Analysis of Nickel and Chromium Levels in the Gingival Crevicular Fluid and Hair of Patients Treated with Fixed Orthodontic Appliances: A Longitudinal Study

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Abstract

The aim of this study was to analyze the levels of nickel and chromium in GCF and hair of orthodontic patients.

Nickel and chromium ion concentrations were measured in GCF and hair of 15 patients (9 females and 6 males, aged 16-28 years old) who had fixed orthodontic treatment using Atomic Absorption Spectroscopy. The samples were taken before treatment (baseline) and four months later during treatment. Data were analyzed using paired t-tests and Pearson correlation test.

GCF nickel level increased from 3.39 µg/g (baseline) to 9.00µg/g (4 months); chromium level increased from 1.88 µg/g (baseline) to 6.85 µg/g (4 months). Both of these increases were significant (paired t-test, *P*=0.000). Hair nickel level increased from 0.14 µg/g (baseline) to 0.42µg/g(4 months); chromium level increased from 0.09 µg/g (baseline) to 0.11 µg/g (4 months). Both of these increases were significant (paired t-test, *P*=0.000) There was no significant correlation between the increase of ion level in GCF with that in hair.

After 4 months of treatment with fixed orthodontic appliances, GCF nickel and chromium levels might increase about 364% and 265%, respectively and hair nickel and chromium levels might increase about 122% and 300%, respectively. There was no significant correlation between the difference (increase) of GCF nickel and chromium levels with the increase of hair nickel and chromium levels.

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Introduction

Nowadays, more adult people are seeking orthodontic treatment and there is an increasing demand for studying the impact of orthodontic appliances on patient's health.[1] Various types ofthefixed orthodontic appliance made of metal have been widely circulated in the market. The stainless steel used for orthodontic brackets, bands, and wires contain approximately 18%chromium (Cr), 8% nickel (Ni), iron, and carbon which can be absorbed in the body. The high elasticity NiTi (nickel-titanium) wire contains approximately 50% nickel and 50% titanium.[2,3]

The appliances placed in the oral cavity experiencing various pressures, such as mastication pressure, orthodontic appliance force, fluctuation of temperature, a variety of food, salivary pH, fluoride-containing toothpaste and mouthwashes, microbiological and enzymatic environment of the oral cavity as well as electrochemical reactions that can cause solubility or the formation of chemical compounds. Previous studies have shown that orthodontic appliances release metalions through the emission of electro-galvanic currents, with saliva acting as the medium for continuous erosion over time.[4,5]

Nickel and chromium have dermatological, toxicological, and possibly mutagenic effects. In addition, they are most common cause of metal-induced allergic contact dermatitis. Exposure to nickel will generate free radicals that may cause oxidative stress and affect the kidneys and liver, breast and lung cancer, miscarriage, birth defect,

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neurological disorders, and cardiovascular collapse.[6,7]

In orthodontics, the release of nickel and chromium ions into the body isamatter of concern and has been studied in various conditions. Previous studies have evaluated the concentration of nickel and chromium in saliva,[8-12], serum,[13-16] and urine[17-19] after different periods of treatment with fixed orthodontic appliances, but the results were still controversial.

Gingival crevicular fluid (GCF) is an inflammatory exudate that follows an osmotic gradient and is similar in appearance to blood plasma.[20] Orthodontic treatment influences systemic exposure which can be measured with exposure biomarkers in GCF. Their presence of trace elements in GCF has been studied by several researchers.[21-23] Absorption into strips of absorbent paper (PerioPaper) is traditionally used and is considered noninvasive and easy to carry out.[24]

The determination of trace elements in hair has been the subject of interest in biomedical and environmental sciences.[25] The content of trace elements in hair reflects the metabolism of minerals in the body. Human hair is an attractive biological material because of the simplicity of sampling, transport, and handling. Hair grows approximately 10 mm per month and may usually reflect average concentrations for previous two or three months exposure. For chronic exposures over a long time, hair is usually suitable, but for studies immediately after acute exposures, urine and blood samples maybe preferable.[26]

The aim of this study was to analyze the levels of nickel and chromium in GCF and hair of orthodontic patients. Moreover, we tried to correlate the mean differences of ion levels in GCF with those in hair.

Materials and methods

This experimental study was conducted on 20 patients (12 females and 8males), age ranged from 16 to 28 years (mean 19.2±2.3 years). The inclusion criteria were: patients were willing to be part of the study and neededa fixed orthodontic treatment in both arches. The exclusion criteria were: any systemic diseases, syndromes, allergies, metal restorations, piercing, and previous orthodontic treatment.

The fixed appliance consisted of bonded 0.018inch slot pre-adjusted Roth prescription stainless steel brackets (Protect, China) on all teeth except the molars, stainless steel orthodontic bands (Protect, China), and NiTi wires (Nitinol; Ormco Corporation California, USA).

The objectives of the study were explained to the participants and written informed consent was obtained before starting the study. The research was approved by the ethics committee of the Medical Faculty, Hasanuddin University.

The patients were asked not to consume nickel- and chromium-rich foods for 48 hours before the sampling visit and not to eat or drink on the morning of the scheduled visit. The sampling was carried out 2 times (before treatment and 4 months later). GCF samples were collected from the gingival crevices of the randomly selected tooth. The tooth surface was dried gently and kept dry with cotton. A standardized absorbent paper (PerioPaper, Oraflow, NY) was gently inserted t gingival sulcus, let it for 60 seconds. Then performed the same procedures with new papers in other three teeth. The paper contaminated with blood was excluded from the study. The 4PerioPapers were kept in a bottle with lid, stored in a freezer until further analysis. The PerioPapers were weighed (Ohaus, UK) before and after GCF sampling.

Hair samples were collected by cutting hair 3-4 cm measured from the scalp with a pair of sterilized stainless steel scissors. Each sample was placed in a sealed plastic bag, transported to the laboratory and washed carefully with carbon tetrachloride to remove lipids and dust.

The measurement of nickel and chromium levels, both in GCF and hair, were performed using atomic absorption spectrophotometry (Buck Scientific 205, USA).

Statistical analysis

The results were tabulated and analyzed using the Statistical Package for Social Sciences (SPSS version 23.0, IBM, USA) A paired t-test was used to analyze statistically significant differences between the groups based on the two- time points. The level of significance was set at 0.05.A Pearson correlation coefficienttest was used to evaluate association between the differences of nickel and chromium levels in GCF with those in hair.

Results

The differences between means of nickel and chromium levels in GCF based on time points are shown in Table 1.

The baseline value for nickel was 3.39 µg/g and after four months it increased to 9.00 µg/g (265%). The baseline value for chromium was 1.88 µg/g and after four months, it increased to 8.99 µg/g (364%)(Table 1). The mean differences were statistically significant ($P<0.05$).

The differences between means of nickel and chromium levels in hair based on time points are shown in Table 2

The baseline value for nickel was 0.14 µg/g and after four months it increased to 0.42 µg/g (300%). The baseline value for chromium was 0.09 µg/g and after four months it increased to 0.11 µg/g (122%) (Table 2). The mean differences were statistically significant ($P<0.05$). Correlation between the difference (increase) of ion levels in GCF with the increase of ion levels in hair was presented in Table 3.

Table 3 shows there is a positive correlation between the difference of nickel and chromium levels in hair with those in GCF, but the correlation is not significant ($P>0.05$).

Discussion

The majority of previous studies regarding the metal ion concentration in patients having fixed orthodontic treatment used saliva, both *in vivo* and *in vitro*. Although there are some similarities between amounts of some markersin blood serum with saliva have been referred in numerous studies,[26,27] the content of metal ion levels might not be the same. Fixed orthodontic appliances, which contain variable amounts of nickel and chromium, can release these metals into the saliva. Nonetheless, it is not clearly identified how much-released nickel and chromium are absorbed by the human. In addition, salivary pH plays a role in the solubility of metals so that the salivary metal ion level fluctuates, and even decreases after the first month of treatment.[13,15]

Gingival crevicular fluid (GCF) is the most relevant biomarker of exposure in theorthodontic study because unlike blood, urine, and hair, it is directly related to the inflammatory response induced by orthodontic forces.[22] In GCF sampling, it cannot be contaminated by saliva because their origin and contents are different. Saliva comes from salivary gland, but GCF originates in the connective tissue surrounding gingival crevice. GCF is very similar in appearance in blood plasma.[20]

In this current study, we used GCF as a biomarker of exposure to assess nickel and chromium ion levels before and after 4 months of orthodontic treatment. It was shown that nickel and chromium levels in GCF increased considerably compared with the baseline after four months of orthodontic treatment (Table 1). Our study result agrees with the findings of previous longitudinal studies on GCF. Aminiet al[22] studied the influence of orthodontic treatment on GCF nickel and chromium levels. They found that compared with the baseline, after one and six months of treatment, nickel increased by 150% and 510%, respectively, and chromium increased by 200% and 700%, respectively. Bhasin & Bhasin[23] assessed the longitudinal changes in GCF after placement of orthodontic appliance, reported hat the mean nickel levels were 3.8±1.32, 5.6±2.6, and 16.7±7.8 mg for every gram, respectively at baseline, month 1, and month 6. Chromium levels were 1.78±0.7, 4.5±1.8, and 11.7±3.5 mg for every gram, respectively.

Previous studies have shown controversial results. Several studies on the saliva of fixed orthodontic wearers found that the nickel and chromium levels increased,[6,28] while others found no significant difference after a period of 30-90 days.[9,10,16,19] In serum, the nickel ion concentration significantly increased after 2 years of orthodontic treatment,[15] while the other reported that serum nickel level initially increased but it gradually decreased after a longer duration;[16] however, serum chromium levels showed no significant changes with time. In urine, the nickel ion levels have been reported to increase significantly two months and longer after placement of an orthodontic appliance.[17,18,29,30]

In our current study, the number of subjects was 15 (9 females and 6 males) but we did not separate based on gender. The previous researcher reported that ion concentrations in males and females were not different.[17] Hair, nail, blood, and urinearecommonly used to analyze systemic exposures. Urine and blood are suitable for acute exposures, while hair can be used for both short-term and chronic exposures. During 4 months of orthodontic treatment, the hair becomes longer for approximately 40 mm, so we used the base of the hair, instead of the tip of hair for samples.

The nickel and chromium levels in hair have significantly increased after 4 months of orthodontic treatment compared with the baseline levels (Table 2).Some of our study results were parallel to the findings of previous researchers. Amini et al.[31] found significant increases from the baseline values for nickel and chromium to the six-month values (from 0.13 µg/g to 0.6715 µg/g for nickel and from 0.1455 µg/g to 0.1683 µg/g for chromium). Jamshidi et al[32] reported their findings that after one year, the levels of nickel and chromium in two groups showed significant differences (0.086±0.007 and 0.258±0.009 mg/g for control group and 0.149±0.010 and 0.339±0.013 mg/g for patient group, respectively for nickel and chromium). Mikulewics et al.[33] found that the peak release of chromium occurred after 4 months of treatment, and the peak release of nickel gradually increased throughout the whole one year of the therapy. Abtahi et al[34] performed their study to measure the hair nickel level of orthodontic patients compared with control group. After 4 months of treatment, nickel increased from baseline value of 0.350 to 0.673 µg/g, while in the control group there was also increased, from 0.245 to 0.382 µg/g. Levrini et al.[35] performed their study on orthodontic patients compared with control group, reported that hair nickel level in the orthodontic group (0.64 mg/g) was significantly higher than the control group (0.50 mg/g).Masjedi et al.[36] in their study on the effect of fixed orthodontic treatment on hair nickel and chromium levels reported a significant increase after 6 months of treatment. Nickel increased from 0.160 to 0.319 µg/g. Chromium increased from 0.165 to 0.306µg/g.

In this study, the maximum value of nickel was 9.00µg/g and chromium was 6.85 µg/g.The values were far below the average dietary intake. Oral daily intake of nickel by food is estimated to be between 300 and 600 µg.[15] While the average daily intake for chromium is between 50 and 200 µg. Although the orthodontic appliances did not have any effect on the general level of nickel and chromium concentrations both in GCF and hair, it cannot be excluded that minor amounts of nickel and chromium dissolved from appliances could be important in cases of hypersensitivity to those metal ion. Clinical abnormalities, such as gingivitis, gingival hyperplasia, lip desquamation, burning sensation in the mouth, metallic taste, angular cheilitis, and periodontitis, may be associated with the release of nickel fromorthodontic appliances. Moreover, even at low ion concentration, nickel can induce biological effects in cells. it can lead to DNA alterations mainly through base damage and DNA strand scission.[37,38]

Different with other previous studies, in this study we have tried to correlate the nickel and chromium levels in hair with those in GCF (Table 3). We found that there was a positive correlation between the differences (increase) of hair nickel and chromium levels of the baseline with the 4th month of orthodontic treatment with those in the GCF. However, the correlation was statistically insignificant. It can be said that the use of hair and GCF as biomarkers of exposures are not interchangeable. The reason might be due to the different routes of metal ion deposition and elimination, so the ion levels in the hair and GCF should not be necessarily correlated.

Conclusions

It can be concluded that after 4 months of treatment with fixed orthodontic appliances, GCF nickel and chromium levels might increase about 364% and 265%, respectively and hair nickel and chromium levels might increase about 122% and 300%, respectively. There was no significant correlation between the difference (increase) of GCF nickel and chromium levels with the increase of hair nickel and chromium levels after 4 months of orthodontic treatment.

Declaration of Interest

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References

1. Aghili H, Yassei S, Eslami F. Evaluation of the effect of three mouthwashes on the mechanical properties and surface morphology of several orthodontic wires: An in vitro study. Dent Res J 2017;14:252-9.
2. Rafeeq RA. Saleem AI. Nissan LM. Ions release from fixed orthodontic appliance in two different mouthwashes. J Baghdad Coll Dentistry 2014;26(4):152-155.
3. Forgacs Z, Massányi P, Lukac N, Somosy Z. Reproductive

toxicology of nickel–review. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2012;47(9):1249-60.
4. Chaturvedi TP, Bansal R. Biocompatibility of dental implant materials: An overview. J Indian Dent Assoc. 2010;2:335-7.
5. Mikulewicz M, Chojnacka K. Trace metal release from orthodontic appliances by invivo studies: a systematic literature review. Biol Trace Elem Res. 2011;137:127-31.
6. de Souza M and de Menezes M. Nickel, chromium and iron levels in the saliva of patients with simulated fixed orthodontic appliances. Angle Orthod 2008;78(2):245-350.
7. Amini F, Khazali M, Hematpour MS. Salivary content of nickel, chromium and cobalt ions in subjects with fixed orthodontic appliances. Iranian J of Orthod 2010; Available from :https://www.researchgate.net/publication/263314648
8. Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. Am J Orthod Dentofac Orthop. 2003;111:595-8.
9. Talic NF, Alnahwi HH, Al-Faraj AS. Nickel and chromium levels in the saliva of a Saudi sample treated with fixed orthodontic appliances. The Saudi Dent J. 2012;25:129-33.
10. Nayak BR, Khanna B, Pasha A, Vinay K, Narayan A, Chaitra K. Evaluation of nickel and chromium Ion release during fixed orthodontic treatment using Inductively Coupled Plasma-Mass Spectrometer: An in vivo study. J Int Oral Health. 2015;7(8):14-20.
11. Yassae S, Dadfamia S, Ahadian H, Moradi F. Nickel and chromium levels in the saliva of patients with fixed orthodontic appliances. Orthodontics. 2013;14(1):e76-81.
12. Neamah ZT. Nickel and chromium Ions levelsIn saliva of patients with fixed orthodontic appliances. Med J Babylon. 2014;11:13-8.
13. Amini F, Rakhsan V, Mesgarzadeh N. Effect of long-term fixed orthodontic treatment on salivary nickel and chromium levels: A 1-year prospective cohort study. Biol Trace Elem Res. 2012;150:15-20.
14. Arash V, Pouramir M, Hajiahmadi M, Mirzafarjooyan S. Measurement of iron, magnesium and chromium concentrations in the saliva of the patients undergoing fixed orthodontic treatment. Caspian J Dent Res. 2012;1(1):27-31.
15. Agaoglu G, Arun T, Izgi B, Yarat A. Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. Angle Orthod. 2001;71:375-9.
16. Rai RV, Shetty S, Ahluwalia R, George S. Quantitative assessment of serum nickel and chromium levels in orthodontic patients: An in vivo study. J Indian Orthod Soc. 2011;45(4):251-5.
17. Amini F, Rakhshan V, Sadeghi P. Effect of fixed orthodontic therapy on urinary nickel levels: A long-term retrospective cohort study. Biol Trace Elem Res. 2012;50(1-3):15-20.
18. Menezes LM, Quintão CA, Bolognese AM. Urinary excretion levels of nickel in orthodontic patients. Am J Orthod Dentofac Orthop. 2007;131(5):635-8.
19. Bengleil MS, Orfi JM, Abdelgader I. Evaluation of salivary nickel level during orthodontic treatment. Int J of Med Health Biomed Bioeng Pharmac Eng. 2013;7(12):809-11.
20. Alencar FG, Kronbauer GC, Gomes SC. Validation of an alternative device for volumetric quantification of crevicular fluid. Acta Odontol Latinoam 2011;24(1);29-34.
21. Meenakshi B, Chandran CR, Aravindhan TR, Devaraj N. A comparative study on the levels of alkaline phosphatase and trace elements in gingival crevicular fluid and serum of periodontitis and gingivitis patients with healthy population. J Dent Med Sci. 2017;16(1):14-7.
22. Amini F, Shariati M, Sobouti F, Rakhsan V. Effect of fixed orthodontic treatment on nickel and chromium levels in gingival crevicular fluid as a novel systemic biomarker of trace elements: A longitudinal study. Am J Orthod Dentofacial Orthop. 2016;149:666-72.
23. Bhasin V, Bhasin M. Assessment of longitudinal changes in gingival crevicular fluid after placement of fixed orthodontic appliances. J Adv Med Dent Sci Res. 2017;5(6):32-5.
24. Silva, FGA, Gomes, SC. Validation of an alternative absorbent paper for collecting gingival crevicular fluid. R.Periodontia 2009;19(3):85-90.
25. Abdulrahman FI, Akan JC, Cellube ZM, Waziri M. Levels of heavy metals in human hair and nail samples from Maiduguri Metropolis, Borno State, Nigeria. World Environment. 2012;2(4):81-9.
26. Olmedo P, Pla A, Hernandez AF, Lopez-Guarnido O, Rodrigo L, Gil F. Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. Anal Chimica Acta. 2010;659:60-7.
27. Barany E, Bergdahl IA, Bratteby LE, Lundh T, Samuelson G, Schutz A, et al. Trace element levels in whole blood and serum from Swedish adolescents. Sci Total Environm. 2002;286(1-3):129-41.
28. Achmad H, Adam M, Oktawati S, Karim Sari R,Thahir H, Pratiwi R, Wicita A. An overjet reduction of class II, division 1 Malocclusion in Twin Block Dentofacial Orthopedic and Fixed Orthodontic Treatment: Case Report. 2017;10(3):1010-6.
29. Achmad H, Tahir H, Adam M, Ramadhany YF. Increased overjet in growing child, problem solving in pediatric dentistry. J Int Dent Med Res. 10(2);374-79.
30. Liamsuwan S, Jaiweerawattana U. Correlation between serum and salivary phenytoin concentrations in Thai epileptic children. J Med Assoc Thailand. 2011;94 Suppl 3:S172-7.
31. Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 2006;28:292-7.
32. Amini F, Mollaei M, Harandi S. Effect of fixed orthodontic treatment on hair nickel an chromium levels: A 6-month of prospective preliminary study. Biol Trace Elem Res 2015;164(1):12-7.
33. Jamshidi S, Kamel MR, Mirzale M, SarrafanA et al. Evaluation of scalp hair nickel and chromium level changes in patients with fixed orthodontic appliances: A one-year follow-up study. Acta Odontol Scand 2018;76(1): 1-5.
34. Mikulewicz M, Wolowicc P, Loster B, Chojnacka K. Metal ion released from fixed orthodontic appliance affect hair mineral content. Biol Trace Elem Res. 2015;163:11-8.
35. Abtahi M, Jahanbin A, Yaghoubi M, Esmaily H, Zare H. Are more nickel ions accumulated in the hair of fixed orthodontic patients? Indian J Dent Res 2013;24:298-301.
36. Levrini L, Lusvardi G, Gentile D. Nickel ions release in patients with fixed orthodontic appliances. Minerva Stomatol 2016;55:115-21.
37. Masjedi MK, Jahromi NH, Niknam O, Hormozi E, Rikhsan V. Effect of fixed orthodontic treatment using conventional (two-piece) versus metal injection moulding brackets on hair nickel and chromium level: A double-blind randomized clinical trial. Eur J Orthod. 2017;17-24.
38. Faccioni F, Franceschetti P, Cerpelloni M, Fracasso ME In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. Am J Orthod Dentofacial Orthop. 2003;124:687-93.
39. Pazzinia CA, Ju´ niorb GO, Marquesc LS, Pereirad CV, Pereirae LJ. Prevalence of nickel allergy and longitudinal evaluation of periodontal abnormalities in orthodontic allergic patients. Angle Orthod. 2009;79:922-7.