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Evaluation of Salivary Nickel, Chromium and Iron Ions in Patients Treated with Fixed Orthodontic Appliances in Vivo Study

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Background and objectives: Most fixed orthodontic appliances are made of stainless steel and Nickel-Titanium alloys, which can release metal ions into the oral cavity. The present study aimed to determine the release of Nickel, Chromium and Iron ions into saliva of patients treated with a fixed orthodontic appliance.

Subjects and methods: Saliva samples from 18 patients (9 males and 9 females) between 15-25 years were taken at three different time points, group A: before placement of the appliance directly (baseline), group B: one month after appliance placement, Group C: four months after appliance placement. The fixed appliance consists of 20 stainless steel brackets, 4 buccal tubes, and superelastic Nickel-Titanium archwires. Level of ions in salivary samples was analyzed by Inductively Coupled Plasma⁄ Optical Emission Spectrometry. Ions recorded in parts per billion. Statistical analysis was performed by nonparametric tests (Friedman) and one way repeated measures ANOVA.

Results: Level of Nickel, Chromium and Iron ions in saliva were highest in group B and lowest in group A. on a pairwise comparison between different groups, it was statistically significant for all groups (< 0.05) except for Iron levels between group A and group C which was not statistically significant.

Conclusion: Nickel, Chromium and Iron levels in saliva were increased after the placement of fixed orthodontic appliance.

Keywords: Ions release; Saliva; Fixed orthodontic appliance; Nickel; Chromium

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Introduction

Most fixed orthodontic appliances such as brackets, bands and archwires are made of stainless steel, which consists of 8-12% Nickel (Ni), 17-22% Chromium (Cr), and different proportions of manganese (Mn), copper (Cu), Titanium (Ti) and Iron (Fe).[1,2,3] Stainless steel has resistance to stain and corrosion, nevertheless, they are not resistant to temperature, or the microbiological and enzymatic environments in the oral cavity. A high percentage of Ni (50%) can be found in NiTi (Nickel-Titanium) wire.[4,5] Fixed orthodontic appliances in the oral cavity are exposed to chemical and mechanical damage increases the susceptibility to corrosion. Corrosion promotes loss of elements from the material, causing alteration in the structural characteristics of the material, or loss of structural integrity as a result of the electrolytic abilities of saliva. Various forms of electrochemical corrosions can occur in the oral cavity.[6,7,8] The corrosion of orthodontic appliances and their consequent release of metal ions in the oral cavity is conducted by two major factors.

The first is the construction procedure, which consists of the type of alloy and the nature of the metals used, the second is environmental factors, like mechanical factors, time of the day, stress, diet, salivary flow rate, health and psychosomatic condition of the person.[9,10,11]

The result of most studies on the amounts of metal ions released from orthodontic alloys had displayed that they were lower than the recommended daily dietary intakes of Ni and Cr, this might be a false positiveness of safety, as long as chronic low levels of metal ions can change cellular metabolism and morphology, and lead to inflammation and even DNA instability. In addition, some in-vivo researches showed biologic toxicity in orthodontic patients.[12, 13]

Few clinical studies on the orthodontic metal ion release lack any clinical trials, thus and in view of the lack any assessments of orthodontic materials in-vitro or clinically, the purpose of the present study aimed to evaluate the salivary amount of Nickel, Chromium and Iron ions in orthodontic patients before treatment, one month and four months after the beginning of treatment with fixed orthodontic appliances.

Subjects and methods

The present study has been carried out at the Department of Pedodontics, Orthodontics and Preventive Dentistry at the College of Dentistry - Hawler Medical University, the ethics of the study approved from the College of Dentistry - Hawler Medical University through a written letter, and written consents have been taken from the subjects or their parents after thorough oral and written explanation.

The samples of this in-vivo study comprised 18 subjects (9 males and 9 females) in the age range of 15–25 years with different malocclusions that needed to be treated by fixed orthodontic appliances. The orthodontic appliance for present study consists of primarily 4 stainless steel buccal tube (Ortho-Cast M-Series, non-convertible (Figure 1), DENTARUM, Ispringen, Germany), 20 stainless steel brackets (equilibrium® 2 "Roth 22", DENTARUM Ispringen, Germany) (Figure 2), upper and lower Nickel-Titanium wires (Super elastic, 0.012, 0.014, 0.16, 0.018 rematitan® “LITE” ideal arch, round, DENTARUM, Ispringen, Germany) (Figure 3). The inclusion criteria for the present study were as follows:

1.Healthy patient with no history of significant medical problems.
2. Patient in the permanent dentition stage
3. Patient has no metallic crowns, bridges, or any other Nickel, Chromium and Iron containing restorations.
4. Patient had not received previous orthodontic treatment

Figure 1: Buccal tubes for first molars.

In order to measure the concentration of metal ions such as Nickel, Chromium and Iron in saliva, unstimulated saliva of these 18 patients were collected in different time points, samples taken before the placement of the appliance, after one and four months of the placement of the appliance.

Figure 2: Stainless steel brackets.

Thus, a total 54 saliva samples were obtained which were divided into three groups:

• Group A: sample was taken before placement of appliance directly.

• Group B: sample was taken one month after appliance placement.

• Group C: sample was taken four months after appliance placement.

Patients were requested to use non-fluoridated one type and one brand of toothpaste (Forever Bright® Toothgel, Forever Bright Sparkling Aloe Vera Tooth gel) for brushing during the study period. The sampling session was scheduled in the morning. The patients were instructed, orally and in writing, to avoid consumption of a given list of foods rich in Nickel, Chromium and iron 24 hours prior to the next visit; they were also told to remain fast in their visit's morning until the sampling time. They were given written and oral instructions for hygiene maintenance.[14]

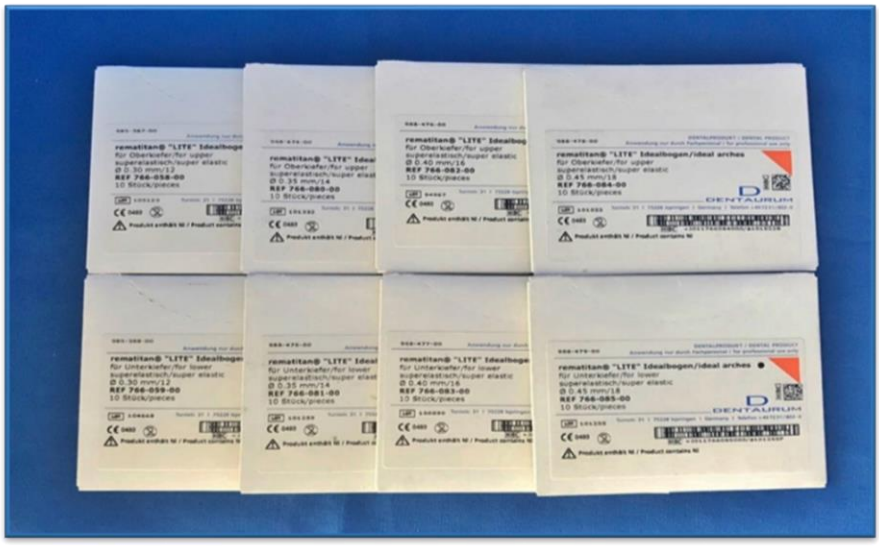

Figure 3: Super elastic Nickel-Titanium arch wire (round 0.012, 0.014, 0.016, and 0.018 for upper and lower arches).

Saliva Collection. Salivary samples had been taken between 9 a.m. and 12 p.m., at least 2 hours after oral hygiene procedures in order to minimize the effects of diurnal variability in salivary composition.[13] Sample collection have been carried out such that, after rinsing with 15 ml of distilled and deionized water for 30 seconds, after that each subject asked to rest and close his or her mouth for 5 minutes in order to collect saliva in his or her mouth without any stimulation, after 5 minutes each subject asked spitting 5 ml of saliva directly into 10 ml sterilize polypropylene tube.[15]

The samples were kept at −20 °C until they were processed and diluted with deionized water to eliminate interference and to reduce the effects of the biological matrix (protein, salt, etc.) as described by Dwivedi, et al.[13]

Salivary samples were analyzed by Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES ARCOS), the concentration of Nickel, Chromium and Iron hd been recorded in Nanogram per milliliter equal to parts per billion (ppb). The analysis of salivary samples had been carried out at Ministry of Higher Education and Scientific Research/ University of Garmian/ Advisory office for analysis.

Statistical Analysis. The statistical analysis has been done by using the Statistical Package

for Social Sciences (SPSS) version 22.0 statistical analysis software. Descriptive statistics were calculated including mean, standard deviation, the Shapiro-Wilk normality test was applied to data to check normality, Nickel and Chromium were not normally distributed so the non-parametric Friedman test used to show the Nickel and Chromium concentrations within subjects in different time points when the result of Friedman test was significant then the post hoc Wilcoxon test applied in order to compare between two time points, the Iron was normally distributed so the one way repeated measures ANOVA used to show the presence of difference of Iron within subjects in different time points then post hoc test applied to compare between two time points of Iron level in saliva, *P* value < 0.05 was considered significant.

Results

Table 1 showed the results of mean, standard deviation and Friedman test of salivary Nickel level of baseline (A), one month (B) and four months (C) after appliance placement. Salivary Nickel level of one month after appliance placement (B) showed the highest mean (34.22 ± 44.85). Friedman test showed overall statistically significant difference of salivary Nickel level at three different time points during the study period ($P<0.05$) and post hoc (Wilcoxon test) showed statistically significant difference of Nickel level between every two time points $P<0.05$ (Table 2).

Table 1: Descriptive statistics and Comparison of salivary Nickel level (ppb) at three time points by Friedman test.

Groups	N	Mean	Standard Deviation	chi-test	Sig.
Ni Baseline (A)	18	3.95	8.193	25.33	<0.001
Ni After 1 month (B)	18	34.22	44.85		
Ni After 4 months (C)	18	9.72	2.27		

P value ≤0.05 is considered significant

Table 2: Comparison salivary Nickel level in between different groups (Wilcoxon test).

Comparison of groups	Z	Sig.
Ni Baseline (A) - Ni After 1 month (B)	-3.595	<0.001
Ni Baseline (A)- Ni After 4 months (C)	-2.733	0.006
Ni After 1 month (B) - Ni After 4 months (C)	-3.683	<0.001

P value ≤0.05 is considered significant

Table 3 showed the lowest mean of Chromium level at the baseline (A) (0.34±1.47). Friedman test showed overall statistically significant difference among three time points of study (P<0.05). The result of post hoc Wilcoxon test showed in Table 4, there was statistically significant difference of Chromium levels between every two time points (P<0.05).

Table 5 showed salivary Iron level at three time points, Iron level at baseline (A) showed lowest mean (45.1±29.3), the result of one way repeated measures ANOVA showed there was statistically significant difference of salivary Iron level of patients with fixed orthodontic appliance at three different time points P=0.002, and the LSD post hoc showed there were statistically significant difference of Iron levels between two pairs (A) with (B) (P<0.05), (B) with (C) (P<0.05). But there was no statistically significant difference between Iron level at the baseline (A) and Iron level at four months after appliance placement (C) P> 0.05 (Table 6).

Table 3. Descriptive statistics and Comparison of salivary Chromium levels (ppb) at three time points by Friedman test.

Groups	N	Mean	Standard Deviation	chi-test	Sig.
Cr Baseline (A)	18	0.34	1.47	36	<0.001
Cr After 1 month (B)	18	17	5.40		
Cr After 4 months (C)	18	8.5	4.66		

P value ≤0.05 is considered significant

Table 4. Comparison salivary Chromium level among different groups (Wilcoxon test).

Comparison of groups	Z	Sig.
Cr Baseline (A) - Cr After 1 month (B)	-3.595	<0.001
Cr Baseline (A)- Cr After 4 months (C)	-2.733	<0.001
Cr After 1 month (B) – Cr After 4 months (C)	-3.683	<0.001

P value ≤0.05 is considered significant

Table 5. Descriptive statistics and Comparison of the salivary Iron level (ppb) at three time points by repeated measure ANOVA test.

Groups	N	Mean	Standard Deviation	F	Sig.
Fe Baseline (A)	18	45.1	29.3	7.7	0.002
Fe After 1 month (B)	18	99.4	65.8		
Fe After 4 months (C)	18	59.7	42.3		

P value ≤0.05 is considered significant

Table 6: Pairwise Comparisons of salivary Iron level among different groups (LSD post hoc).

(I)	(J)	Mean Difference (I-J)	Sig.
Fe Baseline (A)	Fe After 1 month (B)	-54.3	<0.001
Fe Baseline (A)	Fe After 4 months(C)	-14.6	0.220
Fe After 1 month(B)	Fe After 4 months(C)	39.6	0.35

P value ≤0.05 considered significant

Discussion

In the past several in-vitro studies[6,16-18] have been conducted to show the release of metal ions such as Nickel, Chromium, and Iron from fixed orthodontic appliances which have been immersed in the artificial saliva, the result of these studies reported that the concentrations of these metal ions were below the toxic dose to human.

Present study was in vivo because in vivo have many advantages, the first one saliva in the oral cavity has the dynamic composition, the release of an ion is affected by many factors as quantity and quality of saliva, temperature, pH, physical and chemical properties of food and liquid, psychological condition and general oral health condition of person which make results better and reliable. Another advantage in-vitro study used the artificial saliva and this artificial saliva go through precipitation over an extended period of time as saliva during the experiment is not changed or allowed to flow through the system like in the oral cavity.[13,19]

To eliminate any gender bias, an equal number of males and females were included in the present study since the frequency of Nickel hypersensitivity has been reported being 10 times more in females than in males.[9] Patients of present study were requested to use non-fluoridated mouthwash due to the proven effect of fluoride on ion release.[20]

In general, the results in the present study indicated that there were over all significant increase of salivary Nickel, Chromium and Iron level of orthodontic patient after fixed orthodontic appliance placement, release of salivary Nickel was between 3.95 and 34.22 ppb, salivary Chromium between 0.34 and 17 ppb and salivary Iron between 45.1 and 99.4 ppb. There were large variations in present

study, and similarly large variations have been found in previous reports of metal concentrations in saliva.[21,22]

In the present study, the results indicated that the Nickel, Chromium and Iron levels in saliva at one month after fixed appliance placement (group B) was significantly higher than baseline (group A), these findings are in accordance with study by Dwivedi et al.[13] They reported significant increase in Nickel and Chromium levels in the saliva of orthodontic patients at one month after placement of fixed orthodontic appliances in relation to the baseline. Similar results were also reported by Arash[23] et al. and Satija et al.[24] while contrasting results were reported by Matos de Souza and Macedo de Menezes et al.[25] They reported reduction of Nickel, Chromium and Iron in saliva of patients with orthodontic appliances at one month after appliance placement as compared with baseline. The difference among results of these studies might be due to many factors such as differences in temperature, quality and quantity of saliva, plaque, proteins and physical and chemical properties of diet taken and also might be due to different methods used to analyze the levels of the metal ions in saliva or sample selection.

The results of the present study clarified that the levels of Nickel and Chromium in saliva at four months after appliance placement (group C) was significantly higher than baseline (group A) while significantly lower than one month after appliance placement (group B). Since the possibility of releasing metal ions from orthodontic alloys is a time critical phenomenon, so the release of metal ions changes over a period of time.

The results of the present study indicated that the level of Iron in saliva at four months after appliance placement (group C) was higher than baseline (group A) without significant difference, the reason for this might be the high amounts of Iron ion present in the mouth, even before placement of the fixed orthodontic appliances. Whereas, Iron level in group C were significantly lower than at one month after appliance placement (group B). Similar results reported by Matos de Souza and Macedo de Menezes et al.25 They did not find any significant increase in salivary Iron two months after fixed orthodontic appliance. Dissimilar results with the present study reported by Shetty et al.[26] they found an increase of Iron levels in saliva at six months after appliance placement in comparison to baseline with statistically significant difference.

In the present study the amount of metals released from fixed orthodontic appliances in saliva (165 μg/day for Nickel, 150 μg/day for Chromium, 13 mg/day for Iron) are significantly below the average daily dietary intake (The WHO recommended daily doses (RDD) for Nickel, 25–35 μg/day; Chromium, 50–200 μg/day; Iron, 10–18 mg/day)[21] and did not reach toxic levels, which is in agreement with previous researches. Although it can be a false insurance of safety because even non-toxic levels could be adequate to promote biological effects in cells of the oral mucosa.[9]

Conclusion

Nickel, Chromium and Iron levels in saliva were significantly increased after the placement of fixed orthodontic appliances but were below the toxic levels of the ions.

Maximum level of Nickel, Chromium and Iron in saliva were found after one month of appliance placement then they decreased.

Conflicts of interest

The authors reported no conflicts of interest.

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