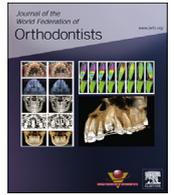


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Research Article

Long-term evaluation of metal ion release in orthodontic patients using fluoridated oral hygiene agents: An in vivo study

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ABSTRACT

Objective: To investigate levels of nickel and chromium in gingival crevicular fluid from subjects undergoing orthodontic treatment with and without using fluoridated toothpaste and mouthwash over a 6-month period.

Materials and methods: Sixty subjects divided into two groups of 30 each served as the experimental group with and without using fluoride agents for oral hygiene care along with fixed appliance treatment. Gingival crevicular fluid was collected in fluoridated and nonfluoridated groups before, and at 7 days, 30 days, and 6 months after commencing orthodontic treatment. Levels of nickel, chromium, titanium, and manganese were evaluated using inductively coupled plasma mass spectrometry.

Results: No changes in metal levels at any time were observed in the untreated control group. Nickel levels were elevated to 101.78 µg/L in the fluoridated group at 30 days posttreatment, indicating heavy leaching from nickel-titanium archwires (Friedman $P < 0.001$). Results returned to baseline in both groups at 6 months. Mean differences for chromium, titanium, and manganese ion release were not statistically significant at 30 days. Titanium showed elevated levels at 30 days in both tested groups, but levels were lesser than nickel.

Conclusions: Archwires are susceptible to corrosion when exposed to fluoride agents during orthodontic therapy causing release of metal ions into the mouth with the possibility of systemic absorption. Long-term consequences of metal ion release into the oral cavity require further investigation.

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1. Introduction

Release of metallic ions, notably nickel and chromium, present in orthodontic fixed appliance attachments like brackets and arch wires has been a cause of concern in recent years [1–3]. Although metals used in the mouth have good biocompatibility and corrosion resistance, they sometimes lose their stable surface oxide layer, leading to leaching of metallic ions when exposed to the intraoral

environment causing allergies, hypersensitivity, and alterations in cellular morphology and characteristics [4–7]. Fluorides prescribed for use during orthodontic treatment have a deleterious effect of promoting leaching of ions by creating an acidic environment [8,9]. Studies comparing in vitro and in vivo orthodontic appliance metal ion leaching have shown variable results. Appreciable increase in amounts of metal ions released in vitro [10–13] and in vivo [14–16] contrast with other in vivo findings [17], and can be attributed to the different experimental methodologies followed. Association between acidic fluoride agents (mouthwashes and toothpastes), used during treatment to minimize tooth decay and white spots, on reduced corrosion potential of orthodontic appliances has been implicated as a possible causative factor in release of metal ions during use of fixed appliances [18,19].

The systemic absorption of leached metal ions usually can be definitively measured using available biomarkers that include blood, hair, urine, saliva, and gingival crevicular fluid (GCF). In orthodontics, analysis of GCF, a biological exudate, could be very useful, as it is very closely related to the cascade of events in the

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Table 1
Nickel release level at different time periods of the study

Group	Time	n	Mean	SD	Min	Max	χ^2	P	Sig. Diff	P
Non-Fl	BF	30	0.49	0.08	0.3	0.6	1.960	0.58		
	7 d	30	0.52	0.09	0.4	0.8				
	30 d	30	13.42	39.42	0.3	134.1				
	6 mo	30	0.51	0.09	0.3	0.7				
Fl	BF	30	0.51	0.09	0.3	0.7	21.320	<0.001 ^a	30 d vs. BF	<0.001 ^a
	7 d	30	0.52	0.09	0.4	0.7				
	30 d	30	101.78	81.20	0.3	206.1				
	6 mo	30	0.51	0.11	0.4	0.8				

BF, before treatment; Fl, fluoride; Max, maximum; Min, minimum; SD, standard deviation; Sig. Diff, significant difference.

^a Denotes statistically significant change.

inflammatory response that causes tooth movement [20]. It is currently being used as a systemic biomarker for periodontal and bone health status due to the presence of several biochemical and cellular elements. The challenge, as far as GCF studies are concerned, is in obtaining enough quantities for analysis. The method of collection also is difficult and time-consuming, with contamination of the samples with blood occurring frequently. GCF can be collected using a variety of techniques, namely use of filter paper strips, gingival washings, or micropipettes [21,22]. Considering the evidence regarding release of metals into the oral cavity during orthodontic treatment [23,24], the present in vivo research was aimed at evaluating amounts of four metal ion constituents found in nickel-titanium archwires, namely nickel, chromium, titanium, and manganese, at definite time periods until 6 months after commencing orthodontic treatment in patients with and without exposure to fluoride prophylactic agents.

2. Material and methods

Sixty subjects, with equal male-to-female distribution, who required fixed appliance treatment were selected and divided into two groups of 30 each and bonded with 0.022 × 0.028-inch slot MBT stainless steel brackets (Mini Twin; Ormco, Glendora, CA) in both arches. The sample size was finalized using G Power software after performing a power analysis. Subjects were recruited with inclusion criteria of requiring nonextraction therapy with all teeth present until second molars without restorations. Patients on medication, who smoked, who consumed alcohol, or who had previous orthodontic treatment were excluded from the study. The institute ethical board approved the study design and written consent was obtained from participants before beginning treatment. Brackets were bonded using light-cured composite resin (Enlight; Ormco), and only nickel-titanium (NiTi) archwires (0.014-inch, 0.016-inch, and 0.016 × 0.022-inch; Tru-Arch Align NiTi; Ormco) were sequentially used to eliminate confounders. Each wire was used for 2 months before replacement with the next. All subjects were taught oral hygiene maintenance and brushed twice daily during the study period. Group 1 patients (n = 30) had and

used only a nonfluoridated toothpaste (Dabur Red; Dabur India Ltd, Ghaziabad, India), whereas group 2 patients were prescribed with fluoridated toothpaste (Colgate Strong Teeth, 1000 ppm fluoride; Colgate Palmolive Co, Mumbai, India) and a mouthwash (Colgate Plax, 225 ppm fluoride; Colgate Palmolive Co.) during the 6-month study period. Subjects were informed not to consume nickel- or chromium-containing foods (printed list of foods to be avoided given to all participants) 3 days before obtaining samples of GCF for metal ion analysis.

2.1. Method of GCF collection and analysis

Samples of GCF were obtained from both the groups before appliance placement, at 1 week, 1 month, and 6 months after appliance placement. GCF was obtained only in the morning for all participants who did not eat or brush before sampling. The maxillary canine region was isolated with cotton, the sulcus area was gently dried using a stream of air, and GCF was collected in a calibrated micropipette (5- μ L capacity, graduated; Sigma-Aldrich Co., St. Louis, MO) placed gently in the gingival sulcus. If contamination with blood was evident, GCF was collected from the opposite side tooth using a new pipette. The tip of the micropipette was gently inserted in the gingival sulcus and enough time was given for the GCF to flow (3 to 5 μ L/3 to 20 minutes). This prolonged time could be primarily because GCF flow rate and quantity is generally lesser in healthy subjects. Once GCF was obtained, the contents were immediately transferred to an Eppendorf tube already containing 2 mL of phosphate buffered saline with a pH of 7.4 (Sigma-Aldrich) and were stored in a refrigerator at -15°C until analysis. Preparation of the GCF samples involved use of concentrated HNO_3 because of its oxidizing nature. Adding HNO_3 converts metal ions into their nitrate salts, which are highly soluble. It also promotes sample digestion, enabling destruction of the organic matrix before introducing the samples to heated plasma, which otherwise would interfere in the analysis.

Samples of GCF were analyzed for four metal ions, namely nickel, chromium, titanium, and manganese with inductively coupled plasma mass spectrometry (Agilent Technologies, Tokyo, Japan) with an octupole reaction system and integrated

Table 2
Titanium release level at different time periods of the study

Group	Time	n	Mean	SD	Min	Max	χ^2	P	Sig. Diff	P
Non-Fl	BF	30	0.49	0.10	0.4	0.8	7.080	0.07		
	7 d	30	0.51	0.11	0.3	0.7				
	30 d	30	40.09	62.30	0.3	157.4				
	6 mo	30	4.80	23.65	0.3	130.0				
Fl	BF	30	0.51	0.09	0.3	0.8	10.920	0.01 ^a	30 d vs. BF	0.001 ^a
	7 d	30	0.50	0.10	0.3	0.8				
	30 d	30	64.69	76.08	0.3	180.0				
	6 mo	30	0.51	0.10	0.3	0.7				

BF, before treatment; Fl, fluoride; Max, maximum; Min, minimum; SD, standard deviation; Sig. Diff, significant difference.

^a Denotes statistically significant change.

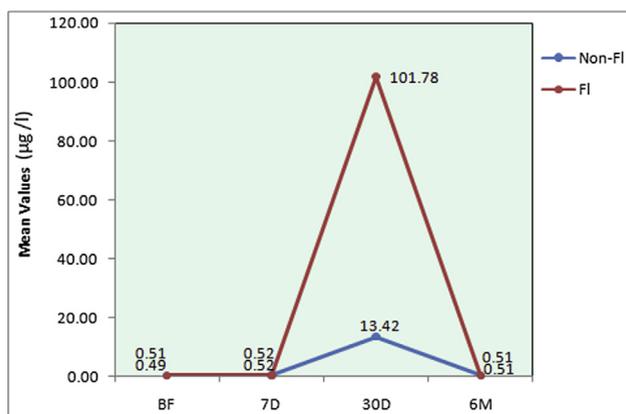


Fig. 1. Release of nickel ions during 6 months of orthodontic treatment. Note the peak levels in GCF observed 30 days after commencing treatment.

autosampler. The octopole sets voltages and frequencies to allow ions of a given mass-to-charge ratio to remain stable and pass through the detector. Ions with different mass-to-charge ratios are unstable and are ejected. The machine also had a very high detection limit up to parts per trillion, ensuring detection and accurate calculation of extremely minute quantities of metals. Inductively coupled plasma mass spectrometry also permits detection of multiple metals rapidly in a single sample unlike atomic absorption spectrometry. Preset detection standards are available for tested metals with the laboratory. Samples of GCF were introduced with a Babington PEEK nebulizer with a double-pass spray chamber that was cooled to 2°C with water for stabilizing the temperature and reducing water vapor.

2.2. Statistical methods

Statistical Package for the Social Sciences (SPSS v.22.0; IBM Corp, Armonk, NY) for Windows was used for data analysis. Descriptive and inferential statistics were used in the study. Descriptive statistics was used for the expression of metal ion release in terms of means and standard deviation. Inferential statistics included the Mann-Whitney *U* test comparing the mean ion release of various metals between two groups at different time intervals. Friedman's test and Wilcoxon signed rank test as post hoc analysis was used for comparing the mean ion release of various metals at different time intervals in both groups. $P < 0.05$ was determined as being significant.

3. Results

The levels of nickel and titanium release from the two groups at different time periods of evaluation is provided as Tables 1 and 2,

Table 3
Metal ion release levels at 30 days and 6 months after treatment

Metal ions	Group	n	Post treatment - 30 d		Mean Diff	Z	P	Post treatment - 6 mo		Mean Diff	Z	P
			Mean	SD				Mean	SD			
Ni	Non-Fl	30	13.42	39.42	-88.36	-4.154	<0.001 ^a	0.51	0.09	0.00	-0.577	0.56
	Fl	30	101.78	81.20				0.51	0.11			
Cr	Non-Fl	30	0.50	0.10	-11.51	-0.414	0.68	0.49	0.07	-0.03	-1.094	0.27
	Fl	30	12.00	35.27				0.53	0.11			
Ti	Non-Fl	30	40.09	62.30	-24.61	-1.072	0.28	4.80	23.65	4.30	-0.606	0.54
	Fl	30	64.69	76.08				0.51	0.10			
Mn	Non-Fl	30	0.50	0.11	0.03	-1.257	0.21	0.49	0.09	0.02	-0.517	0.61
	Fl	30	0.47	0.12				0.48	0.10			

Cr, chromium; Fl, fluoride; Mean Diff, mean difference; Mn, manganese; Ni, nickel; SD, standard deviation; Ti, titanium.

^a Denotes statistically significant change.

respectively. No significant increase in level of any metal ions was observed when fluoridated or nonfluoridated groups were compared before treatment and at 7 days posttreatment. Significant increase in levels of nickel ions released 30 days after treatment commencement in the fluoridated group was evident (Table 1, Fig. 1). The Mann-Whitney test comparing nickel ion release in the two groups after 30 days of treatment revealed significantly lower (13.42 ± 39.42 µg/L) values in the nonfluoridated group compared with the fluoridated group (101.7842 ± 81.20 µg/L) at $P < 0.001$ (Table 3).

Mean chromium ion release was lower (0.50 ± 0.10 µg/L) in the nonfluoridated group as compared with the fluoridated group (12.00 ± 35.27 µg/L). Similarly, mean titanium and manganese release was lower in the nonfluoridated groups (40.09 ± 62.30 µg/L, 0.50 ± 0.11 µg/L) as compared with the fluoridated groups (64.69 ± 76.08 µg/L, 0.47 ± 0.12 µg/L). Even though lower, the mean differences for chromium, titanium, and manganese ion release were not statistically significant (Table 3).

Testing of GCF samples 6 months into treatment revealed no increase in levels of any of the four metals tested (Table 3). Mean nickel ion release in the nonfluoridated group (0.51 ± 0.09 µg/L) and fluoridated group (0.51 ± 0.11 µg/L) was similar. There were no significant increases in any other metals tested (Table 1). Friedman test revealed maximum nickel release in both groups at 30 days posttreatment, which was further substantiated with the Wilcoxon signed rank post hoc test (Tables 1 and 3). Nickel levels were higher in the fluoridated group. Titanium levels also showed increases in both groups (Fig. 2), as can be seen in Tables 2 and 3. The standard deviation showed a higher value than the mean for titanium in the nonfluoridated group.

4. Discussion

The present study was aimed at analyzing possible differences in levels of four metal ions (nickel, titanium, chromium, and manganese) in GCF at four specified time periods in two subject groups, one using nonfluoridated toothpaste and the other exposed to fluoridated mouthwashes and toothpaste. The results showed that there was an appreciable increase in nickel and titanium levels at 30 days into treatment with orthodontic appliances as compared with baseline. In recent years, evaluation of urine, blood, saliva, and hair gave information on systemic absorption of these metal ions [17,25]. However, examination of GCF was found to be more relevant in the orthodontic context, as it is already being used as a systemic biomarker of tooth movement as well as periodontal disease studies [20].

The influence of fluoride ions used during orthodontic treatment to reduce decalcification and white spot lesions on corrosion is well known [26–28]. This study could observe significant increase in nickel levels in GCF at 30 days after treatment in subjects

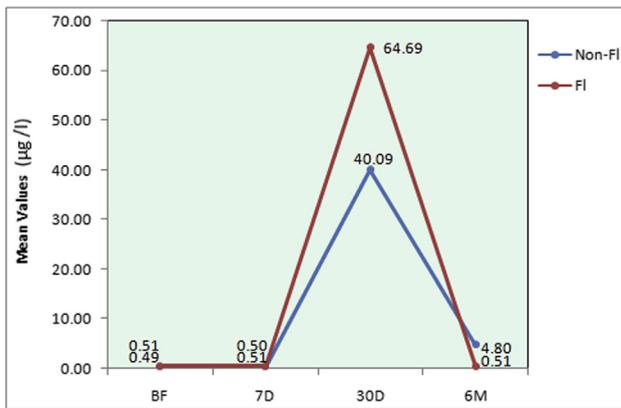


Fig. 2. Release of titanium ions during 6 months of orthodontic treatment. Note the peak levels in GCF observed 30 days after commencing treatment in both fluoride- and non-fluoride-using groups.

using fluoridated mouthwash and toothpaste in comparison with those who were not using it (Fig. 1). An increase was observed in other metal ions also, such as chromium, titanium, and manganese in group 2 (fluoride-using patients) in comparison with group 1 subjects at 30 days of treatment, even though in a statistically insignificant manner. This points to the need of monitoring metal ion release levels at very short intervals to make sure that it never reaches the toxic state. Once released, elimination of these ions could take place through urine, hair, and saliva after systemic absorption, bringing down the levels in GCF at later time periods but creating an alarming situation. These findings were in concordance with that of Petoumenou et al. [16], who could report increase in salivary metal ion levels immediately after archwire placement. The only difference is that we could observe the elevation only after 30 days, with no significant increase at 7 days.

Titanium levels also showed an increase at day 30, but in an insignificant manner statistically (Fig. 2). There was no increase of metal ions in the fluoride-using group at either 7 days or 6 months after beginning treatment in comparison with those who were not using fluoridated agents. The study clearly showed evidence of the corrosive nature of fluoride ions, as release of nickel and titanium showed an increase of greater magnitude in fluoridated groups at 30 days. However, release of metal ions reduces after the 30-day period to baseline levels when tested at 6 months. The effect of fluoride on release of chromium or manganese ions seems to be very less as evidenced by the data gathered. Chromium shows minimal increase in levels at 30 days in the fluoridated group in comparison with the nonfluoridated group, but the result is not significant statistically. Manganese always showed no change in all groups. The possible reason might be the use of AISI type 304L stainless steel, which contains 18% to 20% chromium, 8% to 10% nickel, and minute amounts of manganese and silicon for orthodontic bracket manufacture [29].

The deleterious consequences of fluoride agents on arch wires and brackets causing dangerous leaching of metals into the oral cavity with possible long-term systemic consequences also requires detailed investigation. It is known that nontoxic levels of metals like nickel can cause changes in DNA or inhibit DNA-restoring enzymes [30–32], which could have adverse biological effects in the long term. The effect of an increase in levels of nickel, a known carcinogen, and titanium on oral mucosal cells needs further investigation. Although all tested metals showed nontoxic levels in GCF, long-term evaluation is recommended to better understand exposure effects.

The study had a few limiting factors. It was impossible to verify if GCF was contaminated with plaque before sample collection.

Microorganisms in plaque can absorb and accumulate metals like nickel [33,34] and can show increased metal ion concentrations of GCF. In addition, the flow of GCF, influenced by inflammation associated with orthodontic tooth movement, might show variability in its metal ion presence as well as concentration. The present study collected samples at four time periods, but a continuous monitoring system, as suggested by Eliades et al. [30], might be a better design for proper evaluation. Again, GCF samples were obtained only from the maxillary canine regions, while obtaining samples from more teeth might give more accurate and valid results. This was, however, beyond the scope of the study. Last, even though participants were instructed to avoid nickel- and chromium-rich foods a few days before sample collection, it was impossible to verify.

5. Conclusions

The present study, which evaluated metal ion release in GCF during 6 months of fixed appliance treatment with and without fluoride oral prophylactic agents could demonstrate the following:

1. Presence of metal ions, such as nickel, titanium, chromium, and manganese, in GCF at all time periods from patients undergoing fixed appliance treatment, irrespective of whether they use fluoride oral prophylactic agents.
2. Use of fluoride toothpaste and mouthwashes increased the release of these metal ions in comparison with the non-fluoride-using group, with statistically significant increase only for nickel release.
3. A peak in nickel ion release was observed at 30 days after appliance placement in the fluoridated group, which reduced to baseline by 6 months.
4. Titanium was released to its peak level at 30 days, but in a statistically insignificant level compared with the non-fluoride-using group.
5. Fluoride showed less impact on release of chromium and manganese levels.
6. The study showed release of metal ions to the oral cavity points to further long-term investigation in view of possible systemic absorption of these elements and its aftereffects.

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