



Randomized controlled trial

Effects of fixed orthodontic treatment using conventional (two-piece) versus metal injection moulding brackets on hair nickel and chromium levels: a double-blind randomized clinical trial

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Summary

Introduction: 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 especially those reflected by their best biomarker of exposure, hair. Additionally, metal injection moulding (MIM) brackets are not studied, and there is no study on systemic ion changes following their usage.

Methods: In this double-blind randomized clinical trial, scalp hair samples of 24 female and 22 male fixed orthodontic patients [as two groups of conventional (two-piece) versus MIM brackets, $n = 23 \times 2$] were collected before treatment and 6 months later. Randomization was carried out using a computer-generated random number table. The patients, laboratory expert, and author responsible for analyses were blinded of the bracket allocations. Hair nickel and chromium levels were measured using atomic absorption spectrophotometry. The effects of treatment, bracket types, gender, and age on hair ions were analysed statistically ($\alpha = 0.05$, $\beta \leq 0.02$).

Results: In both groups combined ($n = 46$), nickel increased from 0.1600 ± 0.0890 $\mu\text{g/g}$ dry hair mass (pre-treatment) to 0.3199 ± 0.1706 (6th month). Chromium increased from 0.1657 ± 0.0884 to 0.3066 ± 0.1362 $\mu\text{g/g}$. Both of these increases were significant (paired *t*-test, $P = 0.0000$). Bracket types, age, and gender had no significant influence on ion levels ($P > 0.05$). ANCOVA indicated different patterns of chromium increases in different genders ($P = 0.033$) and ages ($P = 0.056$).

Limitations: Sample size determination should have accounted for the grouping as well.

Conclusion: Hair nickel and chromium levels might increase about 185–200% after 6 months. They might not be affected by bracket types. Gender and age might not influence the baseline or 6th-month levels of both metals. Gender might however interact with orthodontic treatment, only in the case of chromium.

Registration: The research is registered offline (thesis) and online (IR.AJUMS.REC.1394.516).

Protocol: The protocol was pre-determined before any experiments begin.

Funding: The study was self-funded by the authors.

Introduction

Corrosion of orthodontic alloys might release nickel and chromium which might induce contact allergy, asthma, or hypersensitivity (1–15). Chromium oxide forms an anti-corrosive passive film over orthodontic appliances (2, 13, 16). Nevertheless, in clinical situations, this protective layer is disrupted because of mastication (17), brushing (6), thermal stresses (3, 12, 18), saliva flow (4, 6, 18), biofilm microorganisms (1, 3) and their byproducts and enzymatic activities (1, 5, 12, 19–21), recycling of the appliances (3, 21), friction between brackets and wires (12), occlusal loadings (5, 20), as well as acidic drinks, mouthwashes, or toothpastes (1, 4, 6, 10, 13, 18, 20, 21).

Most of studies in this matter are *in vitro* (1, 8, 16, 22), which is not generalizable to complicated and dynamic oral environment in clinical conditions (1–5, 8, 9, 12, 16, 22, 23). The impact of orthodontic treatment on systemic levels of chromium and nickel is disputed, demonstrating lack of changes (10, 24–27) or increases (14, 15, 28, 29). Furthermore, many of these few *systemic* researches have examined the ion level changes in response to acute exposures, only (10, 14, 15, 25, 28).

Systemic exposure to a material can be measured by means of exposure biomarkers, the most accessible ones of which are urine, nail, hair, serum, and blood (15, 28). Serum cannot necessarily reflect ions' blood levels; blood and urine themselves might reveal merely acute exposures and are greatly confounded by the metabolic pathways of ion removal (13, 27, 28, 30). Hair, on the other hand, has numerous advantages: it can show both acute and chronic exposures to metals, is correlated with blood ion levels, and is free of confounding effects of metabolic mechanisms (15, 29–32). It also accumulates rather high concentration of ions (50–100 times more than the blood, urine, and nails) and is convenient and stable for sample storage and transport (15, 29–32). Hence, hair is one of the best biological biomarkers of metal exposure, according to the US Environmental Protection Agency and the International Atomic Energy Agency (15, 29–32). Nevertheless, the effect of orthodontic treatment on hair ion levels is not adequately inspected (15).

Except one recent study (23), all previous researches are performed on conventional brackets, the results of which are not necessarily generalizable to novel orthodontic technologies which might corrode otherwise (23): Metal injection moulding (MIM) brackets are designed to eliminate the intra-bracket galvanic corrosion through their one-piece design with uniform elemental distribution and lack of soldering between bracket parts (23, 33). However, the research on these brackets is limited to a single study on salivary ion release (23); and no other studies (including systemic evaluations) exist in this matter.

Thus, and in view of the lack of clinical trials in the field of orthodontic metal ion release, this study was conducted to evaluate hair concentrations of nickel and chromium in orthodontic patients immediately before treatment, and 6 months after beginning of fixed treatment in two groups of conventional (two-piece) brackets and MIM brackets (23). The primary null hypothesis #1 was the lack of any changes happening to the hair ion concentrations during the 6-month course of the study (or the absence of any difference between hair ion concentrations between the baseline (pre-treatment) and after 6 months of orthodontic treatment). The second primary hypothesis was the lack of differences between hair ion contents of patients enrolled in conventional (two-piece) versus MIM bracket groups. The secondary outcomes were 1. the lack of differences between the extents of time-dependent increases (if any) in two bracket groups, 2. the lack of interactions between the effects

of bracket types and treatment on hair ion content, 3. the lack of any effect of gender and age on the pre-treatment or 4. during-treatment ion levels, and 5. the lack of the interaction of age and gender with treatment (23).

Subjects and methods

Trial design

This was a single-centre double-blind parallel randomized clinical trial of adverse effects on 46 orthodontic patients. The whole study is reported completely in this article and there is no overlap between the patients/procedures/data of the present study and those of any other reports.

Registration

All the experiments were determined before being carried out, as an orthodontics MS thesis, and remained unchanged. The thesis proposal was registered offline at the university archive. The protocol was also registered online by the deputy of research of the university (IR.AJUMS.REC.1394.516).

Ethical considerations and potential harms

The protocol ethics were approved by the research committee of the university according to the Helsinki declaration, and written consents were taken from the subjects or their parents after thorough oral and written explanation. Subjects could leave the study at their wish in any stage. No harms were identified during the study, and the MIM brackets are currently used frequently in orthodontic practice.

Screening for potential subjects

The patients were selected from attendees to the Orthodontics Department of the University during 2014–15. The inclusion of eligible patients began in September 2014 and continued until February 2015. During this period eight originally included patients were dropped out (see below for details) and replaced with new patients, the last one of whom was enrolled in February 2015. The sampling was finished in August 2015.

Subjects and eligibility criteria

The subjects were sequentially acquired and randomly assigned to one of the two groups until two groups of 23 patients (11 males and 12 females) each were pooled. The inclusion criteria comprised the patients' willingness to participate, the indication for fixed orthodontic treatment, no history of previous orthodontic therapy of any kind, 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, smoking or alcohol consumption, the absence of any caries (10), the lack of any metal restorations (e.g., amalgam fillings or fixed prostheses) before or during the treatment, the presence of an adequate length of scalp hair, and the absence of any hair colours or hairdressings. All the inclusion criteria needed to be met during the study period (15, 23). The patients were instructed about the inclusion criteria needing to hold during the study period in order for the patient to remain a participant of the research. The patients were told that if any of the included patients left the study in the middle of the 6-month course (due to any reason such as dying their hair, or receiving dental restorations etc, or simply not wishing anymore to be a part of the study), they would be excluded from the study, their orthodontic treatment would continue as before, and new patients would be screened and included the study.

Randomization

The randomization was done by an orthodontist (NH), based on a random number table generated using Excel (v 2007, Microsoft, USA). The orthodontist was the only person who knew the allocations.

Whenever a randomized patient would be dropped out in the middle of the study, a new patient would be randomly assigned to one of the two groups, based on a new randomization list.

Allocation concealment

The allocations were kept in a sealed file available only to the orthodontist.

Blinding

The laboratory expert, the patients, and the author responsible for statistical analyses were all blinded of the bracket allocations. For this purpose, the hair samples and dataset were all coded, and the patients were not informed of their bracket types. The MIM brackets looked identical in shape and size to the conventional two-piece ones, making it impossible for the patients or orthodontic staff to recognize the groups.

Uniform treatment protocols

The treatments were all bimaxillary non-extraction, and without any bands, in order to use the same number of brackets/tubes in both groups, as well as to exclude the soldered bands, headgears, TPAs, or other metal appliances—potential confounders—from the study. The used archwires in both the control and experimental groups were nickel titanium (NiTi) (G&H, USA) in about the first 4 months and stainless steel (SS) (G&H, USA) in about the next 2 months. The order of the used wires in both groups was: 0.012" round NiTi almost in the first month, 0.014" round NiTi in (2nd month), 0.016" round NiTi (3rd month), 0.017" × 0.025" rectangular NiTi (4th month), 0.017" × 0.025" rectangular SS (4th month), and finally 0.019" × 0.025" rectangular SS wires approximately during the 6th month. This order was the same for all patients with slight deviations if clinically necessary. If the clinical judgment indicated a major deviation from the above sequence, the patient would be excluded from the study either in the beginning or in the middle and would be replaced by a new patient. Wire ligation in both groups was carried out using elastomeric ligatures only (OrthoOrganizer, Encinitas, California, USA). The appliances exclusively employed in the control group were bonded 0.022 inch slot SS Roth prescription conventional brackets (AISI 316L, Stratus, Fairfield Orthodontic, USA) on all teeth except the molars, as well as four conventional orthodontic tubes (Fairfield Orthodontic) placed only on the first molars. The appliances used merely in the experimental group were bonded Roth prescription 0.022 inch slot stainless-steel MIM brackets (AISI 316L, Gem Petit, Fairfield Orthodontic) on all teeth except the molars, and four MIM orthodontic tubes (Fairfield Orthodontic) placed only on the first molars (15, 23).

Hair sampling

The sampling was performed twice, once immediately before the treatment, and the next time after 6 months. In the scheduled sessions, 5–6 cm of hair (which according to the calculations performed previously consisted of hair grown during about the past 5 or 6 months (34)) was cut from the occipital region of the head in the neck proximity, using a SS surgical blade. The hair samples were kept in paper envelopes (15, 31, 32, 35).

Ion level measurement

The hair specimens were shipped to the Chemical Analysis Laboratory for atomic absorption spectrophotometry using a calibrated device [high-resolution continuum source AAS Contra (AA700, Analytik Jena, Germany)], with detection limits of 1 ppb (0.001 µg/g dry hair mass) for nickel and 0.5 ppb (0.0005 µg/g dry hair mass) for chromium, and characteristic concentrations of 0.55 ppb for nickel (0.00055 µg/g) and 0.2 ppb (0.0002 µg/g) for chromium. At the lab, hair pollutions, debris, and proteins were removed by 10-minute application of a surfactant (Triton X100) diluted 1:200. After cleansing with metal-free acetone (10 minutes) and rinsing thrice using a deionized water producer (Direct-Q 3 UV Water Purification System, Millipore, France) (13, 34), the specimens were dried at 80°C for 6 hours in an oven (Binder heating, Germany) (36). From each specimen, 0.5 mg was stored for 12 hours in 2.5 ml of 65% nitric acid and 1.25 ml of 30% hydrogen peroxide at room temperature. Then, in order to remove the organic content, the solution and immersed hair sample was put in an oven (Microwave oven, Merk, Germany) at 80°C for about 2 or 3 hours (34). The prepared 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 ppm or µg per gram of dry hair mass. These procedures were repeated for the 6th month of treatment (15).

Statistical analysis

The sample size was predetermined as 24 patients based on a previous study and power calculations, to obtain test powers above 98% (at a 0.05 level of significance) for the evaluation of the effect of orthodontic treatment (regardless of the brackets used) on hair levels of nickel and chromium. The mean difference between the nickel values measured once at the baseline and once 6 months later was 0.5346 ± 0.1928 µg/g dry hair mass. The mean chromium difference was 0.0229 ± 0.0266 µg/g dry hair mass (in the same patients between baseline and 6th month) (15). The earlier study is published as an independent report (15), and none of the patients or procedures pertaining to it was overlapped with this trial. The total sample size was divided into two bracket groups of equal size. The sample size was augmented to 46 subjects in order to ensure high test powers after presenting new variables.

An independent-samples *t*-test was used to assess whether the randomly assigned groups were similar in terms of baseline levels of nickel or chromium. A Kolmogorov–Smirnov test was used to evaluate the normality of ion levels in groups as well as the normality of paired ion differences between two time points (Tables 1 and 2). The first primary outcome was to evaluate whether or not the extent of hair nickel and chromium had increased during the 6-month course of the study. This was done using a paired *t*-test and a Wilcoxon paired-ranks test, by comparing the ion levels measured at baseline with the values measured after 6 months. As the second primary outcome, the effects of brackets on the ion increase were assessed. For this purpose, the extent of ion increase (i.e. the value measured at the 6th month minus the value measured at the baseline) in the conventional bracket group was compared with the extent of ion increase in the MIM bracket group, using a Mann–Whitney *U*-test. The *post hoc* powers were about 42% and 32% in the case of chromium and nickel, respectively.

As secondary outcomes, the multivariable effects of orthodontic treatment, bracket type, age, and gender as well as their interaction were evaluated using repeated-measures two- and three-way analyses of variance (ANOVA) and repeated-measures two- and three-way analyses of

Table 1. Descriptive statistics for ion values ($\mu\text{g/g}$ dry hair mass, ppm).

Ion	Interval	Bracket	Mean	SD	KS <i>P</i>	Min	Q1	Med	Q3	Max	95% CI	
Ni	Baseline	MIM	0.1698	0.0965	>0.1	0.0639	0.1090	0.1420	0.2210	0.4322	0.1281	0.2116
		Con	0.1502	0.0817	>0.1	0.0030	0.0920	0.1490	0.2030	0.3070	0.1148	0.1855
	6th month	MIM	0.3663	0.2047	>0.1	0.0678	0.2220	0.3560	0.4670	0.9788	0.2778	0.4548
		Con	0.2735	0.1146	>0.1	0.1090	0.1730	0.2450	0.3560	0.4760	0.2240	0.3231
	Difference	MIM	0.1965	0.1985	0.009	0.0039	0.0600	0.1650	0.2460	0.8653	0.1106	0.2823
		Con	0.1233	0.0528	>0.1	0.0150	0.0940	0.1200	0.1610	0.2390	0.1005	0.1462
Cr	Baseline	MIM	0.1792	0.1067	0.046	0.0620	0.0890	0.1398	0.2780	0.4855	0.1331	0.2254
		Con	0.1521	0.0650	>0.1	0.0340	0.1040	0.1730	0.1930	0.2710	0.1240	0.1803
	6th month	MIM	0.3092	0.1688	>0.1	0.0980	0.2160	0.2780	0.3870	0.8534	0.2362	0.3822
		Con	0.3040	0.0971	>0.1	0.1380	0.2220	0.3160	0.4090	0.5010	0.2621	0.3460
	Difference	MIM	0.1300	0.1296	>0.1	-0.1000	0.0260	0.1064	0.1900	0.4499	0.0739	0.1860
		Con	0.1519	0.0955	>0.1	0.0040	0.0640	0.1410	0.2020	0.3860	0.1106	0.1932

No. of subjects per table row = 23 patients. SD, standard deviation; KS *P*, the *P* value calculated for the normality of the sample distribution using the Kolmogorov–Smirnov test; Min, minimum; Q1, 25th percentile; Med, median; Q3, 75th percentile; Max, maximum; CI, confidence interval for the mean; MIM, metal injection moulding; Con, conventional; Ni, nickel; Cr, chromium; Difference, calculated as the values pertaining to the 6th month minus the baseline, per each bracket group.

Table 2. Descriptive statistics for ion values ($\mu\text{g/g}$ dry hair mass, ppm) regardless of the brackets used.

Ion	Interval	Mean	SD	KS <i>P</i>	Min	Q1	Med	Q3	Max	95% CI	
Ni	Baseline	0.1600	0.0890	>0.1	0.0030	0.0938	0.1460	0.2055	0.4322	0.1336	0.1864
	6th month	0.3199	0.1706	>0.1	0.0678	0.1956	0.2840	0.4285	0.9788	0.2693	0.3706
	Difference	0.1599	0.1483	0.0003	0.0039	0.0850	0.1275	0.1928	0.8653	0.1159	0.2040
Cr	Baseline	0.1657	0.0884	>0.1	0.0340	0.0928	0.1635	0.2245	0.4855	0.1394	0.1919
	6th month	0.3066	0.1362	>0.1	0.0980	0.2205	0.2810	0.3925	0.8534	0.2662	0.3471
	Difference	0.1409	0.1131	>0.1	-0.1000	0.0593	0.1410	0.1930	0.4499	0.1074	0.1745

No. of subjects per table row = 46 patients. SD, standard deviation; KS *P*, the *P* value calculated for the normality of the sample distribution using the Kolmogorov–Smirnov test; Min, minimum; Q1, 25th percentile; Med, median; Q3, 75th percentile; Max, maximum; CI, confidence interval for the mean; Ni, nickel; Cr, chromium; Difference, calculated as the values pertaining to the 6th month minus the baseline, in both bracket groups combined.

covariance (ANCOVA). The correlations between gender and ion levels were assessed using a point-biserial correlation coefficient. The correlations between the ion values measured at both intervals as well as the correlations between age and the released ion values measured at each session was evaluated using a Spearman correlation coefficient. The level of significance was set at 0.05. The statistical analyses were carried out using SPSS (v 20.0, IBM, USA) by the author VR. The primary outcomes were defined *a priori*, in the proposal. The other outcomes were defined after the registration of the proposal but before finishing the study.

Results

Participant flow

A total of 60 patients were assessed until 46 patients were included. Of the excluded patients, six did not meet the inclusion criteria and were excluded in the beginning (not randomized): two needed headgears and four needed a different sequence of archwire usage. The rest of excluded patients were randomized but dropped out in the middle of the study (seven women had dyed their hair and a boy had shaved his head due to being drafted to military services). Those eight patients were replaced by new patients who were again randomized based on a new random list (Figure 1).

Sample characteristics

Demographics

There were no missing data. In each of the control or MIM groups, there were 11 males and 12 females. The average age of the included patients was 17.91 ± 3.71 years (range: 12–26). It was

17.70 ± 3.61 years in the conventional group and 18.13 ± 3.88 years in the MIM group.

Baseline ion values

The independent-samples *t*-test indicated a lack of significant differences between the control and MIM groups, in terms of chromium ($-0.028 \mu\text{g/g}$, $P = 0.305$) and nickel ($-0.020 \mu\text{g/g}$, $P = 0.460$) levels measured before the beginning of treatment (Figure 2, Table 1).

Primary outcome 1: changes over the 6-month period of the study

Conventional brackets

In the control group, nickel and chromium increased 0.1233 and $0.1519 \mu\text{g/g}$, respectively. Both differences were significant (both *P* values = 0.0000, paired *t*-test, Table 1).

MIM brackets

In the MIM experimental group, nickel and chromium increased 0.1965 and $0.1300 \mu\text{g/g}$, respectively. Both differences were statistically significant according to the Wilcoxon test ($P \leq 0.0002$, Table 1).

Both MIM/conventional groups combined

In both groups together, mean hair nickel and chromium levels increased about 0.1600 and $0.1410 \mu\text{g/g}$, respectively. Compared to their baseline values, nickel increased 2-fold (compared to its baseline value of $0.1600 \mu\text{g/g}$) and chromium showed an 85% increase

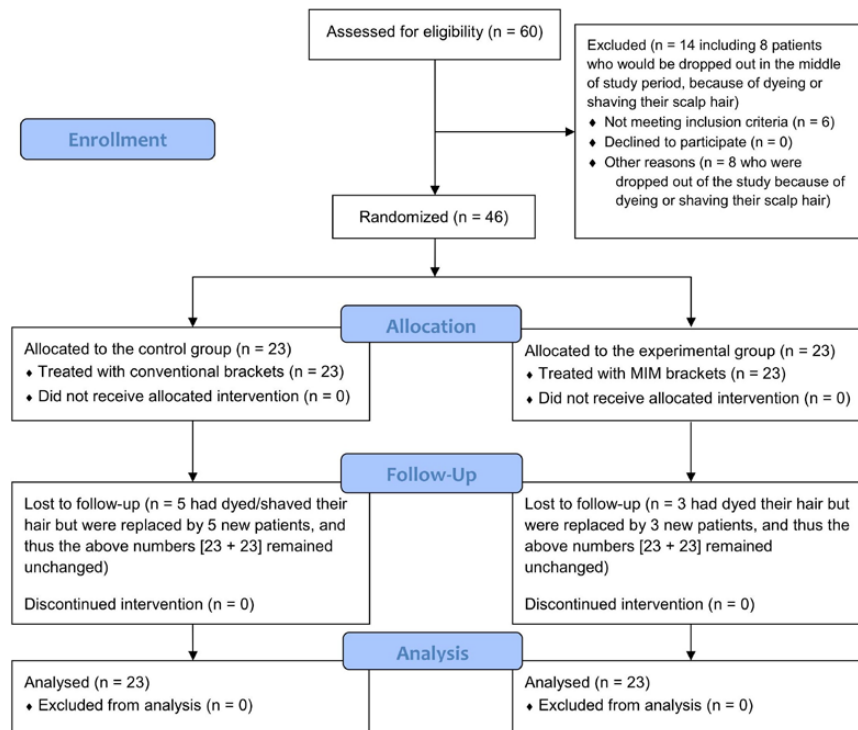


Figure 1. Design and participant flow.

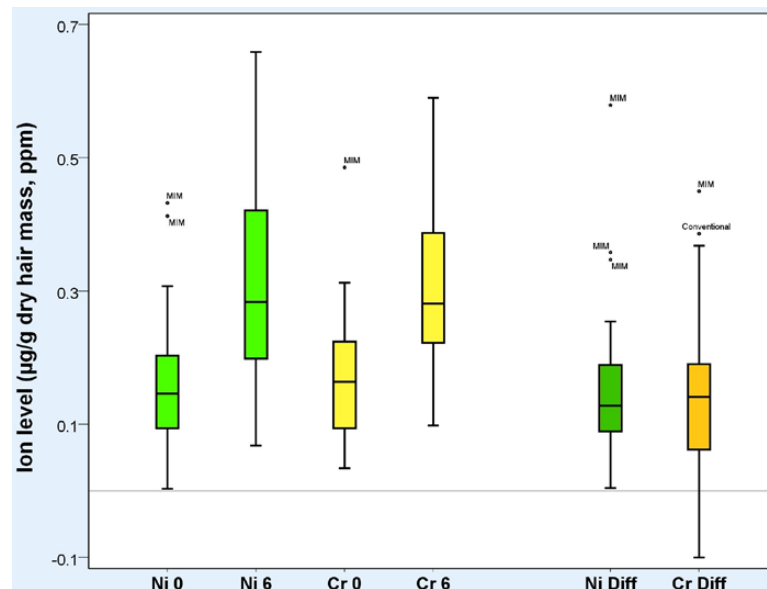


Figure 2. Boxplots illustrating the medians, interquartile distances, and minimums/maximums (excluding the outliers) for hair nickel and chromium levels (µg/g dry hair mass, ppm). Ni, nickel; Cr, chromium; 0, baseline; 6, 6th month; Diff, Difference between the ion levels measured in the 6th month and baseline (6th month minus baseline). The chart was produced using the boxplot feature of SPSS and was edited using Photoshop (Adobe, USA), by VR.

(compared to its baseline value of 0.1657 µg/g). Both increases were statistically significant according to the paired *t*-test (both *P* values = 0.0000, Figure 2, Table 2).

Primary outcome 2: differences between the time-dependent alterations of the conventional versus MIM brackets

Both the MIM and conventional groups showed increases in each of nickel and chromium values. According to the Mann–Whitney

test, the extents of these increases were not significantly different between the MIM and conventional brackets, either in terms of nickel increases (*P* = 0.323) or chromium increases (*P* = 0.398, Table 1).

Secondary outcome 1: differences between conventional versus MIM brackets after 6 months

In the 6th month, no statistically significant differences were observed between the conventional and MIM nickel levels

(Mann–Whitney U -test, $P = 0.119$), and between MIM and control chromium levels ($P = 0.717$, Table 1).

Secondary outcome 2: multivariable analyses of the effect of treatment, bracket types, and their interactions on the extent of ion release

Nickel

According to the two-way repeated-measures ANOVA used to assess the nickel ion, from a holistic perspective, the effects of orthodontic treatment (time) ($F = 55.740$, $P = 0.0000$) on nickel levels was significant. However, bracket types did not significantly affect nickel levels ($F = 2.914$, $P = 0.095$). The interaction of bracket types and orthodontic treatment (i.e. time) was non-significant as well ($F = 2.913$, $P = 0.095$).

Chromium

Similar results were seen, employing two-way repeated-measures ANOVA, for chromium levels: a significant influence of orthodontic treatment (time) ($F = 70.515$, $P = 0.0000$) and a lack of effects of bracket types ($F = 0.295$, $P = 0.590$) and the interaction of bracket types and time ($F = 0.427$, $P = 0.517$) on chromium levels.

Secondary outcome 3: the effect of age and gender on the ion alterations

Correlations

No significant correlations were seen between patients' age and any of the ion concentrations measured at any sessions (all four Spearman P values > 0.098). According to the point-biserial correlation coefficient, there was no significant correlation between the patients' gender with any of the ions measured at any of the intervals (all four P values > 0.087).

Multivariable analyses (taking bracket types and the interactions into account as well)

Nickel. According to the three-way repeated-measures ANOVA, only the effect of time on nickel was significant ($F = 54.780$, $P = 0.0000$). The effects of bracket types ($F = 3.032$, $P = 0.089$), and the interaction of time by bracket type ($F = 3.004$, $P = 0.090$) on nickel levels were close to the significant level, although still non-significant. The rest of variables and interactions including gender ($F = 1.606$, $P = 0.212$) were non-significant (all other P values > 0.310).

Chromium. Chromium levels were affected by time (orthodontic treatment) (ANCOVA, $F = 13.530$, $P = 0.001$) and the interaction of time and gender ($F = 4.846$, $P = 0.033$). The interaction of time and age was marginally significant ($F = 3.883$, $P = 0.056$). The other variables including bracket types ($F = 0.311$, $P = 0.580$), age ($F = 0.002$, $P = 0.962$), gender ($F = 1.409$, $P = 0.242$) and the remainder of interactions were non-significant.

Secondary outcome 4: correlations between pre-treatment and treatment ion concentrations

There were positive significant correlations between the nickel ion levels measured at both time points ($\rho = 0.694$, $P = 0.0000$) and between chromium levels at the two time points ($\rho = 0.410$, $P = 0.0047$).

Discussion

The findings of this study indicated that orthodontic treatment might increase systemic levels of both nickel and chromium about 2-fold. Neither the extent of ion increases during 6 months nor ion levels measured in the 6th month of treatment were different between MIM and conventional brackets. According to the results of this study and the few available ones (15, 24), gender and age might not influence the concentrations of hair nickel or chromium. Nevertheless, they might interact with the increase pattern of chromium, meaning that the pattern of chromium increase might differ between boys and girls, and between older and younger patients. This was consistent with the only other study evaluating the effect of age and gender on the extents of ion increases, which had reported that salivary chromium alterations might follow a different pattern in females compared to males (23). The level of ions observed in this study were within the range reported in other populations, except that of Levrini *et al.* (26) who reported ion concentrations about 1000 times greater than other studies, and perhaps might be due to an error in the writing of the unit of measurement (mg/g instead of $\mu\text{g/g}$).

The systemic ion increases observed in the current research were in line with a prospective study (merely on nickel) (29) which found a significant 2-fold increase from a baseline value of 0.350 to a 4th month nickel level of 0.673 $\mu\text{g/g}$, although their non-orthodontic negative control group as well demonstrated a statistically significant 156% increase of nickel over the 4-month course of their study (29). Another prospective study on both ions (15) demonstrated about 4-fold increase for nickel and about 16% increase for chromium, both being statistically significant (15, 29). Although their results were consistent with this study in terms of significant increases, the extents of increase differed considerably: in the present study, both ions' concentrations became almost doubled after 6 months of treatment while in their study, a much greater increase was observed for nickel and a much smaller increase was seen for chromium (15). Such controversies might be attributable to the differences in the geographical locations of sampling, ethnicities, and sample sizes. On the other hand, retrospective studies on hair nickel/chromium (24, 27) or hair nickel (26) reported smaller increases, all being statistically non-significant: Mikulewicz *et al.* (31) reported about 39% and 2.5% increases in nickel and chromium levels. Even Martin-Camean *et al.* (24) reported insignificant declines in their orthodontic group for both ions. Levrini *et al.* (26) reported a lack of significant difference between nickel levels measured in an 'orthodontic treatment' group compared to a negative control without any treatments. Interestingly, Mikulewicz *et al.* (27) reported a non-significant 145% increase in hair nickel and a significant increase in chromium levels.

According to our findings, the systemic levels of both ions reflected by hair concentrations might increase by using orthodontic appliances. The increases observed by all the prospective studies on hair nickel and chromium were not consistent with the results of studies on salivary nickel or chromium levels, the increases of which were extremely small, if any, compared to the daily intake of those ions (1, 3, 4, 9, 10, 13, 15–17, 23, 37). Such an inconsistency might be justified by some theorems including standard saliva sampling methods which need patients to be abstaining, while the peak of salivary ion release is after eating when the pH drops considerably (10, 12, 13, 15–17). In addition, a considerable part of systemic metal ions might be absorbed through swallowing of plaque which is hidden from salivary examinations, while can considerably accumulate metal ions (1, 3, 5, 8, 14, 15).

Nickel and chromium might have both dose-dependent and dose-independent influences. Small doses of these ions might activate monocytes or endothelial cells and damage DNA or cellular morphology and metabolism (2, 7, 11–13, 15, 16, 23, 38). Nonetheless, most of corrosion artifacts are not toxic or carcinogenic and not all genetic damages are irreversible (2, 11, 14, 15, 23, 25, 38). Hypersensitivity and allergic stomatitis might remain the main concern of dental practitioners regarding the release of these ions, besides the increase in the odds of periodontitis associated with nickel release (13, 15, 19, 23, 25, 28, 38, 39).

There is no study on *systemic* levels of metal ions absorbed after orthodontic treatment with MIM brackets. The only study on MIM brackets (23) had assessed their salivary ion release within 2 months of treatment and had failed to detect a significant difference between MIM versus conventional brackets of the same brand used in this study (23). They had found significant increases in salivary nickel after utilizing either of MIM or conventional brackets, although the increase in the control group was about 20 times greater than that in the MIM group. In contrast, chromium had remained rather unchanged after using conventional brackets, while a small but still statistically significant *reduction* was noted in salivary chromium content (23). MIM brackets are supposed to be less prone to galvanic corrosion (33, 40) while pitting corrosion might be higher in them because of their coarser granular structure and the higher number of micropores of MIMs caused by their different production process (23, 33, 40). Such porosities—besides limitations in detection of very dilute concentrations—might also partially account for the increase of the plaque and its bioaggregation of chromium out of saliva, hence a reduced salivary chromium concentration (23, 33). As stated above, plaque is capable of bioaccumulating metal ions by adhering with glycoproteins or metals stored earlier in the biofilm (2, 7, 8, 12, 13, 17, 38). Therefore, difficulties in plaque removal because of orthodontic treatment plus the porosities of MIM brackets might lead to a more mature and thicker biofilm, leading to reductions in salivary chromium levels (17, 19, 41). The present study showed that regardless of the salivary changes in ions caused by the MIM bracket, the systemic ions might increase in patients; this might imply the previous hypothesis that a considerable amount of metal ions is actually absorbed through plaque and is undetectable by salivary tests (8, 23). There is no other research, either *in vitro* or *in vivo*, on MIM brackets to substantiate our hypotheses; future studies of any type are warranted to evaluate MIM brackets.

Limitations

This study was limited by some factors. Recruiting new patients to compensate for drop outs might bias the randomization. However, the new patients were again randomized, although it might not necessarily eliminate the possibility of randomization bias. Still, this was less likely affecting the results, when the influencing factors were not known (not studied before).

The sample size calculation took into consideration only the longitudinal changes in ion levels of patients under treatment with conventional brackets. It was better to perform a pilot study with both bracket groups, and account for differences between brackets as well. However, this was out of the time and budget projected, and we could only increase the sample size from 24 to 46 in order to hopefully compensate for the grouping. Our *post hoc* powers indicated that in order to reach powers slightly above 80% for detection of differences between nickel and chromium in two bracket groups, we needed samples as large as about 200 and 135 patients, respectively. However, this seems impractical, not to mention that to date,

this was the largest study on systemic metal ion levels in the literature and one of the only two clinical trials in this regard.

It was better to include more intervals and a longer duration. The presence of numerous known or unknown confounding variables as well as biological variations introduced by each patient is major limitations of all *in vivo* studies (4, 9, 12, 13, 28, 42). On the other hand, this randomized clinical trial was advantageous from many aspects: earlier studies were rather small (3, 12, 25, 28, 37), prospective but limited to follow ups of 2 months (13, 23), retrospective (2, 4) or descriptive (10), longitudinal (3, 25, 28, 37), and all except a recent one (23) lacking a clinical trial design. However, as a clinical trial, this study was limited by the use of archwires that could as well release metal ions and reduce the contrast between the two groups. *In vitro* designs seem necessary to compare MIM brackets with conventional ones. The advantages of this study consisted of its RCT design, matching the patients with themselves, its sample size that was both pre-determined based on power calculations and considerably larger than all other prospective studies, a long list of inclusion criteria and treatment requirements, balancing the sample in terms of age and gender, and its follow-up duration. The generalizability of the results was favoured by the use of both SS and NiTi wires, and the capacity of hair samples in reflecting chronic exposures. An inevitable limitation of all *in vivo* studies on biomarkers except hair is that the daily pattern of ionic discharge is not completely known and might fluctuate as a function of pH and other factors (12, 13, 16, 17). However, hair ion levels seem free of the effect of acute fluctuations (4, 13–15, 23). Similar research should be conducted in other populations, as the baseline amounts of ion intake might vary based on differences in numerous sources of metal ions such as air pollutions, quality of food, the diet [e.g., legumes (lentils, peas, and vetch), barley, nuts, and chocolate] difference in bacterial colonization, galvanic currents produced, etc (4, 9, 13, 28, 42).

Conclusions

Hair nickel and chromium levels might increase about 200% and 185%, respectively, after 6 months of treatment with fixed orthodontic appliances consisting of either MIM or conventional brackets. Using MIM brackets might not affect the concentrations of hair nickel and chromium. Gender and age might not influence the baseline or 6th-month levels of nickel or chromium. However, the pattern of changes in chromium levels caused by orthodontic treatment might differ between males and females as well as between older and younger subjects. This needs future studies.

Authors' contributions

MKM searched the literature, hypothesized the primary outcomes, designed and supervised the experiments, mentored the thesis, and interpreted the findings. NHJ searched the literature, hypothesized the primary outcomes, designed and performed the experiments, interpreted the findings, wrote the thesis, and participated in drafting the article. ON and EH participated in the literature search and experiments. VR hypothesized the secondary outcomes, searched the literature, conceived and performed the statistical analyses, interpreted and discussed the findings, and drafted/revised the article.

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