



Salivary Nickel and Chromium Levels in Orthodontic Patients with and Without Periodontitis: a Preliminary Historical Cohort Study

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Abstract

Many periodontal patients may need orthodontic treatment. Alterations in oral environment particularly the reduction of pH in periodontal patients could affect metal ion release from orthodontic appliances. However, there is no study on metal ion release in periodontal patients. The aim of this preliminary study was to comparatively evaluate, for the first time, salivary levels of nickel and chromium in periodontal patients (versus healthy controls) under orthodontic treatment for 2 months. In this *in vivo* study, 40 subjects were evaluated. Patient selection and standardization of orthodontic treatment protocols were prospectively designed and performed. Two groups of $n = 20$ each (control: healthy orthodontic patients, cohort: orthodontic patients with periodontitis) underwent similar protocols of fixed orthodontic treatment for 2 months. After 2 months, salivary nickel and chromium concentrations of the case and cohort groups were measured using inductively coupled plasma mass spectrometry (ICP-MS). The values were compared between the two groups using *t* test. There were 10 men and 10 women in each group. The mean age of patients was 34.6 ± 3.6 years old. The salivary level of nickel was 338.2 ± 235.5 ng/ml and 182.8 ± 116.5 ng/ml in the cohort and control groups, respectively ($P = 0.0118$). The salivary level of chromium was 7.4 ± 3.15 ng/ml in the cohort and 6.35 ± 2.39 ng/ml in the control group ($P = 0.2214$). Salivary level of nickel might be considerably higher in periodontal patients undergoing 2 months of orthodontic treatment compared to orthodontic patients with healthy gingivae.

Keywords Metal ion release · Nickel · Chromium · Periodontitis · Orthodontic treatment · Saliva

Introduction

Release of toxic and carcinogen ions during orthodontic treatment can compromise the biocompatibility of orthodontic appliances [1–5]. Several metals are used in orthodontic alloys, and nickel or chromium is of concern in terms of biocompatibility [1–8]. Stainless steel and NiTi alloys have about 8 wt% and 55 wt% nickel, while their chromium content is about 20 wt% and 0.2 wt%, respectively [6, 9, 10].

Orthodontic appliances are subjected to various disruption forces (such as environmental stresses, foods, thermal

alterations and masticatory forces in the oral environment, tooth brushing, saliva flow, acidic drinks, occlusal loadings, frictions between appliances, mechanical stresses, and microorganisms and their enzymatic activity). These factors can remove the protective chromium oxide coating over the alloys. This facilitates corrosion mechanisms such as galvanic corrosion, which happens when different metals are present in the saliva [3–6, 8, 9, 11–16].

The released nickel and chromium ions can have toxic and mutagenic effects and lead to hypersensitivity reactions, asthma, or allergic contact dermatitis [1, 3–7, 9, 11, 13, 17]. Bearing in mind the possible hazard of these metal ions, it seems necessary to evaluate the release or accumulation of nickel and chromium ions, when using orthodontic appliances [1, 2, 4, 5, 8, 10, 17–20]. *In vitro* studies (which form most of the literature) are not relevant to clinical conditions because they cannot simulate properly the complex environment of the mouth [1–3, 6, 8, 9, 13, 21], and controversy exists over *in vivo* results [4–6, 13]. Therefore, more clinical studies in this regard are needed.

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A considerable percentage of orthodontic patients are adults [22], of whom many suffer from periodontitis, particularly the chronic type [23]. In the USA, about 47.2% of adults suffer from periodontitis [24, 25]. The oral environment of periodontal patients has some differences with that of healthy individuals. For instance, the saliva pH is often lower in periodontal patients than that in healthy individuals [26]. Acidic environments (caused by factors such as dietary habits) might enhance corrosion and increase ion release from orthodontic and dental alloys [27–29].

Therefore, it may be hypothesized that metal ion release from orthodontic appliances might be greater in periodontal patients (who might have more acidic saliva) compared to healthy controls. This hypothesis may have clinical implications in terms of biocompatibility of orthodontic appliances in patients with periodontal inflammations. However, this hypothesis has not been tested in any previous study. Thus, we aimed to compare salivary concentrations of nickel and chromium ions in patients under fixed orthodontic treatment with periodontitis versus in orthodontic patients with healthy gingivae.

Subjects and Methods

This *in vivo* study had two phases. Patient recruitment and orthodontic treatment/standardization were planned and performed prospectively, whereas salivary ion measurements followed a historical cohort design. The study participants were selected prospectively from those presenting to the Orthodontics Department and three private orthodontic clinics in 2017. The sample size of this pilot study was predetermined as two groups of 20 patients each, in line with previous literature. Patients with metal restorations, cigarette smokers, tobacco consumers, mouth breathers, those with systemic diseases (diabetes mellitus, renal conditions, respiratory diseases), and patients taking medications affecting salivary biochemistry were all excluded. The study ethics were approved by the research committee of the university (thesis #25542, ethics code: IR.IAU.DENTAL.REC.1395.7). The study protocol was thoroughly explained to patients, and written informed consents were obtained from them prior to participation in the study.

Patients were prospectively examined by an expert periodontist to assess their eligibility for study inclusion. Control patients had to have healthy gingivae (no bleeding on probing) and probing depths <3 mm. Periodontal patients had to have gingival inflammation, attachment losses with probing depths \geq 3 mm, and radiographically confirmed bone loss in at least 20% of sites (using panoramic radiography). The numbers of males and females in both groups were prospectively determined as similar or preferably equal in each group.

About 4 to 6 weeks before commencement of orthodontic treatment, all patients in both groups entered the first phase of periodontal treatment (receiving scaling and root planning); also, all orthodontic patients in both groups received oral hygiene instructions and were taught the modified Bass tooth brushing technique. They were re-evaluated 4 to 6 weeks later, and the periodontist had to confirm that the disease was under control in the periodontitis group (by confirming the lack of bleeding on probing and a plaque index of zero) in order for the orthodontic treatment to begin. The periodontitis group underwent this treatment for clinical reasons (in order to ensure the inactivity of periodontal disease during the orthodontic treatment and therefore to ensure minimum gingival/periodontal damage caused by orthodontic forces and tooth movements). The control group underwent the same gingival treatments for standardization in terms of initial extents of plaque and calculus. All patients were requested to use the same toothpaste and mouthwash during the first 2 months of orthodontic treatment.

Fixed orthodontic treatment of both arches was then started for patients in both groups using similar brackets for the purpose of standardization (American Orthodontics; Sheboygan, WI, USA), NiTi wires, and no-mix adhesive (3M ESPE, St. Paul, MN, USA).

Saliva samples were collected from the patients 2 months after the beginning of their fixed orthodontic treatment (at the NiTi wire phase). Saliva was collected from patients in both groups from 9 am to 12 pm because time of the day and time passed since the last meal can change the amount of released ions [27]. Patients were requested not to consume acidic or carbonated beverages and to refrain from eating foods containing sodium chloride for 1 week prior to saliva collection because all these factors can increase the susceptibility of metals to corrosion and change the concentration of metal ions. Patients were also requested to refrain from eating and drinking in the morning prior to saliva collection.

Nickel-free vials were rinsed with distilled water (Merck, Germany) and filled and emptied with acetone to eliminate any moisture. Afterwards, 5 ml of unstimulated saliva samples were collected from each patient in each vial using the spitting method, and the vials were kept in a freezer.

The collected samples were analyzed within 1 week of their collection. The samples were centrifuged at 8000 rpm. Next, 1 ml of the supernatant was collected and transferred to 2 ml microtubes; 50 μ l of 20% nitric acid and 50 μ l of 1% Triton X-100 were added; the samples were centrifuged again after 60 min in order to deposit proteins. The clear supernatant was transferred to another microtube [30]. The concentrations of nickel and chromium ions were then measured using inductively coupled plasma mass spectrometry (ICP-MS, AA280Z GTA 120; Varian, Mulgrave, Australia) and tabulated. The average ion concentration was recorded in parts per billion (ppb, ng/ml).

Descriptive statistics and 95% confidence intervals (CI) were calculated for ion levels. Data normality was assessed and confirmed using the D'Agostino and Pearson omnibus normality test. Ion levels were compared using the independent samples *t* test. The level of significance was predetermined as 0.05.

Results

This study was performed on 40 patients under fixed orthodontic treatment including 20 males (10 in each group) and 20 females (10 per group) with a mean age of 34.6 ± 3.6 years (range 25 to 42 years). None of the patients had any systemic disease or history of medication intake or smoking and had no dental metal restoration.

The salivary level of nickel was 156 ppb or 85% higher in the test group than that in the control group. The *t* test showed a significant difference ($P = 0.0118$) between nickel values of orthodontic patients with and without periodontitis (Tables 1 and 2). However, the salivary concentration of chromium was only 1.1 ppb or 17.3% higher in the test group compared to that in the control group, and this difference between chromium levels of the groups was not significant ($P = 0.2214$, Tables 1 and 2).

Table 1 Salivary levels of nickel and chromium ions in all patients (ng/ml)

Patient	Ni		Cr	
	Control	Periodontitis	Control	Periodontitis
1	88	60	8	5
2	2	282	7	10
3	242	220	4	6
4	200	608	11	5
5	258	670	11	9
6	180	237	8	14
7	413	118	6	8
8	10	181	9	4
9	140	350	4	10
10	151	311	7	7
11	164	797	7	4
12	195	109	7	5
13	179	135	6	6
14	275	626	4	12
15	364	49	4	6
16	145	309	5	10
17	55	613	8	4
18	206	70	5	7
19	20	595	3	4
20	368	423	3	13

Discussion

This study showed that the salivary level of nickel but not chromium could be higher in orthodontic patients with periodontal disease compared to that in orthodontic patients with healthy periodontal tissues. No previous study has assessed metal ion release from orthodontic appliances in patients suffering from periodontitis. Increased salivary level of metal ions in periodontal patients under orthodontic treatment can be explained as follows: Lower pH decreases the resistance of alloys to corrosion and enhances the release of metal ions from orthodontic appliances, such that pH reduction from 6.75 to 3.5 can increase the ion release by 100-folds [6, 27, 31]. Although this study did not directly assess the pH, numerous studies have demonstrated that periodontal patients have a lower saliva pH than healthy individuals [26, 32–36]. Huang et al. [37] showed that ion release from metal brackets increases in acidic solutions compared to neutral pH, and the process of ion release accelerates over time [37]. Kuhta et al. [27] reported that acidity has a direct correlation with ion release from orthodontic appliances. Moreover, Takahashi et al. [32, 33] showed growth and proliferation of *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* at acidic pH levels. These microorganisms together with their enzymatic activity and their products might have as well contributed to the increased level of nickel observed in the periodontitis group of this study. In the current study, we standardized the two groups with respect to the aforementioned parameters in order to eliminate or minimize the effects of confounders on the results. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are the major culprits responsible for chronic periodontitis, which destruct the periodontal tissue and cause bone loss [26, 38]. Efficient plaque control accompanied by mechanical plaque and calculus removal can decrease the signs/symptoms of inflammation. Thus, periodontal patients in our study underwent scaling and root planning and received oral hygiene instructions prior to fixed orthodontic treatment in order to improve the orthodontic treatment results. However, this could reduce the inflammation and perhaps reduce the pH contrast between the groups. Nevertheless, saliva was collected 2 months after the beginning of orthodontic treatment; in the presence of orthodontic appliances, this time period is probably sufficient for re-accumulation and colonization of microorganisms resulting in increased inflammation in both groups [18, 39–42]. Overbrushing and overuse of mouthwashes and toothpastes, due to great emphasis placed on oral hygiene by orthodontists, can be other reasons for the significant increase observed in salivary level of nickel ions in periodontal patients under orthodontic treatment compared to healthy controls, because such chemical solutions might increase metal ion release through reducing the pH and mechanical disruption of the chromium oxide film by brushing can

Table 2 Descriptive statistics for ion levels (ng/ml) and the results of the D'Agostino and Pearson omnibus normality test

Ion	Group	Mean	SD	Min	Q1	Med	Q3	Max	95% CI	<i>P</i>	
Ni	Control	182.8	116.5	2	101	179.5	254	413	128.2	237.3	0.840
	Periodontitis	338.2	235.5	49	122.3	295.5	604.8	797	227.9	448.4	0.212
Cr	Control	6.35	2.39	3	4	6.5	8	11	5.231	7.469	0.631
	Periodontitis	7.45	3.15	4	5	6.5	10	14	5.974	8.926	0.317

SD, standard deviation; *Min*, minimum; *Q1*, 25th percentile; *Med*, median; *Q3*, 75th percentile; *Max*, maximum; *CI*, confidence interval for the mean; *Ni*, nickel; *Cr*, chromium

expose the alloy to the corrosion [43, 44]. Also, increased saliva flow in periodontal patients [45] could be another explanation for increasing ion release in periodontal patients.

Most previous studies [4, 13, 15, 21, 46–49] showed that salivary nickel and chromium concentrations in orthodontic patients are very low compared to the normal doses already taken daily through diet (100 to 800 µg for nickel and 50 to 280 µg for chromium) [6, 48, 49]. However, this study found high concentrations especially for nickel. This was in line with a few other studies which reported considerably higher concentrations [47, 50]. Various factors can contribute to the controversy, such as differences in sampling methods (such as stimulated versus non-stimulated, or timing of sampling), the extent of galvanic currents, bacterial colonization, salivary compositions, health status (i.e., affliction with systemic diseases such as diabetes mellitus, or smoking), or diet [4, 21, 48, 50–53]. Noting that about 1.5 l of saliva might be swallowed daily [54, 55], it seems that if the concentrations of salivary metal ions were hypothetically constant throughout the day, the average levels observed in this study could sum up to doses about 274 µg and 9.5 µg daily, respectively, for nickel and chromium in orthodontic patients without periodontitis and doses about 507 µg and 11.2 µg daily, respectively, for nickel and chromium in orthodontic patients with periodontitis. Although the averages were still below toxic doses, nickel doses in some periodontitis patients might reach toxic levels; this might be of clinical significance (in terms of the biocompatibility and toxicity of fixed orthodontic treatment in periodontal patients) if verified in future larger studies. Such elevated concentrations might also worsen the other adverse effects of these ions attributed to chronic release of them, even if at a low dose, namely DNA damage, inflammation, and changes in cellular morphology or metabolism [3, 4, 21]. It is not known yet if metal ion release is constant or changes throughout the day, but it is suggested to peak when the pH drops, e.g., after the meal [3, 4, 21, 49]. However, the main health concerns with these highly sensitizing trace elements might be hypersensitivity and periodontal damage (which can be of a greater concern to patients already having periodontitis) [4, 50, 52, 56–58]. These issues especially the periodontal damage happened in some cases can have clinical implications in terms of biocompatibility of orthodontic appliances,

especially for patients already suffering from periodontitis, in whom an additional periodontal damage could be even more undesirable.

There were some limitations in this study, most of which were common to all earlier in vivo research and discussed extensively in some of them [4, 8, 20]; these include the lack of total control over many variables in the complex oral environment and the probably complicated pattern of metal ion release that cannot be reflected by a single and brief period of sampling [3, 4, 8, 20, 48]. Another limitation of this pilot study was the lack of sample size calculations which might have contributed to the lack of a significant contrast between the chromium levels of both groups; also, measuring the baseline concentrations was out of the budget for this pilot study. Due to budget constraints of this preliminary study, it was not possible to measure baseline ion levels in this retrospective cohort design. Future studies should adopt more comprehensive setups such as clinical trials and measure baseline values as well (in larger samples) in order to be able to also assess the changes in each group over time. Another limitation was the lack of assessment of salivary pH in this pilot research. We only *assumed* (based on previous studies) that the cohort group with periodontitis would have more acidic pH levels. However, the pH should have been measured directly, and the correlations between the pH and metal ion leach should have been established. Furthermore, future studies are recommended to assess the correlations between pH with the salivary level of ions in periodontal patients under orthodontic treatment. It should also be researched whether rinsing with alkaline mouthwashes can aid periodontal patients under orthodontic treatment in terms of reducing their relatively high nickel release.

Conclusions

Within the limitations of this preliminary study, it was found for the first time that salivary nickel levels would be considerably higher in periodontal patients under 2 months of orthodontic treatment compared to orthodontic patients with healthy gingivae. Results pertaining to chromium values were inconclusive. Given the significance of biocompatibility of

orthodontic appliances in periodontal patients, future studies with more comprehensive designs and larger samples are warranted to assess this further.

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Conflict of Interest The authors declare that they have no conflict of interest.

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