Original Article

Release of nickel and chromium ions in the saliva of patients with fixed orthodontic appliance: An *in-vivo* study

Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, [1]Department of Analytical Chemistry, Indian Institute of Toxicology Research Centre, Lucknow, Uttar Pradesh, India

Anoop Dwivedi, Tripti Tikku, Rohit Khanna, Rana Pratap Maurya, Geeta Verma, R. C. Murthy[1]

ABSTRACT

Introduction: Various components of fixed orthodontic appliances are continuously interacting with saliva and other fluids in the mouth releasing various metal ions including nickel and chromium that can cause damaging effects if their concentration exceeds above the toxic dose. **Aim:** To determine and compare the level of nickel and chromium in the saliva of patients undergoing fixed orthodontic treatment at different time periods. **Materials and Methods:** The sample of saliva of 13 patients was taken at different time periods that is: Group 1 (before appliance placement), Group II, III, and IV (after 1-week, 1-month, and 3 months of appliance placement respectively). The fixed appliance comprised of brackets, bands, buccal tubes, lingual sheath, transpalatal arch and wires composed of Ni-Ti and stainless steel. The level of ions was determined using graphite furnace atomic absorption spectro-photometry. The data thus obtained were statistically analyzed using SPSS Statistical Analysis Software (Version 15.0). **Results:** Level of nickel and chromium in saliva was highest in Group II and lowest in Groups I for both the ions. On comparison among different Groups, it was statistically significant for all the groups (<0.001) except between Group III and Group IV. **Conclusion:** The release of nickel and chromium was maximum at 1-week and then the level gradually declined. These values were well below the toxic dose of these ions. The results should be viewed with caution in subjects with Ni hypersensitivity.

Address for correspondence:
Dr. Anoop Dwivedi,
Babu Banarasi Das College of Dental Sciences, BBD City, Faizabad Road,
Lucknow - 227 105,
Uttar Pradesh, India.
E-mail: dranoop.dwivedi@gmail.com

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Introduction

In orthodontics, the various components of fixed appliances are fabricated by the use of varying materials which have their own physical and mechanical properties.[1-2] Stainless steel is most commonly used for the construction of these components such as wires, brackets, bands, buccal tubes, and other auxiliaries due to its low cost, high strength, resistance to corrosion, and biocompatibility.[2] According to the clinical needs, besides stainless steel wires, other wires such as Ni-Ti, beta titanium, cobalt chromium, and teflon polyethylene coated wires are also used.

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Various factors such as temperature, pH variation, salivary conditions, mechanical loads, microbiological and enzymatic activity, physical and chemical properties of food and oral health conditions provide an environment for the corrosion of dental materials.[3,4] This results in weakening of the appliance and the release of nickel, chromium, and iron, etc., Nickel and chromium are trace minerals or micronutrients, and they play an important part in the overall health of the human body. The average dietary intake of nickel is 200–300 μg/day. Nickel aids in iron absorption, as well as adrenaline and glucose metabolism. It also helps in improving bone strength and may play a role in the production of red blood cells. The primary route of eliminating nickel is through the urine. The dietary intake of chromium is 50–200 μg/day. The capacity of humans to absorb chromium is greatly influenced by the oxidation state of the chromium ion. Chromium regulates the functioning of insulin hormone and also facilitates the processing of carbohydrates and fats in the body. Excretion of absorbed chromium occurs mainly via the urine.

In higher doses, both Ni and Cr have been found to be harmful. Nickel has been systematically studied for detrimental effects at cell, tissue, organ, and organism levels. In higher doses, Ni can be an allergen or carcinogenic and act mutating substance by causing alteration in DNA. Higher doses of chromium are also capable of inducing side effects which may include insomnia or irregular sleeping, headaches, vomiting, diarrhea, and irritability.[5,6] Many *in-vitro*[1,5,7-17] and *in-vivo* studies[18-28] have been conducted in the past to show the release of nickel and chromium from orthodontic appliance in saliva but none of them considered auxiliaries like the transpalatal arch, which is frequently used in fixed orthodontics to reinforce anchorage and can be the major source of nickel and chromium release. Hence, the present *in-vivo* study was conducted to evaluate the level of nickel and chromium in the saliva of the patients with fixed orthodontic appliances incorporating transpalatal arch at different time intervals.

Materials and Methods

The sample for this *in-vivo* study comprised of 13 subjects, including 6 males and 7 females in the age range of 15–33 years, who reported to the Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Lucknow for Orthodontic treatment. Fixed orthodontic appliance was placed in the mouth. The unstimulated saliva of these 13 patients was collected at different time intervals that is, before placement of appliance, after 1-week, 1-month, and 3 months of placement of appliance. Thus, a total of 52 saliva samples were obtained which were divided into four groups each:

- Group I: Sample taken before placement of appliance
- Group II: Sample taken 1-week after appliance placement
- Group III: Sample taken 1-month after appliance placement
- Group IV: Sample taken 3 months after appliance placement.

Sample selection criteria

- Patients were in the permanent dentition period
- Patients did not have any amalgam fillings and metal restorations, which could cause any galvanic corrosion in the mouth
- Patients had brackets on the incisors, canines, and second premolars
- Patients had bands with double buccal tube in mandibular first molars and triple buccal tube in maxillary first molar with transpalatal arch attached to the lingual sheath.

Appliance placement

After cleaning the tooth surface with pumice, the etching was done with 37% phosphoric acid. MBT preadjusted brackets with 0.22″ slot were bonded in position on incisors canine and second premolars in both maxillary and mandibular arches using Transbond XT light cure adhesive paste according to the chart given by MBT prescription. Preformed bands with the bucaal tube were cemented on first molars using glass ionomer cement. A transpalatal arch made of 0.8 mm stainless steel wire was placed in the lingual sheath which was welded on the palatal aspect of the maxillary molar band. 0.014″ Ni-Ti wire was placed after banding and bonding of maxillary and mandibular arch. 0.016″ Ni-Ti and 0.016″ × 0.022″ stainless steel wire were ligated with elastic ligature tie after 1st and 2nd month of treatment respectively.

Sample collection

The saliva sample was taken in the morning before breakfast. The patients were asked to rinse the mouth with deionized and distilled water before the sample collection. Patients were also instructed to clean the appliance with water and toothbrush before collection of second, third and fourth sample. Approximately 5 ml of saliva was collected after 5 min with mouth closed without stimulation and transferred to sterilize plastic container by direct spitting. The samples were transferred to Indian Institute of Toxicology and Research Center, Lucknow for determination of the level of nickel and chromium in saliva where the samples were kept at −20°C till the time of processing. Graphite furnace atomic absorption spectrometry was used for determination of nickel and chromium ions in saliva by machine Zeenit 700 P, Analytik Jena AG, Konrad-Zuse-Str. 1Jena/Germany. This analytical technique is designed to perform the quantitative analysis of metals in a wide variety of samples. Due to enhanced sensitivity, it allows measurements in picogram.

Statistical analysis

The statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL) statistical analysis software. Mean and standard deviation were determined for Cr and Ni level in saliva. Tukey - honestly significant difference test was used to compare the level of Ni and Cr among different groups. $P < 0.05$ was considered as significant.

Results

Table 1 shows the level of nickel and chromium in saliva in different groups, where it was found highest in Group II and lowest in Groups I for both the ions. On comparison of nickel and chromium level in saliva among different Groups [Table 2], it was found statistically significant for all the groups except between Group III and Group IV.

Discussion

Many *in-vitro* studies[1,5,7-17] have been conducted in the past to show the release of nickel and chromium from stimulated fixed orthodontic appliance immersed in artificial saliva, which was found below the toxic dose to humans. To confirm the validity of the result of *in-vitro* studies, several *in-vivo* studies[18-28] were carried out. The advantage of *in-vivo* study is that the artificial saliva used *in-vitro* study undergoes precipitation over a long period of time as saliva during the experiment is not replaced or allowed to flow through the system as in the oral cavity. The other advantage is that in the oral cavity, the release of any ion is affected by many factors as temperature, quantity and quality of saliva, pH, physical and chemical properties of food and liquid and general oral health condition which give a better and reliable result. The method of saliva collection can be by stimulated and unstimulated method. In the present study, the saliva sample was collected by unstimulated method because by this method about two-third of whole saliva is produced by the submandibular gland. On the contrary, the stimulated saliva collected by chewing gum or paraffin has different compositions because all the salivary glands are stimulated, and at least half of the whole saliva is released by the parotid gland. Thus, stimulation could change the protein composition of saliva and nickel has a tendency to rapidly combine with protein, and this will affect the nickel concentration in saliva.[29]

Table 1: Nickel and chromium level of saliva (μg/L) in different groups

Groups	Mean ± SD (μg/L)	
	Nickel	Chromium
I	1.156 ± 0.675	11.570 ± 2.145
II	6.841 ± 1.326	70.386 ± 6.884
III	3.403 ± 1.631	21.254 ± 5.099
IV	3.124 ± 1.321	20.002 ± 3.684

SD: Standard deviation

In the present study, the level of Ni and Cr in saliva nickel level was found maximum after 1-week of appliance placement (Group II). The possible reason for this sudden increase during the first 7 days of the appliance placement was due to the corrosion of Ni and Cr present on the surface of bands, brackets and wires.[8] Nickel being cathodic in nature is prone to corrosion thus resulting in the release of Ni ions in saliva.[20] Barret *et al*.[2] suggested that Ni on the surface of the stainless steel corroded quickly during first 7 days and then decreased as the surface Ni is depleted. The passivating layer of chromium oxide formed on brackets, bands, etc., leached Cr on exposure to potentially damaging physical and chemical agents in the oral cavity. A significant increase of Ni and Cr ions was reported in the present study as compared to the previous studies, which may be due to the inclusion of the transpalatal arch inserted in the lingual sheath welded to the molar bands. Besides corrosion of lingual sheath and stainless steel wire, the welded joint is also an area more prone to corrosion as welding occurs at increased temperature that may increase the propensity for corrosion and adds to increased Ni and Cr levels.

In the previous studies, it was found that the levels of Cr and Ni in saliva increased to a maximum from 7th day to 14th day, and thereafter, the level decreased gradually.[18,30] Hwang *et al*. found that release of Ni in saliva was significantly increased up to 3rd week but was then constant till the 12th week whereas Cr level increased until the 4th week and continued to increase slightly from 8th week to 12th week.[31] Agaoglu *et al*.[19] in his study evaluated that the level of Ni and Cr reached their highest levels in the 1st month and then decreased to their initial levels in saliva. Satija *et al*.[20] noted a significant increase

Table 2: Comparison of nickel and chromium level in saliva among different groups (Tukey-HSD test)

Comparison among different groups	Nickel			Chromium		
	Mean difference	SE	*P*	Mean difference	SE	*P*
I versus II	5.685	0.505	<0.001***	58.817	1.877	<0.001***
I versus III	2.247	0.505	<0.001***	9.684	1.877	<0.001***
I versus IV	1.969	0.505	0.002**	8.433	1.877	<0.001***
II versus III	−3.439	0.505	<0.001***	49.133	1.877	<0.001***
II versus IV	−3.717	0.505	<0.001***	−50.384	1.877	<0.001***
III versus IV	−0.278	0.505	0.946	−1.251	1.877	0.909

$P<0.01$: **Significant, $P\leq0.001$: ***Highly significant. SE: Standard error, HSD: Honestly significant difference in Ni and Cr level in saliva and it reached the highest level in 1st week.

In this study, the level of Ni and Cr in saliva at 1-month after appliance placement (Group III) was significantly higher than Group I whereas, significantly lower than Group II. As the potential of releasing elements from orthodontic alloys is a time-dependent phenomenon, thus the releasing of elements changes over a period of time. Similar to results of the present study, Sahoo *et al*.[18] and Matos de Souza and Macedo de Menezes *et al*.,[32] Arash *et al*.[25] and Satija *et al*.[20] reported a decrease of Ni and Cr levels at 30 days of appliance placement in comparison to values obtained at 1-week. Agaoglu *et al*.,[19] Kocadereli *et al*.[33] and Kerosuo[5] showed a continued increase from pretreatment values to 1-week to 30 days. Variable results might be due to the effect of various factors like difference in temperature, quality of saliva, plaque and proteins and physical and chemical properties of food and liquids taken. Saleem *et al*.[34] had observed the similar findings for their study group with fixed appliance in both the arches.

Level of Ni and Cr in saliva at 3 months after appliance placement (Group IV) was significantly higher than Group I whereas, significantly lower than Group II in this study. The results of the present study were supported by Amini *et al*.[35] who found increased level of both the ions after 3 months in comparison to pretreatment values.[23] Other studies by Matos de Souza and Macedo de Menezes,[32] Kocadereli *et al*.,[33] Eliades *et al*.,[10] and Kerosuo *et al*.[5] did not demonstrate significant increase from 1-month to 2 months.

Though the wire was changed from NI-Ti to stainless steel 1-month before the collection of sample for Group IV, but the level of Ni and Cr did not show a significant difference with Group III. The reason for this could be that the saliva collection for Group IV was 1-month after the placement of S.S. wire and not immediately or within 1-week when the release of Ni and Cr would have been maximum. In addition, the major release of ions is from bands, brackets, lingual sheath, transpalatal arch, and not from the wire. Grimstottle and Pettersen[36] suggested that negligible level of Ni and Cr was released from archwires. Though most of the orthodontic attachments are made of highly corrosion resistant metals and metal alloys but their electrochemical breakdown is observed in the oral environment, thereby contributing to the release of metal ions. The psychological stress can also alter the oral environment by decreasing the pH value, decreasing salivary flow and increasing the protein content of saliva. Nickel binds to salivary proteins, thereby influencing its concentration. The variation in the values of the present study from the other studies could be because of varying concentration of Ni and Cr ions in air, soil; food and water that the subjects were taking; and the type of utensils being used for cooking.

Even with this additional leaching of Ni and Cr ions in saliva, the levels were below the toxic doses recommended by WHO and will not cause adverse reactions at cell, tissue, and organ level. However, if the patient is allergic to Ni or Cr and shows even mild signs and symptoms of nickel allergy, appliance should be removed immediately. Such patients can be treated with alternative materials such as ceramic, polycarbonates, metal coated with epoxy resins or components fabricated with other metals such as titanium, vanadium, Co-Cr, and aluminum.

Future research should be carried for a longer time to study the effect of corrosion process and mechanical phenomenon such as wear and fatigue on the release of Ni and Cr in the oral cavity. In addition, nickel and chromium should be observed for different combination of brackets and wire and also from recycled brackets.

Conclusion

The following conclusions can be drawn from this *in-vivo* study:

- Nickel and chromium level in saliva were significantly increased after the appliance placement but were below the toxic level
- Nickel and chromium level in saliva were found to be maximum after 1-week of appliance placement then gradually decreased.

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