant and important method for the assessment of nickel release is analysis of saliva since it is the first diluent of the human body and allows long periods of analysis. Thus, the effects of material aging and fatigue on the ion release could be investigated.[30] In the present study, the release of nickel in saliva and serum was initially slow and increased steadily at the end of 1.5 years. However, it was still within the dietary limits and did not reach toxic levels. The salivary nickel concentration has shown a large variation in the past studies.

Nickel concentration in saliva varied from 0.07 to 3.32 μg/mL in a study done by Kocadereli *et al.*,[29] and the release was about 40 μg/mL per day according to Park and Shearer[31] and was not significant when compared to before insertion of the appliance.

A study by Gjerdet *et al.*[32] showed increase in nickel levels in saliva immediately after placement of orthodontic appliance. In another study by Ağaoğlu *et al.*[22] showed that nickel level in saliva reached the highest level in the 1st month and then decreased during treatment and showed a significant increase in serum nickel level in the 2nd year of treatment. However, a study by Bishara *et al.*[33] did not find any difference in nickel levels in serum after 4–5 months of treatment.

The variation could be due to differences in dietary habits, geographic location, and method of saliva collection. Furthermore, some studies have been conducted on simulated conditions which will be different from the patient's oral condition. Furthermore, in the present study, a significant difference was found in nickel level in saliva among control and study groups at the end of treatment. A similar result was reported by Amini *et al.*[34] who found higher levels of nickel in saliva in patients with orthodontic appliances. In a study by Petoumeno *et al.*,[35] nickel levels in saliva increased immediately after placement of orthodontic appliance but decreased after 10 weeks. However, no significant difference was found in saliva among participants with and without appliances in a study by Staffolani *et al.*,[36] Kerosuo *et al.*,[37] and Eliades *et al.*[38]

Ousehal and Lazrak[1] reported that orthodontic appliances release nickel ions mainly at the start of orthodontic treatment. Nayak *et al.*[3] reported a statistically significant increase in the nickel after the initial aligning phase, and a net decrease of 1.58 ppb was found after 10–12 months of treatment. This decrease in nickel ion concentration was, however, found to be statistically insignificant. A positive correlation was found in the initial rise in nickel concentration. However, no correlation was found for the change in nickel ion concentration at the end of 10–12 months.

Amini *et al*.[39] have shown that nickel might increase in patients undergoing treatment with both bracket types – conventional and metal injection molding (MIM) brackets although the rate of increase might be greater in patients undergoing treatment with conventional brackets. Still, ion levels leached from conventional versus MIM brackets might not show a difference after 2 months. Amini *et al*.[40] showed that induction of stress led to a significant increase in nickel release from orthodontic appliances into saliva. A study by Singh *et al*.[41] showed that salivary nickel concentrations significantly increased after insertion of fixed orthodontic appliances as compared to baseline levels, with the maximum concentration seen in the 1st week after placement of fixed orthodontic appliances.

Downarowicz *et al*.[42] have shown that the most biocompatible material used in the production of fixed orthodontic appliances is titanium, and the least biocompatible material is steel, which releases the largest amount of nickel. Metal ions are released from fixed orthodontic appliances only in the first phase of treatment. Orthodontic appliances release nickel ions which induce localized genotoxic effects, but these changes come to normal on the removal of the appliances as reported by Natarajan *et al*.[43]

In the present study, the release of chromium in saliva and serum was initially fluctuating and increased at the end of 1.5 years. Comparison of mean levels of chromium in saliva and serum of participants in study and control groups showed no significant difference with $P > 0.05$ before the insertion of appliance and showed the statistically significant difference with $P < 0.05$ at the end of 1.5 years. However, it was still within the dietary limits and did not reach toxic levels. A study by Eliades *et al.*[20] found no significant difference between study and control groups in salivary chromium levels.

A study by Ağaoğlu *et al*.[22] showed that chromium in saliva reached the highest level in the 1st month and then decreased to the initial level at the end of treatment and showed statistically significant increase in chromium levels in serum in the 2nd year groups. Kerosuo *et al*.[37] found no significant increase in chromium levels in saliva before and after 1 month of orthodontic treatment. Whereas, a study by Kocadereli *et al*.[29] found a significant increase in salivary chromium level during the first 2 months of treatment.

Nayak *et al*.[3] reported a statistically significant increase in chromium ion concentration after the initial aligning phase and a net increase of 17.92 ppb was found at the end of 10–12 months, which were statistically significant. A positive correlation was found for the increase in chromium ion concentration after the initial alignment and at the end of 10–12 months.

Amini *et al*.[39] have shown that using MIM brackets might reduce salivary chromium for a trivial but generalizable amount. Still, ion levels leached from conventional versus MIM brackets might not show a difference after 2 months. Chromium concentrations significantly increased after 1 week of insertion of fixed orthodontic appliances have been reported by Singh *et al*.[41]

Downarowicz *et al*.[42] have shown that chromium released from fixed orthodontic appliances only in the first phase of treatment. Chromium alloys released from orthodontic appliances have known to cause localized genotoxic effects, but these changes come to normal on the removal of the appliances as reported by Natarajan *et al*.[43] A study by Matos de Souza *et al*.[30] found the highest chromium levels in saliva immediately after placement of appliance. A study by Amini *et al*.[34] showed an increase in salivary chromium levels in patients as compared to the controls. However, this difference was not statistically significant.

In the present study, the levels of zinc in saliva and serum also showed a steep increase in its concentration level at the end of 1.5 years. Comparison of mean levels of zinc in the saliva of participants in study and control groups showed no significant difference with $P > 0.05$ before the insertion of appliances and showed the statistically significant difference with $P < 0.05$ at the end of 1.5 years. However, it was still within the dietary limits and did not reach toxic levels. A study by Mikulewicz *et al*.[44] showed higher concentration of zinc in artificial saliva in which bands and brackets were incubated. However, this study was done outside the oral cavity, and hence, the result cannot be directly compared to the present *in vivo* study. Since no studies have been conducted to assess the zinc levels in saliva and serum of orthodontic patients, the results of the present study cannot be compared.

In most of the studies, metals released from orthodontic appliances in saliva and serum were significantly below the average dietary intake and did not reach toxic levels. There is a high variation in the levels of metal ions in saliva among patients undergoing orthodontic treatment shown by studies in the past. These variations can be attributed to the difference in techniques of preparation of samples as well as different analytical methods. Furthermore, most of the studies conducted are *in vitro* which do not exactly reflect the oral environment.

Other factors which change the levels of metals in the *in vivo* studies could be related with eating, drinking, or toothbrushing. The WHO guideline values for drinking water currently accept the concentration for nickel at 70 µg/mL and chromium at 50 µg/mL which are higher than saliva of patients as found in many studies. The normal range of metal ions in the body is 300–600 µg for nickel and 50–200 µg for chromium which is higher than saliva and serum of patients as found in many studies. A similar result has been found in the present study.

CONCLUSION

Orthodontic appliances used to treat patients release considerable amounts of nickel, chromium, and zinc into saliva and serum during different periods of treatment as analyzed in our study. However, the levels were well below the average dietary intake and did not reach toxic levels.

Financial support and sponsorship

The authors are thankful to the Indian Council of Medical Research, Government of India, for financial support. (Sanction No: 5/4/2-12-2009-NCD-II).

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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