Randomized controlled trial

Effects of fixed orthodontic treatment using conventional versus metal-injection molding brackets on salivary nickel and chromium levels: a double-blind randomized clinical trial

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Summary

Introduction: Despite the importance of nickel and chromium release from orthodontic brackets, there are no in vivo or in vitro studies on this issue in the case of metal-injection molding (MIM) brackets.

Methods: Saliva samples were collected from 30 orthodontic patients divided randomly into two groups of conventional and MIM brackets, before treatment and 2 months later. Approved attendees with odd and even numbers were, respectively, assigned to the control and treatment groups. For blinding, the patients were not informed of their bracket types, and the saliva samples were coded. Nickel and chromium levels were determined using atomic absorption spectrophotometry. Data were analysed using repeated-measures two-way analysis of covariance, independent-samples t-test, chi-squared, Spearman and point-biserial correlation coefficients, Mann–Whitney, and Wilcoxon tests ($\alpha = 0.05$).

Results: Mean nickel level increased from $7.87 \pm 8.14$ (pre-treatment) to $12.57 \pm 9.96$ (2nd month) in the control group, and from $8.62 \pm 9.85$ (pre-treatment) to $8.86 \pm 6.42 \mu g/l$ in the MIM group. Both of these increases were significant (Wilcoxon $P < 0.03$). Average chromium level changed from $0.25 \pm 0.56$ (pre-treatment) to $0.35 \pm 0.62$ and from $0.42 \pm 0.48$ to $0.26 \pm 0.57 \mu g/l$ in the MIM group. Only the reduction observed in the MIM group was significant (Wilcoxon $P = 0.0438$). Age and gender had no significant influence on ion levels ($P > 0.1$). The differences between both ions’ levels measured in the 60th day in both bracket groups were not significant (Mann–Whitney $P > 0.05$). The extents of changes over time were not significantly different between the bracket types (Mann–Whitney $P > 0.05$).

Limitations: The sample size was not predetermined based on power calculations. The spectrophotometer was limited to detecting chromium concentrations above $0.25 \mu g/l$. Ion discharge from brackets might continuously change. The current in vivo methods are unable to take such fluctuations into account.

Conclusion: Nickel might increase in patients undergoing treatment with both bracket types, although the rate of increase might be greater in patients under treatment with conventional brackets. Using MIM brackets might reduce salivary chromium for a trivial but generalizable amount. Still, ion levels leached from conventional versus MIM brackets might not show a difference after 2 months. Age and gender might not affect the ion levels in normal people or orthodontic patients.

Registration: The protocol is registered offline at the university library.

Protocol: The protocol was not published before the trail commencement.
Introduction

Biocompatibility of orthodontic appliances might be compromised by the potential discharge of toxic and carcinogenic ions during orthodontic treatment (1–5). Orthodontic alloys are made of 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 damages (1–16). Corrosion of orthodontic alloys might lead to the release of considerable amounts of nickel and chromium ions into saliva (4–6, 9, 11, 13). Chromium is added to these alloys to form an anticorrosive passive chromium oxide film (4, 9, 17). Nevertheless, in clinical situations, this protective layer is disrupted via several mechanisms such as thermal stresses, saliva flow, mastication, brushing, biofilm layer and its biochemical activities, recycling of the appliances, friction between brackets and wires, occlusal loadings, and acidic solutions (3–6, 10–13, 15, 18–22). The exposed surface can be corroded by mechanisms such as galvanic corrosion, which occurs when different metals (e.g., brackets’ wings, base, and brazing alloys) are joined in the construction of conventional brackets or appliances (6, 11, 13, 23, 24).

Considering the potential danger of nickel and chromium, assessment of these ions’ release from orthodontic appliances is essential (1, 2). The majority of the studies in this regard are in vitro (1, 2, 6, 17), which is not a method relevant to clinical conditions, since it cannot reproduce dynamic and complicated oral environment with the presence of alloy biodeterioration (1–3, 6, 9–12, 14, 17). Furthermore, some of the few in vivo works have evaluated short-term exposures to orthodontic alloys (i.e., maximum 1 month) (25, 26), and the controversial reports are quite confusing to the orthodontists (4–6, 10).

Besides being debated, previous studies are all carried out on conventional brackets only. Whereas, there are novel systems that might corrode differently, to which previous results are not generalizable. For instance, metal-injection molding (MIM) brackets are single-unit brackets with uniform elemental distribution, designed to eliminate the intrabracket galvanic corrosion, although they might be more prone to pitting corrosion instead (23). However, there are no in vitro or in vivo studies on metal ion release from these brackets.

Furthermore, the few clinical studies on orthodontic metal ion release lack any clinical trials, which are of the highest impact among in vivo designs. Thus, and in view of the lack of any assessments of MIM brackets in vitro or clinically, we aimed to evaluate the salivary amounts of nickel and chromium in orthodontic patients immediately before treatment, and 2 months after beginning of fixed treatment in two groups of conventional brackets and MIM brackets.

The null hypotheses were: 1. Salivary ion levels (for each of nickel and chromium) would not differ between the baseline (pre-treatment) and after 2 months of orthodontic treatment with either conventional or MIM brackets, or within both bracket groups combined. 2. There would be no significant difference between the ion concentrations in patients undergoing 2 months of treatment with conventional versus MIM brackets (for each of nickel and chromium). 3. There would be no difference between the changes of the ions in the two bracket groups. 4. There would be no interactions between treatment and bracket types, meaning that their ion release patterns would be similar. 5. Age and gender would have no impact on the pre-treatment or during-treatment ion levels. 6. Age and gender would not interact with treatment, meaning that the ion level changes (if any) would follow a similar pattern between males and females and among people at different ages.

Subjects and methods

Trial design

This double-blind randomized clinical trial was performed on 60 saliva specimens sampled from 30 orthodontic patients at two time points. The patients were divided into two groups of conventional (control) and MIM (experimental) brackets (n = 15 each), as well as two time groups of 30 observations each: baseline (pre-treatment) and 60 days after the treatment initiation. The sample size was predetermined based on the previous in vivo literature.

Registration

The experimentations done in this trial were all designed and predetermined before being carried out, as an MS thesis. The thesis proposal is registered offline at the university archive as #81.

Ethical considerations and potential harms

The protocol ethics were approved by the research committee of the university, 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.

Screening for potential subjects

The patients were selected from attendees to the Orthodontics Department of the Tehran Dental School of Azad University during 2013–2014. The subjects were sequentially enrolled and randomly assigned to one of the two groups until two groups of 15 patients each were acquired.

Subjects and eligibility criteria

The inclusion criteria comprised the subjects’ willingness to participate, the indication for bimaxillary non-ext fixed orthodontic treatment, subjects being 11–26 years old, having all the permanent teeth fully erupted (no semieruptions, 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, as well as no history of previous orthodontic treatment of any kind. All the included patients also needed to have 24 teeth (12 in each arch, 6–6) continuously (and at the same time) under fixed orthodontic treatment for the whole period of the study.

Randomization

The randomization was done by an orthodontist who was the only person knowing the allocations. The method of randomization based on the attendance order of the approved patients. Approved attendees with odd and even numbers were respectively assigned to the control and treatment groups.

Funding: Self-funded (S. H.).
Conflict of interest: None to declare.
Blinding
The patients and the laboratory expert were all blinded of the bracket allocations. For this purpose, the patients were not informed of their bracket types, and the saliva samples were all coded.

Uniform treatment protocols
The used brackets in the control/experimental groups were macroscopically similar to each other in shape and size. In the control group (n = 15 patients), bonded 0.022 inch slot stainless steel conventional brackets (AISI 316L, Stratus, Fairfield Orthodontics, USA) on all teeth except the molars, as well as four conventional orthodontic tubes (Fairfield Orthodontics) and nickel titanium (NiTi) archwires (G&H, USA) were used for both arches. In the experimental group (n = 15 patients), bonded 0.022 inch slot stainless steel MIM brackets (AISI 316L, Gem Petit, Fairfield Orthodontics) on all teeth except the molars and four MIM orthodontic tubes (Fairfield Orthodontics) and NiTi archwires (G&H, USA) were used for bimaxillary treatment. The treatments were all non-ext, 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 (which can confound the ion release) from the study.

Saliva sampling
The sampling was performed twice, once immediately before the treatment, and once after 60 days. The patients were instructed, orally and in written, to avoid consumption of a given list of foods rich in nickel and chromium from 24 hours prior to the next visit; they were also told to remain fast in their visit’s morning until the sampling time (15). They were given written and oral instructions for hygiene maintenance. They were all given a single type and brand of toothpaste (Crest® Regular, Proctor & Gamble, USA) and were asked to use it for brushing during the treatment and at the night before their visit, which was scheduled in the morning. They were requested not to brush their teeth in their visit’s morning.

In the scheduled sessions, the participants were asked to rinse their mouth for 30 seconds with distilled water, then wait for 2 minutes and eject the unstimulated saliva into nickel-free 5-ml polyethylene tubes that were washed with distilled water and lab acetone beforehand.

Ion level measurement
In maximum a week, the saliva specimens were shipped to the Central Chemical Analysis Laboratory of Tehran University of Medical Sciences for atomic absorption spectrophotometry using a calibrated device (AA280Z GTA120, Varian, Mulgrave, Australia). One milliliter of saliva was centrifuged at 5000–8000 rpm; its debris and proteins were removed by Triton X100 surfactant; and it was diluted 1:5 with 0.1 per cent nitric acid. Each specimen was examined thrice, and the average ion concentration and its standard deviation and relative standard deviation (RSD = the standard deviation divided by the mean) were recorded for nickel and chromium metals in each specimen. These procedures were repeated for the 60th day of treatment.

Statistical analysis
There were no missing data. The difference between gender distributions of the two groups was evaluated using a chi-squared test. The difference between the average ages was evaluated using an independent-samples t-test. A repeated-measures two-way analysis of covariance (ANCOVA) was used to assess the effects of treatment and bracket types on the salivary ions. The difference between the control and experimental groups was assessed using a Mann–Whitney U 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. The correlations between gender and ion release were assessed using a point-biserial correlation coefficient. The ion changes over time were assessed using a Wilcoxon signed-ranks test. The before–after differences between the two time points were calculated for each ion in each patient. The before–after changes in the ion levels were compared between the two bracket types for each ion, using a Mann–Whitney U test. The level of significance was set at 0.05.

Results
Participant flow
A total of 48 patients were assessed until 30 patients were enrolled. Of the excluded patients, 15 did not meet the inclusion criteria, and three who had been included first, did not attend the second session (so were dropped out of the study and replaced by three new patients assessed from the beginning, Figure 1).

Sample characteristics
Demographics
The mean age of the included patients was 19.36 ± 5.56 years (19.20 ± 5.28 in the control group and 19.53 ± 5.93 in the MIM group). The average ages were similar between the two groups, according to the t-test (P = 0.752, Table 1). In the conventional group, there were nine males and six females; whereas, in the MIM group, 5 males and 10 females existed. The difference between the gender distributions of the groups was not statistically significant (chi-squared P = 0.269).

Baseline ion values
The Mann–Whitney U test showed no statistically significant differences between the control and MIM nickel (−1.5 µg/l, P = 0.901) and chromium (0.17 µg/l, P = 0.110) levels measured before the beginning of treatment.

Primary outcome 1: changes over time
Control group
After 2 months, in the control group (conventional brackets), nickel significantly increased for 5.45 µg/l (P = 0.0034, Wilcoxon). The 0.1 µg/l increase in the chromium level was not significant (P = 0.843, Wilcoxon).

Experimental group
In the MIM experimental group, nickel significantly increased for 0.24 µg/l (P = 0.0353, Wilcoxon) after 2 months. Chromium significantly reduced for 0.16 µg/l (P = 0.0438, Wilcoxon) after 2 months (Figure 2; Table 2).

Both groups combined
Overall, mean salivary nickel had a significant increase of 2.85 µg/l after 2 months (P = 0.0005, Wilcoxon) in both groups combined. However, chromium did not show a significant overall change (a 0.03 µg/l decrease, Wilcoxon P = 0.343, Table 3).
Primary outcome 2: differences between the two groups: control versus MIM

After 2 months of treatment

In the 60th day, no statistically significant differences between the control and MIM nickel (−3.7 µg/l, Mann–Whitney U P = 0.455) and chromium (0.09 µg/l, P = 0.720, Figure 1; Table 2) were observed.

Overall

Overall (both sessions combined), there were no significant differences between the nickel (P = 0.702) and chromium (P = 0.417) levels between the patients undergoing treatment with conventional (control) versus MIM (experimental) brackets, according to the Mann–Whitney U test.

Primary outcome 3: differences between the time-dependent changes of the two groups: control versus MIM

When the before–after changes in the two bracket groups were compared, a 5.211 ± 2.988 µg/l (95 per cent CI = −0.9434 to 11.337) difference between the means of the changes of nickel levels in the two bracket groups was observed. According to the Mann–Whitney U test, this difference was not statistically significant (P = 0.263). The difference between the time-dependent changes in chromium levels in the two bracket groups was 0.2613 ± 0.1877 µg/l (95 per cent CI = −0.1232 to 0.6458), which was not significant as well (Mann–Whitney P = 0.194, Table 4).

Secondary outcome 1: the effect of age and gender on the ion alterations

Bivariate correlations

There were no significant associations between patients’ age and any of the ion concentrations at any sessions (all four Spearman P > 0.28). As well, there was no correlation between the patients’ gender with any of the ions measured at any of the intervals (all four point-biserial correlation P > 0.17).

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

Nickel: According to the two-way repeated-measures ANCOVA used to assess the nickel ion, from a holistic perspective, the effects of treatment (F = 0.258, P = 0.616), bracket types (F = 0.011, P = 0.916), age (F = 1.061, P = 0.313), and gender (F = 0.887, P = 0.355) on nickel levels were not significant. The interactions were mostly non-significant, except for the interaction of treatment and bracket type that was significant (F = 4.808, P = 0.038), meaning that the pattern of nickel release differed between conventional and MIM brackets. The interaction of bracket type and gender was borderline significant (F = 3.677, P = 0.067).

Chromium: Chromium levels were not affected by treatment (ANCOVA’s F = 0.064, P = 0.803), bracket types (F = 0.089, P = 0.768), age (F = 1.464, P = 0.238), and gender (F = 0.035, P = 0.853). Most of the interactions were non-significant. However, the interaction of treatment and gender effects on chromium concentration was significant (F = 4.559, P = 0.043).
Table 1. Mean (µg/l), standard deviation (SD, µg/l) and relative standard deviation (RSD, per cent) of nickel and chromium levels in each patient immediately before treatment and 2 months after treatment initiation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age</th>
<th>Baseline (pre-treatment)</th>
<th>After 2 months of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nickel</td>
<td>Nickel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>1 M</td>
<td>24</td>
<td>1.45</td>
<td>0.03</td>
<td>2.3</td>
</tr>
<tr>
<td>2 F</td>
<td>11</td>
<td>5.09</td>
<td>0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>3 M</td>
<td>24</td>
<td>1.82</td>
<td>0.11</td>
<td>6.2</td>
</tr>
<tr>
<td>4 M</td>
<td>15</td>
<td>33.69</td>
<td>0.74</td>
<td>22.2</td>
</tr>
<tr>
<td>5 F</td>
<td>17</td>
<td>5.73</td>
<td>0.10</td>
<td>1.7</td>
</tr>
<tr>
<td>6 M</td>
<td>16</td>
<td>12.57</td>
<td>0.20</td>
<td>1.6</td>
</tr>
<tr>
<td>7 F</td>
<td>13</td>
<td>9.84</td>
<td>0.19</td>
<td>1.9</td>
</tr>
<tr>
<td>8 F</td>
<td>16</td>
<td>5.99</td>
<td>0.13</td>
<td>2.2</td>
</tr>
<tr>
<td>9 F</td>
<td>17</td>
<td>4.07</td>
<td>0.08</td>
<td>2.0</td>
</tr>
<tr>
<td>10 M</td>
<td>23</td>
<td>1.39</td>
<td>0.06</td>
<td>4.6</td>
</tr>
<tr>
<td>11 F</td>
<td>27</td>
<td>0.51</td>
<td>0.00</td>
<td>0.5</td>
</tr>
<tr>
<td>12 M</td>
<td>22</td>
<td>5.31</td>
<td>0.38</td>
<td>7.1</td>
</tr>
<tr>
<td>13 M</td>
<td>24</td>
<td>7.3</td>
<td>0.36</td>
<td>4.9</td>
</tr>
<tr>
<td>14 F</td>
<td>13</td>
<td>9.47</td>
<td>0.51</td>
<td>5.4</td>
</tr>
<tr>
<td>15 M</td>
<td>26</td>
<td>2.5</td>
<td>0.06</td>
<td>2.2</td>
</tr>
</tbody>
</table>

MIM brackets (experimental)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Nickel</th>
<th>Chromium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>16 F</td>
<td>12</td>
<td>0.92</td>
<td>0.07</td>
<td>7.2</td>
</tr>
<tr>
<td>17 F</td>
<td>16</td>
<td>1.42</td>
<td>0.03</td>
<td>2.1</td>
</tr>
<tr>
<td>18 F</td>
<td>18</td>
<td>7.54</td>
<td>0.08</td>
<td>1.0</td>
</tr>
<tr>
<td>19 F</td>
<td>13</td>
<td>12.15</td>
<td>0.07</td>
<td>0.6</td>
</tr>
<tr>
<td>20 F</td>
<td>23</td>
<td>1.5</td>
<td>0.11</td>
<td>7.4</td>
</tr>
<tr>
<td>21 M</td>
<td>15</td>
<td>1.69</td>
<td>0.15</td>
<td>7.2</td>
</tr>
<tr>
<td>22 F</td>
<td>25</td>
<td>1.37</td>
<td>0.05</td>
<td>3.3</td>
</tr>
<tr>
<td>23 F</td>
<td>26</td>
<td>3.82</td>
<td>0.32</td>
<td>8.3</td>
</tr>
<tr>
<td>24 M</td>
<td>20</td>
<td>3.77</td>
<td>0.33</td>
<td>8.7</td>
</tr>
<tr>
<td>25 F</td>
<td>12</td>
<td>1.94</td>
<td>0.19</td>
<td>9.6</td>
</tr>
<tr>
<td>26 M</td>
<td>21</td>
<td>36.6</td>
<td>0.88</td>
<td>2.4</td>
</tr>
<tr>
<td>27 F</td>
<td>23</td>
<td>13.26</td>
<td>0.70</td>
<td>5.3</td>
</tr>
<tr>
<td>28 M</td>
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<td>20.21</td>
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<td>2.3</td>
</tr>
<tr>
<td>29 F</td>
<td>31</td>
<td>15.7</td>
<td>0.35</td>
<td>2.1</td>
</tr>
<tr>
<td>30 M</td>
<td>25</td>
<td>7.35</td>
<td>0.30</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Mean, SD, and RSD are calculated based on three measurements per saliva specimen from each patient. ND, not detectable by the device (i.e. when all three chromium values per specimen were below detectable level, which was <0.25 µg/l). These values were considered zero in the statistical analyses.

Secondary outcome 2: correlations between pretreatment and treatment ion concentrations

There was a significant positive correlation between the nickel ion amounts measured at both time points ($r = 0.612, P = 0.000$) but not between chromium levels at the two time points ($r = 0.226, P = 0.229$).

Discussion

Nickel and chromium can be corroded through various mechanisms. A simple redox reaction with the environment might corrode the metal; this can be enhanced by pH reduction after ingesting acidic diets or caused by anaerobic conditions induced by the biofilm (4, 6, 11, 13, 21). In this study, nickel ion increased significantly in both bracket groups, and the increase caused by the conventional brackets was slightly greater. Similar statistically significant but subtle increases have been shown in other studies after appliance placement.
## Table 2. Descriptive statistics for the ion values (all in µg/l units) in both groups and intervals.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Interval</th>
<th>Bracket</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Q1</th>
<th>Med</th>
<th>Q3</th>
<th>Max</th>
<th>95% CI</th>
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</thead>
<tbody>
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<td>Ni</td>
<td>Baseline</td>
<td>Control</td>
<td>7.12</td>
<td>8.14</td>
<td>0.51</td>
<td>1.82</td>
<td>5.31</td>
<td>9.47</td>
<td>33.69</td>
<td>2.61 - 11.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MIM</td>
<td>8.62</td>
<td>9.85</td>
<td>0.92</td>
<td>1.5</td>
<td>3.82</td>
<td>13.26</td>
<td>36.6</td>
<td>3.16 - 14.07</td>
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<td></td>
<td>60th day</td>
<td>Control</td>
<td>12.57</td>
<td>9.96</td>
<td>1.2</td>
<td>4.1</td>
<td>12.94</td>
<td>14.97</td>
<td>36.6</td>
<td>7.05 - 18.08</td>
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<td></td>
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<td>MIM</td>
<td>8.86</td>
<td>6.42</td>
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<td>4.21</td>
<td>5.42</td>
<td>13.9</td>
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<td>5.3   - 12.41</td>
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<tr>
<td>Cr</td>
<td>Baseline</td>
<td>Control</td>
<td>0.25</td>
<td>0.56</td>
<td>0</td>
<td>0</td>
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<td>2.16</td>
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<td>0.15</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>60th day</td>
<td>Control</td>
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<td>0.53</td>
<td>1.89</td>
<td>0.01</td>
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<td></td>
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<td>0.27</td>
<td>1.8</td>
<td>-0.06</td>
<td>0.57</td>
</tr>
</tbody>
</table>

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

## Table 3. Descriptive statistics for the ion values (all in µg/l units) in both intervals regardless of the brackets used.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Interval</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Q1</th>
<th>Med</th>
<th>Q3</th>
<th>Max</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>Baseline</td>
<td>7.87</td>
<td>8.91</td>
<td>0.51</td>
<td>1.64</td>
<td>5.2</td>
<td>10.42</td>
<td>36.6</td>
<td>4.54 - 11.19</td>
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<td>8.44</td>
<td>1.2</td>
<td>4.19</td>
<td>7.51</td>
<td>14.46</td>
<td>36.6</td>
<td>7.56 - 13.86</td>
</tr>
<tr>
<td>Cr</td>
<td>Baseline</td>
<td>0.34</td>
<td>0.52</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.48</td>
<td>2.16</td>
<td>0.14 - 0.53</td>
</tr>
<tr>
<td></td>
<td>60th day</td>
<td>0.3</td>
<td>0.59</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.28</td>
<td>1.89</td>
<td>0.08 - 0.52</td>
</tr>
</tbody>
</table>

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

## Table 4. Descriptive statistics for the before–after differences of ion levels (all in µg/l units) between the two intervals for each bracket type.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Bracket</th>
<th>Mean diff</th>
<th>SD</th>
<th>Min</th>
<th>Q1</th>
<th>Med</th>
<th>Q3</th>
<th>Max</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>Control</td>
<td>5.45</td>
<td>6.95</td>
<td>-2.25</td>
<td>0.57</td>
<td>3.47</td>
<td>9.24</td>
<td>22.75</td>
<td>1.61 - 9.3</td>
</tr>
<tr>
<td></td>
<td>MIM</td>
<td>0.24</td>
<td>9.26</td>
<td>-32</td>
<td>0.94</td>
<td>1.93</td>
<td>3.49</td>
<td>7.2</td>
<td>-4.89 - 5.37</td>
</tr>
<tr>
<td>Cr</td>
<td>Control</td>
<td>0.1</td>
<td>0.65</td>
<td>-0.91</td>
<td>-0.33</td>
<td>0</td>
<td>0.26</td>
<td>1.89</td>
<td>-0.26 - 0.46</td>
</tr>
<tr>
<td></td>
<td>MIM</td>
<td>-0.16</td>
<td>0.33</td>
<td>-0.73</td>
<td>-0.48</td>
<td>0</td>
<td>0</td>
<td>0.46</td>
<td>-0.35 - 0.02</td>
</tr>
</tbody>
</table>

The differences are calculated by subtracting the ion levels in the first interval from the ion levels in the second interval.

CI, confidence interval for the mean of before–after difference; Cr, chromium; Mean diff, the mean of paired differences between two intervals (the second interval’s result minus the first interval’s result) for each ion in each patient; Max, maximum; Min, minimum; Ni, nickel; SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile.

...or a month later (10, 15, 27). Nickel showed large interindividual variations that might be due to changes in saliva formulation under the influence of physiologic factors such as pH and flow rate, health conditions, diet, the time of day, psychological conditions, and adhesion of nickel to epithelial cells, bacteria, and salivary macromolecules (1, 4, 10, 11, 14, 15, 17, 21).

Most of previous studies found either no significant changes in ion levels (4, 14, 15, 17, 21, 25, 27), or a higher release rate in the treatment group compared to the pre-treatment group (similar to our results pertaining to nickel) (10, 11, 26). A few reports showed small declines in nickel (10) or chromium levels (4, 10, 15). In this study, chromium did not show any notable change in the control group (conventional brackets). In the MIM group however, chromium reduced for a trivial but still significant amount. The difference between the two bracket types might be justified by a higher corrosion resistance of MIM brackets (23, 28). The elemental composition of MIM might vary slightly from conventional brackets (23, 28), although metal ion release might not be necessarily associated with the metal ratios in the alloys (29). When used with NiTi archwires, MIM might show a less galvanic corrosion tendency than do conventional brackets (23, 28), although it might exhibit a higher rate of pitting corrosion because if its porosities (23). Nevertheless, the differences of these bracket types cannot justify the observed decline to levels below pre-treatment, in the same MIM group of this study. Dropping below the pre-treatment threshold implies that chromium ion was absorbed by the added brackets or the plaque covering them. It is not known why this happened in the MIM group only; however, it might be related to the larger granular structure and the greater number of micropores of MIMs (that undergo a different production process) (23, 28). A rough bracket surface might allow better accumulation of microorganisms and disallow effective cleansing. It is known that plaque microorganisms can considerably aggregate metal ions by adhering with glycoproteins or older ions in the biofilm (1, 3, 4, 8, 9, 21), and might thicken because of difficulties in oral hygiene maintenance during the treatment (21, 22, 30). Therefore, a thickened and more mature plaque potentially available on rougher MIM brackets might absorb more ions (21). It should be noted that the proposed mechanism (if correct) might not affect the increases in the systemic intake. In spite of decreases in salivary ions (or the absence of notable increases in other studies) (14, 15, 17, 21, 25, 27), the additional ions accumulated in the biofilm might still be adsorbed into the bloodstream through intake of plaque (1). Nevertheless, the chromium changes in the MIM group and the increases in salivary nickel in both bracket groups were still far...
below dietary levels (4). Another reason for the decreases observed might be the limitation of the spectrophotometer in identifying chromium levels less than 0.25 µg/L; since these values are considered zero, this can add some false negative artefacts and reduce the average values more than their real (undetected) values. Nevertheless, the non-parametric tests used might be robust to this limitation.

Nickel as well exhibited a statistically different pattern of increase over time between the two bracket types. Although both bracket groups had significant increases over time, their increase pattern significantly differed: the increase in the MIM group was much smaller than the increase in the conventional group. This can be attributable to a combination of factors such as a higher rate of nickel absorption by a thicker plaque on MIM brackets as well as potentially lower rates of nickel release from them. This cannot be clarified unless in vitro studies evaluate salivary and plaque ion concentration simultaneously. Still, it is shown that plaque nickel concentrations might be much higher than filtered saliva, and that the biofilm formed over teeth with metal surfaces is richer in nickel content compared to enamel (21). Moreover, although our aim was to compare the bracket types, the source of ion release was not exclusively the brackets, but was a combination of brackets and archwires. The same archwires shared between the two (conventional/MIM) groups could overshadow the actual discrepancy existing between the brackets’ results. This might be a reason for the lack of significance observed between measured ions of the two bracket groups in the 60th day. Future in vitro studies should compare these brackets, in the absence of any archwires.

This study as well as earlier research (4, 10, 11, 14, 15, 17, 21, 25, 27) confirmed that orthodontic appliances might not increase nickel and chromium to daily intake levels (50–280 µg/day for chromium and 100–800 µg/day for nickel) (4, 6, 14, 21). This might imply the lack of toxicity. Nevertheless, low but chronic release of corroded metal ions might still produce inflammation, or inflict cellular or DNA damage (3, 4, 9, 16, 17). Besides the allergic effects, cytotoxicity and genotoxicity are also assigned to nickel and, to a lesser degree, chromium (8, 16), which should be the main clinical concern (3). These ions might have both dose-dependent and dose-independent effects. It is not clearly known that how much cytotoxic metals released from orthodontic appliances is absorbed by the organism (3, 9, 11, 15, 31, 32). Metals are not biodegradable, and their sustained release and accumulation in the tissues might leave irreversible toxic influences (3). Even the current trivial dose of corroded nickel and chromium ions is sufficient to damage the DNA, activate endothelial cells or monocytes or to modify cellular metabolism and morphology, especially in long-term exposures (3, 9, 16, 17). Besides, dosage-independent mutagenic effects of nickel and chromium such as induction of single-strand DNA breakage, DNA fragmentation, increases in DNA migration and comet formation, inhibiting DNA restoring enzymes and several other known or unidentified mechanisms should be taken into consideration (3, 8, 9, 16, 17). Nevertheless, most of corrosion products of nickel and chromium are unlikely to be carcinogen or toxic, apart from hexavalent oxides of chromium and more insoluble compounds of nickel (9, 31). Additionally, the DNA damage might be reversed (after appliance removal) in patients with intact DNA repair mechanisms (16).

Contact allergy has been generally considered the most common adverse effect of nickel in orthodontics. Nevertheless, even more than its sensitizing effects, nickel can exercise an influence over periodontal status in sensitive patients, predisposing them to periodontitis (7, 22, 26, 32). Nickel is the most common source of short- or long-term sensitivity through type-IV cell-mediated immune response. Up to 28.5 per cent of people worldwide might have hypersensitivity to nickel, including 2–5 per cent of males and 20–30 per cent of females (7, 22, 26, 32). Second in frequency is chromium with about 3 per cent allergy prevalence in women and 10 per cent in men (15, 26). It is well understood that corroded nickel may trigger soft tissue inflammation, leading to dermatitis and irritation (1, 6, 20). The allergic stomatitis caused by orthodontic appliances needs immediate treatment by removing the appliance (7). It characterizes by lip desquamation, gingivitis, gingival hyperplasia, changes in colour, multiform erythema, bleeding upon probing, periodontitis, angular cheilitis, burning sensation in the mouth, and metallic taste (4, 7, 22, 31). The allergic influence of corroded nickel is cumulative and appears mostly after 9 or 12 months of treatment (22); however, not all investigators consider the time factor as a major variable (6, 7). Some authors have postulated that orthodontic treatment does not necessarily cause allergy. Instead, it might even desensitize patients by improving the tolerance of their immune system (6, 12). Moreover, certain researchers suggest that short-term use of orthodontic appliances neither will sensitize the patients nor will induce tolerance to nickel (33).

Nickel has been usually found to be much more concentrated than chromium (3, 4, 9–11, 15, 16), except in few studies where chromium was at higher levels (14, 17, 25). The results of this study pertaining to the ion levels in random subjects not undergone orthodontic treatment were similar to the mostly low rates reported as 0.53 (14) to 11.9 (11) µg/L for nickel and 0.64 (10) to 3.9 µg/L (4) for chromium, except some and very high concentrations (25, 26). The controversy might root in the salivary composition dynamism, genetics, difference in bacterial colonization, patients’ dietary and smoking habits, galvanic currents produced, air and water trace elements, or sampling details such as the time between rinsing with distilled water and saliva collection as well as the stimulation of saliva secretion (4, 11, 14, 17, 21, 26, 32, 34). The same factors might also contribute to the different pattern of chromium alteration in males compared to females.

Limitations and generalization

This study was limited by some factors. A sample size based on power calculations might favour the reliability of the results. However, this controlled randomized trial with its long list of exclusion criteria had a sample size comparable or larger than almost all other available clinical studies. As an advantage, in vivo designs allow the assessment of the effect of appliances in their natural functional conditions (which this might improve the generalizability). However, the existence of several known and unknown confounding variables can reduce the accuracy (4, 11, 14, 32, 34). Biologic variations introduced by each patient might confound the standardization of the methods and render the interpretation of the results quite difficult (3). On the other hand, this study benefited from precise inclusion criteria, which ruled out many variations. Some of the exclusion criteria were unique to this study and allowed a more balanced design and a better control over the confounders, while the before–after nature of the clinical trial allowed matching of patients with themselves. Besides, balancing the ages and genders between the two groups was another advantage. Moreover, the short sampling period in clinical studies and continuous flow of saliva might not give time sufficient for release of a considerable dissolution of nickel and chromium from the fixed appliances (14). Another unavoidable limitation of all in vivo studies to date is that the daily pattern of ionic discharge is not completely...
known; it is possible that the pattern of ion release is not steady and constant, probably peaking after ingesting the food, because of the pH reduction (3, 4, 17, 21). However, most of in vivo studies have avoided sampling immediately after eating in order to reduce its confounding effect, however at the cost of reducing the generalizability (4). As a limitation of all prospective studies including this clinical trial, almost no prospective research had assessed patients for durations beyond 2 months (4). On the other hand, this randomized clinical trial (unique to the literature) was advantageous over previous designs from other aspects, as the earlier studies were rather small (3, 10, 27, 31, 32), retrospective (9, 11) or descriptive (15), longitudinal (10, 27, 31, 32), and all lacking a clinical trial design. However this study was limited by the use of archwires that could as well release metal ions and reduce the contrast between the two groups. Finally, the spectrophotometer was limited in detecting extremely low chromium levels (below 0.25 μg/l) which could introduce some error to the results of parametric (but not non-parametric) analyses. On the other hand, the device's accuracy was confirmed by the very small relative standard deviations.

Conclusions

Within the limitations of this study, it was shown that chromium ion might reduce in saliva after 2 months of initial orthodontic treatment with MIM brackets. Nonetheless, chromium might not differ after 2 months, if conventional brackets are used. Nickel might increase after 2 months, regardless of the bracket type in use. However, the pattern of increase depends on the bracket type and its increase would be smaller if MIM brackets are used. Despite the higher rate of increase in the conventional brackets, 2 months of nickel ion increase at different rates might not end in a considerable difference. Overall, the ion changes were trivial in terms of clinical significance, and might confirm the safety of orthodontic treatment as long as only the ion concentrations matter.

Age and gender might have no influence on metal ion levels of normal people or orthodontic patients undergoing the initial stage of treatment for 2 months. Chromium changes might be affected by the patient's gender.

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References


