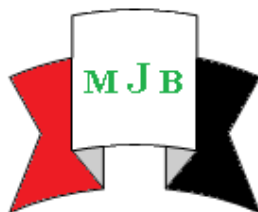


Nickel And Chromium Ions Levels In Saliva Of Patients With Fixed Orthodontic Appliances

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Abstract:

Objective : The aim of this study was to test the hypothesis that there is no difference in salivary metal ion content between subjects with fixed orthodontic appliances and their same-gender sister or brother without any orthodontic appliance.

Materials and Methods: Fifty orthodontic patients were included in this study. The first group consisted of 25 patients (14 female, 11 male) with fixed appliances placed in their upper and lower arches. The second group consisted of 25 patients (13 female, 12 male) with a fixed appliance placed only in the upper arch. In order to limit the effects of dietary and hygiene habits on salivary metal ion concentration, a same-gender brother or sister (total of 50 subjects) was selected as a control (25 female, 25 male) who were not undergoing orthodontic treatment. Four samples of stimulated saliva were collected from each patient before insertion of the fixed appliance, 1 month after insertion of the appliance, 2 months after insertion of the appliance and 6 months after insertion of the appliance. The 4 samples of saliva were collected from each control patient at the same time intervals as for the fixed-appliance groups. Approximately 5 ml of saliva was collected from each subject. Saliva samples were analyzed for nickel, chromium, by electrothermal atomic absorption spectrophotometry. The detection limit of the method for sample solutions was 1 ng/ml. Statistical analysis was performed by nonparametric tests (*Mann-Whitney U* and *Wilcoxon W*). The *Wilcoxon matched pairs* signed ranks used to test differences between samples before and after insertion of the orthodontic appliances. A *Kruskal Wallis 1-way* analysis of variance was used to test differences in nickel and chromium concentration among the 3 test groups. A *Kruskal Wallis 1-way* analysis of variance (*ANOVA*) was used to test differences in nickel and chromium concentrations among the 3 test groups.

Results: The mean salivary nickel (Ni) content in subjects with and without a fixed orthodontic appliance was 16.8 ± 12.2 ng/ml and 10.3 ± 11.7 ng/ml, respectively. A statistically significant difference ($P < 0.041$) was found between the two groups. The mean salivary chromium (Cr) ion level recorded was 2.4 ± 1.8 ng/ml in the study group and 2.1 ± 1.6 ng/ml in the control group. The difference, however, was statistically insignificant.

Conclusion: Nickel and chromium ion concentrations increased immediately after placement of the appliance in the mouth for all study groups. There were no significant differences in the nickel ,chromium levels released by the three groups of appliances at all study periods. Within the limits of this in vivo study, it can be concluded that the presence of fixed orthodontic appliances leads to an increased concentration of metal ions in salivary secretions

Key Words: Nickel; Chromium ;saliva ; Fixed orthodontic appliances.

مستويات ايونات النيكل والكروم في لعاب المرضى الحاملين للاجهزة القويمية الثابتة
(دراسة مقطعية مقارنة في النماذج الحية)

الخلاصة:

هو اختبار الفرضية بأنه لا يوجد فرق في محتوى ايون الفلز اللعابي بين الاشخاص الحاملين لجهاز التقويم الثابت بالمقارنة مع اشقائهم من نفس الجنس بدون أي جهاز تقويم.

المواد والطرق:

تم تضمين ٥٠ مريض تقويم في الدراسة. المجموعة الاولى تكونت من ٢٥ مريض (١٤ اناث و ١١ ذكور) مع جهاز التقويم الثابت في الفك العلوي والسفلي. المجموعة الثانية تكونت من ٢٥ مريض (١٣ اناث و ١٢ ذكور) مع جهاز تقويم ثابت في الفك العلوي فقط. ولغرض تحديد تأثيرات الطعام والعادات الصحية على تركيز ايون الفلز اللعابي تم المقارنة مع ٥٠ شخص قياسي (٢٥ اناث و ٢٥ ذكور) وهم اشقائهم من نفس الجنس بدون أي جهاز تقويم. تم جمع اربع عينات من اللعاب المحفز من كل مريض الاولى قبل ادراج الجهاز والثانية بعد شهر من ادراج الجهاز والثالثة بعد شهرين من ادراج الجهاز والرابعة بعد ستة اشهر من ادراج الجهاز. نفس الاربع عينات جمعت من كل مريض قياسي في نفس الفترات الزمنية لمرضى الجهاز الثابت. تم جمع تقريبا ٥ مل من اللعاب من كل مريض تقريبا. تم تحليل عينات من اللعاب للنیکل والكروم بواسطة كهروحرارية المقياس الطبقي للامتصاص الذري. كان الحد من الكشف عن طريقة ايجاد حلول عينة ١ نانوغرام/مل. تم اجراء التحليل الاحصائي عن طريق اختبارات اللامعلمية. *Wilcoxon matched pairs signed ranks* استعمل لاختبار الفروقات بين العينات قبل و بعد ادراج اجهزة التقويم. تحليل التباين *A Kruskal Wallis 1-way* استعمل لاختبار الفروقات في تركيز النیکل والكروم في المجاميع الثلاثة.

النتائج:

معدل محتوى النیکل اللعابي في الاشخاص مع وبدون جهاز التقويم الثابت 12.2 ± 16.8 نانوغرام/مل و 11.3 ± 10.3 نانوغرام/مل تتابعيا. وجد هناك فرق احصائي هام بين المجموعتين. المستويات المسجلة لمعدل الكروم اللعابي كانت 1.8 ± 2.4 نانوغرام/مل في مجموعة الدراسة و 1.6 ± 2.1 نانوغرام/مل في مجموعة القياس. لكن الفرق احصائيا كان ضئيلا. ضمن حدود هذه الدراسة الحية ممكن الاستنتاج بأنه وجود اجهزة التقويم الثابتة تؤدي الى زيادة تركيز ايونات الفلز في الافراز اللعابي.

الاستنتاجات:

تركيز ايون النیکل والكروم زادت مباشرة بعد وضع الجهاز في الفم لكل مجاميع الدراسة. لم يكن هنالك فروقات هامة في مستويات النیکل والكروم الصادرة عن المجاميع الثلاثة للجهاز طيلة فترات الدراسة.

Introduction

Materials used in dentistry must have specific characteristics such as biological safety and functionality, adequate tissue response, and resistance to corrosion. As such, metal alloys have been extensively used in orthodontic dentistry because of their elasticity, shape memory, hardness, and stress resistance [1]. Metals used as components of these alloys, i.e., nickel and chromium, have been identified as cytotoxic, mutagenic, and allergenic [2,3]. The biocompatibility of orthodontic materials is widely discussed in recent scientific literature. In vitro and in vivo studies aimed to investigate whether a patient is exposed to toxic doses of metals from orthodontic appliances have not yet been published [4, 5]. The relationship

between the potential dose of toxic elements released from orthodontic appliances and the response of an organism has not been evaluated. Also, the effect of treatment time and the type of appliance used on the amount of released metal ions as a consequence of corrosion has not been evaluated. This is related with difficulties associated with the use of invasive vs. noninvasive biomarkers [6, 7]. Nickel is the most common cause of contact allergy. Orthodontic brackets, bands, and archwires are universally made with an alloy, which contains approximately 6% to 12% nickel and 15% to 22% chromium. In addition to the allergic issue, carcinogenic, mutagenic, and cytotoxic effects have been assigned to nickel and, to a lesser extent, chromium. The introduction of metal ions into the human body is an additional risk to

health since these ions may be released in different places and at different levels, depending on the characteristics and solubility of the products containing them [8]. Consequently, biological functions are affected, which may lead to systemic and local effects [9]. Several in vitro tests have demonstrated the corrosion and release of nickel and chromium ions from orthodontic brackets. However, the results of these tests are limited and extrapolation to the clinical situation difficult because the methodologies used are unable to precisely reproduce the highly complex and dynamic oral environment [10]. Kocadereli et al [11] evaluated the salivary concentrations of nickel and chromium on 45 patients treated with fixed orthodontic appliances (1) before, (2) after 1 week, (3) after 1 month, and (4) after 2 months. The results of this study did not indicate statistically significant differences between metal concentrations before and after placement of the appliance. Fors and Persson [12] compared the salivary concentration of nickel in young patients who did wear and did not wear fixed orthodontic appliances. The average period since appliance insertion was 16 months at the time of sample collection. No significant difference in the nickel content of filtered saliva was found between the test and the control samples; the median values of nickel content were 0.005 and 0.004 $\mu\text{g/g}$ saliva, respectively. On the other hand, a significant difference was found for the filter-retained fraction; the median values for nickel were 25.3 and 14.9 $\mu\text{g/g}$, respectively. The most significant method for measurement of nickel release before and after onset of orthodontic treatment is salivary analysis since it is the first diluting of the human body and allows long periods of analyses [13]. Thus, the

effects of material aging and fatigue on the ion release could be investigated.

The aim of this study was to evaluate the concentrations of nickel and chromium ions in salivary samples from patients treated with fixed orthodontic appliances. A second aim of this study was to determine any significant changes in these concentrations during any period of the treatment time.

Materials and Methods:

The objectives of the study were explained to the participants and informed consent was obtained before salivary collection. A total of 100 subjects were included in this study. The study used salivary samples collected from new patients starting orthodontic treatment. A total of 50 patients participated in the study. Twenty five (14 female, 11 male) with a mean age of 18.6 years ($\text{SD} \pm 1.2$ years) had upper and lower fixed appliances. Twenty five patients (13 female, 11 male) with a mean age of 19.7 years ($\text{SD} \pm 1.1$ years) had only maxillary fixed appliances. To limit the effect of food and oral hygiene habits on salivary metal ion concentration, a same-gender sister or brother without any orthodontic appliance formed the control group. Their age range was from 14 to 22 years (mean 18.2 ± 3.9 years). All patients were from the clinic of the author. Criteria of patient inclusion were as follows:

- Patients having a same-gender sister or brother.
- Patients were in the permanent dentition period
- good health and medication-free; and absence of any systemic diseases
- Patients did not have any amalgam fillings and metal restorations, which could any galvanic corrosion in the mouth,

- Patients had standard edgewise brackets on the incisors, canines, and premolars and standard edgewise bands on the first molars,
- Patients did not have any palatal or lingual appliances welded to the bands (ie, rapid maxillary expansion appliance) or extraoral auxiliary orthodontic appliances (ie, head gear) .

good health and medication-free; and absence of any systemic diseases. Since placement of nickel titanium (NiTi) archwires can temporarily cause an increase in Ni concentration [14], none of the patients had a NiTi archwire in their set-up for at least 1 month prior to sample collection. Similarly, no subjects in the control group had any piercings, metal restorations, systematic diseases, or were receiving any medication. In the upper and lower fixed orthodontic appliance group ,the patients had maxillary molar bands with Roth triple buccal tubes (ORTHO-organizers REF 702-399Q langenhagen,Germany) and second and first premolar, canine, and lateral and central incisor direct-bonded brackets Roth .022 ELITE OPTIMIM SET (5 x 5 HOOKS)QPK(ORTHO-organizers REF 702 - 399 Q langenhagen,Germany). In the mandible, these patients had mandibular first molar bands with Roth double rectangular buccal tubes with vertical ball hook(ORTHO-organizers REF 702-399Q langenhagen,Germany) and first and second premolar, canine, and lateral and central incisor direct-bonded brackets(Roth .022 ELITE OPTIMIM SET (5 x 5 HOOKS)QPK(ORTHO-organizers REF 702 - 399 Q langenhagen,Germany). In the upper-fixed-appliance-only group, the patients had the same maxillary

attachments mentioned above. In both of the groups, there were no buttons either on the molar bands or on the other teeth .

Sampling of saliva:

Three samples of stimulated saliva were collected from each orthodontic patient at the following times: before insertion of the fixed appliance, 1 month after insertion of the appliance, 2 month after insertion of the appliance and 6 months after insertion of the appliance. The 4 samples of saliva were collected from each control patient at the same time intervals as for the fixed appliance groups.

Saliva collection:

Sample collection was carried out such that after rinsing with 15 ml of distilled and deionized water for 30 seconds .After mouth rinsing ,the patient used a piece of paraffin(BATOOK CHEWING GUM produced by IND.LTD.SAUDI ARABIA) as a chewing gum for stimulation of the salivary secretion. Approximately 5 ml of saliva was collected from each subject and transferred to an assigned cold polypropylene tube. The samples were kept at -20°C until they were processed and diluted with Zolal deionized water (BahreZolal Tehran Co., Tehran, Iran) to eliminate interference and to reduce the effects of the biological matrix (protein, salt, etc.). The same person collected all the salivary samples from the subjects.

Salivary preparation and analysis:

Chromium and nickel concentrations of saliva are stable for 6 months when stored at -20°C. Extraction methods can be used for isolation and purification of elements from biological materials. The use of an atomic absorption spectrophotometer permits the analysis of metals in biological samples without any separation of the metal from its biological matrix. By using the spectrophotometric method,there is no

necessity for extraction procedures to analyze the elements [15]. The only dilution of the samples was enough to eliminate the interference and effects of the biological matrix (protein, salts, and others). A volumetric flask was used to dilute 1 ml of saliva in 10 ml of deionized water and the samples were analysed using an electrothermal atomic absorption spectrophotometer (Model UV1600/1800 UV/VIS Scanning Spectrophotometer). The results were considered as nanograms per millilitre (ppb). The detection limit of the method for sample solutions was 1 ng/ml. Determination of the metal content was performed at the Analytical Chemistry Department, College of Agriculture/Baghdad university/Iraq. The same batch of glass tubes and diluting agent were used for both groups.

Analysis of data and statistics:

Normal distribution of data was examined using the non-parametric *Kolmogorov-Smirnov* test. Since some variables were not normally distributed, the non-parametric tests (*Mann-Whitney U* and *Wilcoxon W*) were used for statistical analysis. Statistical significance was set at $P < 0.05$. The *Wilcoxon matched-pairs* signed ranks test was used to test

differences between samples before and after insertion of the orthodontic appliances. A *Kruskal Wallis 1-way* analysis of variance (*ANOVA*) was used to test differences in nickel and chromium concentrations among the 3 test groups.

Results

A large variation of Ni and Cr concentration was observed in both the study and control groups. The Ni concentration varied from 1 to 44.0 ng/ml in the controls and from 1 to 46.0 ng/ml in the study group. The mean salivary Ni content was 10.3 ± 11.7 and 16.8 ± 12.2 ng/ml in the controls and study subjects, respectively. Statistically significant differences were found between the groups ($P < 0.041$; Table 1). The salivary concentration of Cr varied from 1.1 to 6.2 ng/ml in the controls and from 0.80 to 6.2 ng/ml in the subjects with an appliance. The mean level of Cr ion was 2.1 ± 1.6 ng/ml in the controls and 2.4 ± 1.8 ng/ml in the study group. However, the minimal increase in Cr concentration in the study group was not statistically significant (Table 1).

TABLE 1. Salivary nickel and chromium content in subjects with and without orthodontic appliances (ng/mL).

With out appliance (N = 50)						With appliance (N = 50)						P
Ions	Mean	SD	Min	Max	Variance	Mean	SD	Min	Max	Variance		
Ni	10.3	11.7	1.0	44.0	151.6	16.8	12.2	1.0	46.0	117.1		0.041
Cr	2.1	1.6	1.1	6.2	2.3	2.4	1.8	0.8	6.2	2.6		0.242

nickel (Ni), chromium (Cr), standard deviation (SD), nanograms per milliliter (ng/mL).

The mean salivary nickel and chromium concentrations (ng/mL) of the upper and lower fixed appliance group, the upper fixed appliance-only group, and the control group before

insertion, after periods of 1 month, 2 month, and 6 months are shown in (Table 2). A large variation in the concentrations of both nickel and chromium was present in the saliva. The nickel concentration varied from 1

to 46.0 ng/ml, and the chromium concentration varied between 0.80 to 6.2 ng/ml. No significant differences were found between the no-appliance group and the fixed appliance groups tested by the *Wilcoxon* test (Tables 3

and 4). *The Kruskal Wallis 1-way ANOVA* showed that the differences between the mean concentrations of nickel and chromium in the different appliance groups were not statistically significant ($P > .05$).

TABLE 2. Mean Salivary Nickel and Chromium Concentrations and Standard Deviations (ng/mL).

Time	Control Group		Upper F.A Group		Upper and Lower F.A Group	
	Ni	Cr	Ni	Cr	Ni	Cr
Before insertion	11.4±10.6	2.2±1.7	16.9±12.4	2.6±1.65	17.4±12.8	2.4±1.68
1 mo later	10.6±11.4	2.2±1.6	17.1±13.1	2.6±1.71	16.8±11.9	2.6±1.78
2 mo later	11.8±10.9	2.3±1.6	16.4±12.8	2.6±1.89	16.5±12.3	2.7±1.54
6 mo later	10.4±11.3	2.3±1.8	16.5-13.4	2.7±1.76	16.8±11.7	2.7±1.74

F.A (fixed appliance)

TABLE 3. The Statistical Differences of Salivary Nickel Concentrations in Control, Upper Fixed Appliance, and Upper and Lower Fixed Appliance Groups.

Nickle	Control Group <i>P</i>	Upper F.A Group <i>P</i>	Upper and lower F.A Group <i>P</i>
Before insertion/1 mo later	.345	.685	.482
Before insertion/2 mo later	.421	.062	.501
Before insertion/6 mo later	.342	.250	.184
1 mo later/2 mo later	.618	.592	.428
1 mo later/6 mo later	.813	.148	.548
2 mo later/6 mo later	.477	.328	.739

F.A (fixed appliance)

TABLE 4. The Statistical Differences of Salivary Chromium Concentrations in Control, Upper Fixed Appliance, and Upper and Lower Fixed Appliance Groups.

Chromium	Control Group <i>P</i>	Upper F.A Group <i>P</i>	Upper and lower F.A Group <i>P</i>
Before insertion/1 mo later	.088	.594	.091
Before insertion/2 mo later	.854	.311	.239
Before insertion/6 mo later	.169	.734	.256
1 mo later/2 mo later	.812	.405	.084
1 mo later/6 mo later	.067	.129	.498
2 mo later/6 mo later	.098	.286	.570

F.A (fixed appliance)

Discussion

Most of the metals used in the oral cavity can be expected to undergo some type of corrosion. Most orthodontic appliances are made of stainless steel and NiTi alloys [16,17] which can release metal ions into the oral cavity [18,19,20]. The corrosion of orthodontic appliances and their subsequent metal ion release in the oral environment is governed by two main factors. The first is the manufacturing process, which includes the type of alloy and the characteristics of the metals used [21]. The second is environmental factors, such as mechanical stress, diet, time of the day, salivary flow rate, and health and psychosomatic condition of the individual [22]. If the salivary pH is reduced from 6.75 to 3.5, it can increase the release of metal ions from orthodontic appliances up to 100-fold [23]. Low pH values also reduce the resistance of dental alloys to corrosion [24]. The effects of levels of anxiety and stress on salivary pH, found a statistically significant direct correlation between increasing levels of anxiety and stress and increases in salivary pH [25]. Hypotheses of shared genetic aetiologies as a potential basis for stress and anxiety have been tested and there is clear scientific evidence that anxiety runs in families [26, 27]. To overcome or at least to reduce the effects of emotional behaviour and dietary intake on salivary ion concentration and to obtain a closer match between the study and control group, the same-gender sibling was selected as the control in the present study. Several previous studies have reported on the corrosive behavior of orthodontic wires, brackets, bands, or appliances separately. In this study, we preferred to use saliva samples obtained from orthodontic patients in order to analyze the release in the dynamic oral cavity environment.

Artificial salivary solutions are not commonly used in long-term studies based on their precipitation disadvantage. Fixed orthodontic treatment generally lasts about 2 years, so there was a necessity for long-term research of the release from the fixed orthodontic appliances. In our study, we didn't separate the appliance components under in vitro conditions because, in orthodontic treatment, wires, brackets, and bands are used together in the oral environment. The use of an electrothermal atomic absorption spectrophotometer permits analysis of metals in biological samples without separating the metal from its biological matrix. By utilizing this technique, there was no necessity for extraction procedures to analyse the elements. Large variations have also been found in previous reports of metal concentrations in saliva [28]. The findings of the current study are in accordance with the study by Kerosuo et al, who did not find any significant increase in nickel and chromium concentration in saliva of orthodontic patients after insertion of different fixed appliances [28]. The present study is also in accordance with the study by Gjerdet et al, who did not find any differences in nickel amounts in saliva before and 3 weeks after insertion of fixed appliances [29]. The continuous flow of saliva in the mouth and short sampling period may not give time enough for a detectable dissolution of nickel and chromium to separate from the fixed appliances. In the current study, to eliminate the risk of contamination, maximum care was taken, and the sterile paraffin wax used as a chewing gum for stimulation of the salivary secretion did not contain any nickel. The concentrations of salivary nickel and chromium, with and without appliances, were somewhat higher in this study compared with earlier reports of these

metals in saliva. The design of some of the studies is similar, but they used unstimulated saliva samples. On the other hand, nickel release in vivo in the oral cavity has been more difficult to demonstrate. The literature includes some in vivo studies evaluating the ion release in saliva. Kerosuo et al evaluated the salivary concentrations of nickel and chromium in patients wearing different types of appliances. The study sample was composed of 47 patients, and four saliva samples were collected: (1) before placement of the appliance, (2) after 2 days, (3) after 1 week, and (4) after 1 month. The mean salivary concentration was 55 ng/mL for nickel and 61 ng/mL for chromium, similar to the values observed before placement of the appliance²⁸. Kocadereli et al evaluated the salivary concentrations of nickel and chromium on 45 patients treated with fixed orthodontic appliances (1) before, (2) after 1 week, (3) after 1 month, and (4) after 2 months. The results of this study did not indicate statistically significant differences between metal concentrations before and after placement of the appliance[30]. Fors and Persson[12] compared the salivary concentration of nickel in young patients who did wear and did not wear fixed orthodontic appliances. The average period since appliance insertion was 16 months at the time of sample collection. No significant difference in the nickel content of filtered saliva was found between the test and the control samples. In general, this study showed an increase in salivary Ni and Cr concentration in patients with fixed orthodontic appliances compared with their same-gender control sister or brother. This finding is in agreement with those of Ağaoğlu et al. , who reported an increase in salivary Cr and Ni concentration 1 year after appliance insertion [31]. Fors and Persson

(2006) also showed that the amount of Ni in saliva debris retained on filters was significantly higher in orthodontic patients compared with controls when collecting the salivary sample after an average period of 16 months [12]. On the other hand, Eliades et al. and Gjerdet et al. failed to show increased levels of metal ions in the saliva of orthodontic patients [10,22]. This difference may be due to diverse methods for analysing the levels of the metals or sample selection. Other studies, however, were either carried out over a short period of time of 1 week to 3 months [11 14 ,31, ,32] or were in vitro investigations. Currently, the concentration of metal ions at a specific time point cannot be applied to full-term treatment, so the results could not be directly compared.

Conclusions

- There is a large variability among individuals in the concentrations of nickel, chromium, in saliva.
- There is an increase in nickel and chromium ions immediately after placement of the appliance in the mouth.
- There were no significant differences among the nickel and chromium levels released by the three groups at all study periods.

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