

Effects of Fixed Orthodontic Treatment on Hair Nickel and Chromium Levels: A 6-Month Prospective Preliminary Study

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Abstract Although nickel and chromium are known as allergen and cytotoxic orthodontic metals, very few and controversial studies have assessed the effect of orthodontic treatment on their *systemic* levels reflected by their best biomarker of exposure, hair. This prospective preliminary study was conducted to evaluate hair nickel and chromium levels in fixed orthodontic patients. Scalp hair nickel/chromium concentrations of 12 female and 12 male fixed orthodontic patients were measured before treatment and 6 months later, using atomic absorption spectrophotometry. The effects of treatment, gender, and age on hair ions were analyzed statistically ($\alpha=0.05$). The patients' mean age was 18.38 ± 3.98 years. The mean nickel levels were 0.1380 ± 0.0570 and 0.6715 ± 0.1785 $\mu\text{g/g}$ dry hair mass, respectively, in the baseline and sixth month of treatment. Chromium concentrations were 0.1455 ± 0.0769 and 0.1683 ± 0.0707 $\mu\text{g/g}$ dry hair mass, respectively. After 6 months, nickel increased for 387 % (paired *t* test $P=0.0000$) and chromium increased for 16 % ($P=0.0002$). No significant correlations were observed between any ion levels with age or gender (Spearman $P>0.2$). Within the limitations of this preliminary study, it seems that 6 months of fixed orthodontic treatment might increase levels of hair nickel and chromium. Future larger studies are necessary to validate these results.

Keywords Atomic absorption spectrophotometry · Orthodontic treatment · Scalp hair · Systemic nickel and chromium concentrations

Introduction

Orthodontic alloys are made from various metals, among which chromium and nickel are of major concern [1–8]. Both of these genotoxic, mutagenic, and cytotoxic metals might induce contact allergy, asthma, hypersensitivity, birth defects, and reproductive damage [1–7, 9–16]. Corrosion of orthodontic alloys might lead to release of considerable amounts of nickel and chromium ions into saliva [1, 5–7, 9, 11, 13].

The systemic absorption of such metals is an extra health risk [9–11, 17, 18]. Despite its importance, systemic nickel changes during orthodontic treatment have been assessed merely in a few controversial studies [6, 15, 19–25] showing systemic increases [6, 19, 21] or lack of any significant changes [15, 20, 22–24]. Systemic chromium changes are assessed in even fewer researches [15, 24, 25]. Moreover, many of these few studies have assessed only the systemic ion levels in response to acute exposures [6, 15, 21, 22]. Measurement of systemic exposure to a substance can be performed using biomarkers of exposure, of which hair, nail, blood, and urine are the most accessible ones [21]. Biomarkers of exposure such as urine and blood can show only acute exposures to metals. Besides, serum metal ions cannot necessarily reflect blood concentrations. They are also highly confounded by the metabolic pathways of ion removal [5, 21, 24, 26].

Hair can show both short-term and chronic exposures to metals, is correlated with blood ion levels, and is free of confounding effects of metabolic mechanisms [19, 25–27]. These as well as other factors such as the high ion concentration in hair (50 to 100 times more than the blood, urine, and nails) or stability and convenience of sample storage/transport

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make hair one of the best biological biomarkers of exposure to metals, according to the US Environmental Protection Agency and the International Atomic Energy Agency [19, 25–27]. Nevertheless, the literature on the role of orthodontic therapy on hair ion levels is scarce.

In view of the mentioned shortcomings and controversies, we aimed to evaluate comparatively the hair nickel and chromium concentrations in fixed orthodontic patients in a prospective design.

Materials and Methods

This prospective preliminary study was performed on 48 hair specimens sampled from 24 orthodontic patients (12 males, 12 females) at two time points: baseline (immediately before treatment) and 6 months later. The sample size was predetermined based on a pilot study and power calculations. The protocol ethics were approved by the research committee of the university (as the proposal # 80/t 167), and written consents were taken from the subjects or their parents after thorough verbal and written explanation. The subjects were sequentially acquired until two groups of 12 male and 12 female patients were included.

The patients were selected from attendees to the Orthodontics Department of the Azad University during the years 2013–2014. The inclusion criteria comprised the subjects' willingness to participate, the indication for bimaxillary non-ext fixed orthodontic treatment, the age range of 11 to 26 years old, having all the permanent teeth fully erupted (no semi-eruptions, no missing, or extraction) excluding the third molars, the absence of any systemic diseases, any history of allergic reactions, medication intake, alcohol consumption or smoking, the absence of any caries [15], any metal restorations such as amalgam fillings or fixed prostheses placed before or during the treatment, any hair colors or hairdressings, as well as no history of previous orthodontic treatment of any kind. All the inclusion criteria needed to be met during the study period [7].

Uniform Treatment Protocols

Bonded stainless steel conventional MBT 22 brackets (Dentaurum, Germany) with 0.022×0.028 " bracket slots, on all teeth except the molars, as well as four conventional orthodontic bands (Fairfield Orthodontics, USA) were used for both arches. The used archwires were nickel titanium (NiTi) (G&H, USA) and stainless steel (SS) archwires (G&H, USA) in both jaws. The order of the used wires was as follows— 0.016 " round NiTi, 0.018 " round NiTi, 0.019×0.025 " rectangular NiTi, and finally, 0.019×0.025 " rectangular SS wires. The wire replacement intervals were about 4 to 6 weeks for each wire, depending on the treatment plan.

Hair Sampling

The sampling was performed twice, once immediately before the treatment and once after 6 months. In the scheduled sessions, 5–6 cm of hair (which consists of hair grown during about the past 5 to 6 months [28]) was cut off from the occipital region of the head in the neck proximity, using a stainless steel surgical blade. The hair samples were put in paper envelopes [25, 27, 29].

Ion Level Measurement

The hair specimens were shipped to the Central Chemical Analysis Laboratory of the Tehran University of Medical Sciences for atomic absorption spectrophotometry using a calibrated device (high-resolution continuum source AAS Contra (AA700, Analytik Jena, Germany)). The detection limits were 0.5 ppb ($0.0005 \mu\text{g/g}$ dry hair mass) for chromium and 1 ppb ($0.001 \mu\text{g/g}$ dry hair mass) for nickel. Characteristic concentrations were 0.2 ppb ($0.0002 \mu\text{g/g}$) for chromium and 0.55 ppb for nickel ($0.00055 \mu\text{g/g}$).

Hair pollutions, debris, and proteins were removed by the application of Triton X100 surfactant diluted 1:200 for 10 min. Then it was cleansed with metal-free acetone (10 min) and finally rinsed thrice using a deionized water producer (Direct-Q 3 UV Water Purification System, Millipore, France) [5, 28]. The specimens were then dried in an oven (Binder heating, Germany) at 80°C for 6 h [30]. From each specimen, 0.5 mg was immersed in 2.5 mL of 65 % nitric acid and 1.25 mL of 30 % hydrogen peroxide for 12 h at room temperature. Then the solution and immersed hair sample was stored for about 2 or 3 h in an oven (microwave oven, Merck, Germany) at 80°C , in order to remove the organic content [28]. The resulted transparent solution was diluted by adding 25 mg of deionized water (Millipore).

Each hair specimen was examined thrice, and the average ion concentration was recorded in parts per million or micrograms per gram of dry hair mass. These procedures were repeated for the sixth month of treatment. The *r*-squared values were 0.999 for chromium and 0.998 for nickel, indicating the excellent precision of the spectrophotometer.

Statistical Analysis

The sample size was calculated based on a pilot study of 5×2 patients to obtain test powers >0.9 . The pilot sample was not included in the study sample. The difference between the average ages was evaluated using an independent-samples *t* test. The associations between the ion values at both intervals as well as the correlations between age and the released ion values at each session was assessed using a Spearman correlation coefficient. Metal ion data were analyzed using a paired-samples *t* test of the SPSS statistical package (version

20, IBM, USA). The 95 % confidence intervals (CI) for the means and differences were computed as well. The differences between the two time points were calculated for nickel and chromium. The nickel and chromium levels and their changes were evaluated in males and females, separately. The gender-dependent difference between the time-dependent changes of nickel and chromium were assessed using an independent-samples *t* test and a Mann–Whitney *U* test, respectively. The level of significance was set at 0.05.

Results

The patients' mean age was 18.38 ± 3.98 years (18.92 ± 4.27 years in males and 17.83 ± 3.76 years in females). The average ages were similar between the two genders, according to the *t* test ($P=0.517$). There was a significant positive correlation between the chromium ion amounts measured at both time points ($\rho=0.908$, $P=0.000$) but not between nickel levels at the two time points ($\rho=-0.081$, $P=0.706$). There were no correlations between nickel and chromium levels at the baseline ($\rho=-0.020$, $P=0.924$) or 6 months later ($\rho=-0.36$, $P=0.084$). There were no significant associations between patients' age and any of the ion concentrations at any of the two intervals (all four Spearman *P* values >0.32). There was no significant correlation as well between the patients' gender with any of the ions measured at any of the sessions (all four point-biserial correlation *P* values >0.22).

After 6 months, nickel increased for $0.53 \mu\text{g/g}$ ($P=0.0000$, paired *t* test) and chromium increased for $0.02 \mu\text{g/g}$ ($P=0.0002$, paired *t* test, Table 1). These values accounted for about fourfold (387 %) increase in the nickel value and about 16 % increase in the chromium value (Fig. 1, Table 1).

Nickel increased to $0.5509 \pm 0.1902 \mu\text{g/g}$ after 6 months of treatment in females. In males, it increased to $0.5161 \pm 0.2011 \mu\text{g/g}$ after 6 months (Table 2). The difference between the increases of nickel in men versus the increase in women was not significant ($P=0.667$, independent-samples *t* test).

Chromium increases were 0.0212 ± 0.0203 and $0.0244 \pm 0.0302 \mu\text{g/g}$ in females and males, respectively (Table 2). The difference between the alterations of chromium in men versus women was not significant, according to the Mann–Whitney *U* test ($P=0.686$).

Discussion

The baseline values in this study were similar to previous studies on hair nickel and chromium [24, 25] or hair nickel [19, 23]. All the baseline or control nickel values have been higher than chromium values. The differences observed in this study were consistent with a prospective study (merely on nickel) [19] which found a significant increase. However, they were greater than the retrospective studies on hair nickel [23] and hair nickel/chromium [20, 24], all of which reported non-significant increases. Levrini et al. [23] compared hair nickel levels in two groups of orthodontic patients and matched controls. They found a significantly lower level of hair nickel in the control group ($0.50 \text{ mg/g}=500 \mu\text{g/g}$) compared with the orthodontic group ($0.64 \text{ mg/g}=640 \mu\text{g/g}$) [23]. Their reported ion levels were much greater than all the values reported in the literature [23]. Abtahi et al. [19] conducted a clinical trial to assess the effect of 4 months of orthodontic treatment on hair nickel. In their orthodontic group, nickel increased from a baseline value of 0.350 to $0.673 \mu\text{g/g}$ while, in their control group of those who were not under orthodontic treatment, nickel increased from 0.245 to $0.382 \mu\text{g/g}$. The nickel levels were not significantly different at the baseline but became significantly different after 4 months of treatment, being greater in the orthodontic group [19]. Also, their orthodontic patients showed a significant increase of about 190 % after 4 months of treatment, similar to our findings [19]. One interesting finding was that their non-orthodontic group as well showed a significant 156 % increase of hair nickel over time [19]. Mikulewicz et al. in 2011 [25] showed that hair nickel and chromium levels were 0.3642 and $0.1298 \mu\text{g/g}$, respectively, in individuals not being treated with orthodontic appliances. In their experimental group

Table 1 Descriptive statistics for the ion levels and their differences (all in micrograms per gram dry hair mass)

Ion	Time	Mean	SD	CV (%)	Min	Q1	Med	Q3	Max	95 % CI
Ni	Baseline	0.1380	0.0570	41.3	0.063	0.0885	0.1185	0.1925	0.240	0.1139 0.1620
	Sixth month	0.6715	0.1785	26.6	0.408	0.5333	0.5980	0.8788	0.925	0.5961 0.7468
	Difference	0.5346	0.1928	36.1	0.240	0.3725	0.4900	0.7350	0.830	0.4532 0.6160
Cr	Baseline	0.1455	0.0769	52.9	0.062	0.0778	0.1180	0.2080	0.290	0.1130 0.1779
	Sixth month	0.1683	0.0707	42.0	0.080	0.1043	0.1525	0.2230	0.304	0.1384 0.1981
	Difference	0.0229	0.0266	116.2	0.000	0.0100	0.0150	0.0350	0.110	0.0117 0.0342

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

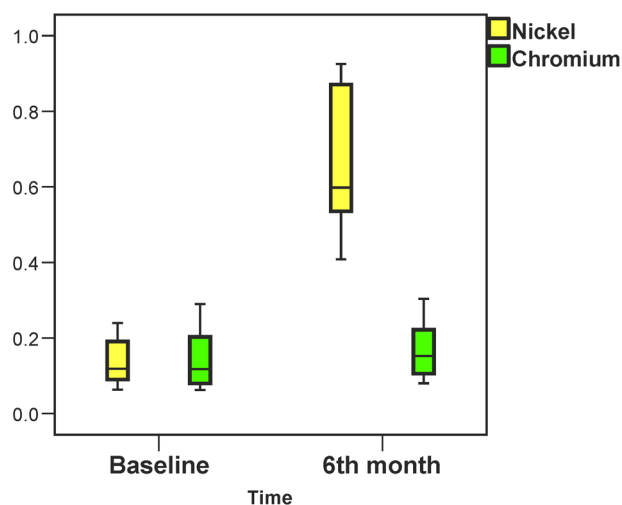


Fig. 1 Box plots showing hair nickel and chromium levels (micrograms per gram dry hair mass)

consisting of orthodontic patients, the values were 0.5073 and 0.1331 $\mu\text{g/g}$, respectively. Hence, similar to the current study, nickel had increased for about 39 % in their experimental group, while chromium had increased for 2.5 %. However, unlike this study, none of their increases was statistically significant. Martin-Camean [20] performed a similar but larger study in 2014 and found hair nickel and chromium concentrations to be 0.47 and 0.36 $\mu\text{g/g}$, respectively, in the control group. They observed about 2–3 % insignificant declines in their orthodontic group for both ions (0.46 and 0.35 $\mu\text{g/g}$ for nickel and chromium, respectively), which contradicted our results showing increases only. Similar to our findings, they failed to observe any link between age and gender, with the ion levels. They found a positive link between all their ion levels, although no correlations were significant between nickel and chromium. In contrast, the correlations between the two ions were negative at both intervals of this study, becoming marginally significant at the second session. Mikulewicz et al. [24] recorded the ion levels immediately before beginning the treatment and in the fourth, eighth, and

12th months of treatment. The nickel values changed from 0.288 at the baseline to 0.366, then to 0.320, and finally to 0.422 $\mu\text{g/g}$. The chromium content declined continuously from 0.0201 at the baseline to 0.184, then to 0.179, and finally to 0.158 $\mu\text{g/g}$. They identified significant results for hair chromium level of orthodontic patients but failed to find significant changes in hair nickel [24], in contrast to our study in which both ions had only increases and not reductions, both being significant. According to our findings, the systemic levels of both ions reflected by hair concentrations might increase by using orthodontic appliances. In comparison to the baseline values, the increases were considerable, particularly in the case of nickel, which had a four-times increase. This was interesting, as previous studies on salivary ion levels had shown small increases of salivary concentrations after placing orthodontic appliances, compared with the daily intake (i.e., 100–800 $\mu\text{g/day}$ for nickel and 50–280 $\mu\text{g/day}$ for chromium) [1, 5, 7, 10, 11, 14, 15, 31–33]. The inconsistency might be partly explained by a combination of the following suggestions: In order to standardize the conditions of in vivo studies, saliva is sampled not before, during, or after eating/drinking. Therefore, assuming that salivary ion release does not follow a steady pattern, such studies usually do not measure the ion levels at the peak of metal ion discharge. Hence, they might underestimate the total influence of orthodontic appliances [4, 5, 7, 15, 31, 33]. Furthermore, metal elimination routes vary greatly, and levels of blood, urine, and hair ions should not be necessarily correlated. For instance, the routes of nickel aggregation and exertion can be affected by the path of absorbed nickel as well as the presence of other metals available in orthodontic appliances such as cadmium [6, 18, 34]. In addition, biofilm might increase the rate of the corrosion procedure and solubilize nickel ions, increasing its systemic absorption through plaque ingestion [1, 3, 6, 10, 12]. The absorbed nickel is mostly eliminated by kidneys at a 28-h half time [6, 21, 22, 31, 35], which might stabilize its blood levels [6]. It also eliminates through sweat, saliva, and keratin tissues such as hair and nails [3, 6, 21, 35, 36]. Furthermore, a

Table 2 Descriptive statistics for the ion levels in genders (all in micrograms per gram dry hair mass)

Sex	Ion	Time	Mean	SD	CV (%)	Min	Q1	Med	Q3	Max	95 % CI	
Female	Ni	Baseline	0.1237	0.0513	41.5	0.063	0.0818	0.1105	0.1760	0.221	0.0911	0.1563
		Sixth month	0.6746	0.1796	26.6	0.408	0.5100	0.6540	0.8750	0.904	0.5605	0.7887
	Cr	Baseline	0.1440	0.0760	52.8	0.073	0.0778	0.1080	0.2080	0.290	0.0957	0.1923
		Sixth month	0.1652	0.0692	41.9	0.080	0.1035	0.1585	0.2150	0.300	0.1212	0.2091
Male	Ni	Baseline	0.1523	0.0610	40.0	0.074	0.0948	0.1370	0.2143	0.240	0.1135	0.1910
		Sixth month	0.6683	0.1854	27.7	0.431	0.5368	0.5960	0.8848	0.925	0.5506	0.7861
	Cr	Baseline	0.1469	0.0812	55.3	0.062	0.0713	0.1180	0.2288	0.281	0.0953	0.1985
		Sixth month	0.1713	0.0752	43.9	0.100	0.1045	0.1425	0.2435	0.304	0.1236	0.2191

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

difference between the metal ion levels in different media might be justifiable by the attempt of the organism to exclude the toxic content. Findings pertaining to systemic ion increases reflected by hair were in contrast to the stable systemic ions indicated by blood [15, 22]. However, they were in line with the increases in systemic nickel levels indicated by urine [6, 21]. This might be explained by efficient mechanisms of metal excretion, which might stabilize blood levels and might imply the biocompatibility of these metals [6]. Nevertheless, low but chronic release of metals might still produce inflammation, or inflict cellular/DNA damage [4, 5, 8, 9, 16, 33]. Moreover, even low levels of systemic metal ions cannot indicate an absence of selective binding of these ions to organs such as kidneys [3, 6, 12, 18, 21]. Nickel and, to a lesser degree, chromium might be cytotoxic and genotoxic [2, 5, 16]. These ions might have both dose-dependent and dose-independent influences. Small doses of these ions might damage DNA, alter cellular morphology and metabolism, and activate monocytes or endothelial cells [4, 5, 9, 16, 33]. Dosage-independent effects of nickel and chromium consist of increased DNA migration and comet development, DNA breakage and fragmentation, and inhibiting DNA-restoring enzymes [2, 4, 5, 9, 16, 33]. Nevertheless, except chromium hexavalent oxides and some nickel compounds, most of corrosion artifacts are not toxic or carcinogen [9, 22]. Besides, some of genetic damages might be reversible [6, 16].

The advantages of this study consisted of its prospective design, its sample size pre-determined by power calculations, the rather long list of exclusion criteria, the removal of several confounders, balancing the sample in terms of age and gender, and matching the patients with themselves. On the other hand, a randomized clinical trial as well as a larger sample might favor the reliability. The study might continue by adding more subjects and including a control group. Still, excessively large samples can increase the rate of false-positive error, and thus sample sizes should be determined based on power calculations, unless more flexible statistical approaches are considered [37]. The generalizability of the results was favored by the rather broad range of age ranges, the use of both types of orthodontic archwires for the enrolled patients (nickel titanium and stainless steel), and the capacity of hair samples in reflecting chronic exposures.

Conclusions

Within the limitations of this preliminary study, it seemed that hair nickel and chromium levels might increase after 6 months of treatment with fixed orthodontic appliances. Gender and age influence neither the baseline nickel or chromium values nor their time-dependent alterations. This study should continue with a larger sample and a control group.

Authors' Contributions Fariborz Amini searched the literature, conceived the study, supervised the experiments, mentored the thesis, interpreted the findings, and critically reviewed the article draft. Mobina Mollaei searched the literature, designed, funded, and performed the experiments, interpreted the findings, and drafted the article. Saghar Harandi participated in the literature search and experiments. Vahid Rakhshan suggested evaluating the role of age and gender, ion correlations, and gender-specific ion differences, searched the literature, specified and performed the statistical analyses, interpreted the findings, and drafted the article.

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