


## Nickel and Chromium Ion Levels in Hair and Gingival Crevicular Fluid with the Corrosion of Brackets in Orthodontic Patients: A Longitudinal Study


Susilowati Mudjari<sup>1</sup>, Muhammad Harun Achmad<sup>2</sup>, Marhamah F. Singgih<sup>3</sup>, Irene Edith Rieuwpassa<sup>4</sup>, Fuad Husain Akbar<sup>5</sup>

<sup>1</sup>Department of Orthodontics, Hasanuddin University, Makassar, Indonesia.  0000-0003-4678-5272

<sup>2</sup>Department of Pediatric Dentistry, Hasanuddin University, Makassar, Indonesia.  0000-0003-3124-2064

<sup>3</sup>Department of Pediatric Dentistry, Hasanuddin University, Makassar, Indonesia.  0000-0003-1842-4234

<sup>4</sup>Department of Oral Biology, Hasanuddin University, Makassar, Indonesia.  0000-0001-6316-0472

<sup>5</sup>Department of Dental Public Health, Hasanuddin University, Makassar, Indonesia.  0000-0003-4819-4820

Author to whom correspondence should be addressed: Muhammad Harun Achmad, North Hertaning Street III Block F.24 Number 11, Makassar, Indonesia. 90222. Phone: +62 852-4273-9400. E-mail: [harunachmader@gmail.com](mailto:harunachmader@gmail.com).

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### Abstract

**Objective:** To assess the levels of nickel and chromium ions in hair and Gingival Crevicular Fluid (GCF) of orthodontic patients and to evaluate the corrosion of orthodontic bracket surfaces. **Material and Methods:** Nickel and chromium ion concentrations were measured in hair and GCF 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), 4, 8, and 16 months later during treatment. Along with ionic sampling, microscopic sampling was done. One of each patient brackets was removed to get 15 brackets per group. Five brackets were taken randomly from each group to be examined under scanning electron microscope (SEM). Data obtained were analyzed using paired t-tests. **Results:** After 16 months, compared with the baseline, average hair nickel level changed from 0.125 µg/g to 0.956 µg/g with statistically significant difference ( $p=0.00$ ); average chromium level changed from 0.090 µg/g to 0.295 µg/g but no significant difference ( $p>0.05$ ); average GCF nickel level changed from 3.335 µg/g to 10.410 µg/g; average chromium level changed from 1.859 µg/g to 9.818 µg/g. Both of these increases were significant ( $p=0.000$ ). SEM examinations showed that the corrosion on brackets was seen in the fourth month, and more severely visible after 8 and 16 months of uses. **Conclusion:** After 16 months of treatment, compared with the baseline, the hair nickel level was increased by 7.7 times; while for chromium was by 3.3 times. Gingival crevicular fluid nickel level was increased by 3.1 times and chromium level was by 5.3 times. The longer time of treatment, the more ions released and the more corrosion of brackets will be.

**Keywords:** Orthodontics; Metals, Heavy; Orthodontic Brackets; Corrosion.

## Introduction

Fixed orthodontic appliance in the mouth for a long time (1-2 years), experiencing continuous interaction with physiological fluids. Oral tissues are subjected to chemical and physical stimuli, as well as the metabolism of various bacterial species [1]. The salivary pH ranges from 5.2-7.8. Factors that influence the occurrence of metal corrosion processes in the mouth such as quantity and quality of saliva, temperature, plaque, pH, protein, physical/chemical properties of food and drink, and oral conditions [2].

In orthodontic treatment, hypersensitivity reactions often occur due to the content of nickel and chromium in brackets, bands, and stainless steel wire. Alloys in orthodontic metals contain about 6-12% nickel and 15-22% chromium. Nickel elements function to provide good properties in the bracket for formability, hardness, and heat resistance. Allergic, carcinogenic, mutagenic and cytotoxic effects often with nickel and to a lesser extent, chromium. Allergic reactions include edema, mouth lining, and anaphylaxis [3].

Nickel and chromium ions released from fixed orthodontic appliance have been a matter of concern to the researchers. The results of previous studies with nickel and chromium levels varied. Several studies on saliva of fixed orthodontic patients found that nickel and chromium levels increased [4]; while others reported that there were no significant differences after 1-3 months of treatment [5-7]. After two years of treatment, it was reported that the nickel ion level significantly increased [8]. It was reported that serum nickel level increased in a short time but it decreased again afterward [9]; however, there was no significant difference for serum chromium level after orthodontic treatment. On the contrary, nickel ion levels in urine have been reported to increase significantly two months and longer after insertion of the orthodontic appliance [10,11].

Fixed orthodontic appliances have several basic components such as orthodontic wire and brackets. The orthodontic bracket serves to deliver the necessary force on the teeth, therefore the bracket used must be produced accurately, both in terms of shape, strength, and level of corrosion resistance and biocompatibility [12]. A material is defined biocompatible if it does not have a negative influence on its biological environment, i.e. there are no toxic reactions, allergies or carcinogenic can be noticed. In addition, the physical properties of the material cannot be changed when used in vivo. Corrosion resistance and fundamental aspects of biocompatibility are influenced by several factors. First, it depends on the manufacturing process, alloy type, and bracket surface characteristics. Second, it refers to the environment in which the bracket is used. Third, it relates to the use of alloys, which emphasize side effects such as pressure, thermal handling, and the recycling process of components [13,14].

It is considered that corrosion can influence the physical-chemical properties of stainless steel brackets and arch wires, which may act on the clinical performance of these materials, increase the friction between arch-wire and slot, and release metal or alloy ions, which consequently can result in discoloration of enamel and soft tissues, local pains, allergic reactions in predisposed patient, and lead to caries [15-17].

Orthodontic treatment may influence systemic exposure, which can be measured with exposure biomarkers in Gingival Crevicular Fluid (GCF) [18]. GCF is relevant to orthodontic treatments and might reflect systemic changes associated with the inflammatory response induced by orthodontic forces. Therefore, it might also be used to show metal ion changes. Hair can be sampled to determine the trace elements that are most desirable because sampling, handling, and transportation are easy to do. Hair can reflect the average of trace elements, suitable for chronic exposure in a long time such as orthodontic treatment [19].

The aim of the study was to assess the levels of nickel and chromium ion in hair and GCF of orthodontic patients at different treatment times and to evaluate the corrosion of orthodontic bracket surfaces.

## Material and Methods

### Study Design and Sampling

This study was performed on 15 orthodontic patients (9 females and 6 males) who visited Dental Hospital of Hasanuddin University, Indonesia between the month of April 2017 to September 2018. The range of age was 16-28 years ( $19.2 \pm 2.3$  years). The patients had the following inclusion criteria: they need to be treated orthodontically in both arches and willing to be part of the study. The following conditions of patients must be excluded: has experience of orthodontic treatment, has systemic diseases, syndromes, allergies, metal restorations and piercing.

### Data Collection

The fixed appliance comprised of stainless steel orthodontic bands (Zhejiang Protect Medical Equipment Co., Zhejiang, China), bonded 0.018 inch slot pre-adjusted Roth prescription stainless steel brackets (Zhejiang Protect Medical Equipment Co., Zhejiang, China) on all teeth except the molars, NiTi wires (Nitinol; Ormco Corp., Orange, CA, USA), and stainless steel wires (Remanium; Dentaureum GmbH & Co., Ispringen, Germany).

The patients were asked not to drink on the morning of the scheduled visit and not to consume nickel- and chromium-rich foods for 48 hours before the sampling visit. The sampling was done 4 times (before treatment/baseline, 4 months, 8 months, and 16 months later). The teeth were selected randomly for collecting GCF from the gingival crevices. The tooth surface was dried gently and kept dry with cotton [20]. A standardized absorbent paper (PerioPaper®, OraFlow Inc., New York, NY, USA) was gently inserted into the gingival sulcus, let it for 60 seconds [21]. Then performed the same procedures with new papers in the other three teeth. The paper contaminated with blood was excluded from the study. The 4 PerioPapers® were kept in a bottle with a lid, stored in a freezer until further analysis. The PerioPapers® were weighed (Ohaus; Fisher Scientific UK Ltd., Loughborough, UK) before and after GCF sampling.

For collecting hair samples, a pair of sterilized stainless steel scissors was used to cut hair 2 mm from the scalp with a maximum length of 4 mm [19]. The samples were kept individually by keeping them in a sealed plastic bag and transported to the laboratory.

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

#### Scanning Electron Microscopy Analysis of Samples

The sample was composed of 60 stainless-steel brackets. The brackets were randomly selected and removed from the same patients who have been sampled for nickel and chromium ion levels. The brackets were divided into four groups ( $n = 15$  per group): group Control (without treatment) and groups T4, T8, and T16 (brackets after a treatment time of 4, 8 and 16 months, respectively). The removed brackets were replaced by new brackets.

The bracket was carefully removed with a “pistol” type pair of pliers and stored in deionized water. After that, they were brushed for 10 seconds and rinsed with deionized air, then stored in a sterile container until the time of analysis [2].

Then, orthodontic brackets were randomly selected for analysis by scanning electron microscopy (JSM-6510 JEOL Ltd., Tokyo, Japan). A total of 20 randomly selected brackets, with five brackets of each group, were examined. The bracket surface examined by SEM was the surface facing the oral cavity. The images obtained by SEM were acquired at various magnifications (25–500 x). Secondary electron images (SEI) for topography were recorded at 15-kV accelerating voltage.

#### Data Analysis

Data were analyzed using IBM SPSS Statistics for Windows Software, version 23 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate the mean and standard deviation. A paired t-test was used to assess statistically significant differences between the groups based on the two-time points. The level of significance was set at 0.05.

#### Ethical Aspects

The research was approved by the ethics committee of the Medical Faculty, Hasunuddin University. Written informed consent was obtained before starting the study.

#### Results

Table 1 shows the mean value of nickel and chromium ion levels ( $\mu\text{g/g}$ ) in hair and GCF at different treatment times. Nickel ion in the hair was found to increase from 0.125 (before treatment) to 0.420 (month 4), 0.610 (month 8), and 0.956 (month 16). Chromium ion level in the hair increased from 0.090 (before treatment) to 0.116 (month 4) but decreased to 0.090 at month 8, then increased again to 0.295 (month 16). Nickel ion level in the GCF increased from 3.335 (before treatment) to 8.965 (month 4), increased again to 10.260 (month 8) and then to 10.410 (month 16). Chromium ion level in GCF also increased from 1.859 (before treatment) to 6.819 (month 4), but decreased to 5.459 (month 8), and increased again to 9.818 (month 16).

**Table 1. Average nickel and chromium ion levels in hair and GCF.**

Samples	Ion	Ion level ( $\mu\text{g/g}$ )			
		Baseline Mean $\pm$ SD	4 Months Mean $\pm$ SD	8 Months Mean $\pm$ SD	16 Months Mean $\pm$ SD
Hair	Nickel	0.125 $\pm$ 0.044	0.420 $\pm$ 0.018	0.610 $\pm$ 0.053	0.956 $\pm$ 0.031
	Chromium	0.090 $\pm$ 0.006	0.116 $\pm$ 0.013	0.090 $\pm$ 0.033	0.295 $\pm$ 0.029
GCF	Nickel	3.335 $\pm$ 0.157	8.965 $\pm$ 0.335	10.260 $\pm$ 0.788	10.410 $\pm$ 0.675
	Chromium	1.859 $\pm$ 0.864	6.819 $\pm$ 0.333	5.459 $\pm$ 0.514	9.818 $\pm$ 0.451

Table 2 presents a multi-comparison test of nickel ion level with different orthodontic treatment period using paired sample t-test. All tests showed a statistically significant difference found in hair ( $p < 0.05$ ). In the GCF it was found that almost all observations had a statistically significant value ( $p < 0.05$ ), except for observations T8 to T16 with a value of 0.194 ( $p > 0.05$ ), which meant there was no significant difference between them. Nearly all ions have increased with a minus (-) result on statistical findings, which means that there is an increase in ion levels from observations T0, T4, T8, and T16.

**Table 2. Multi-comparison test of nickel ion levels at different period of orthodontic treatment.**

Nickel Level	Group Compared		Mean Difference	95% CI (min – max)	p-value
Hair	T <sub>0</sub>	T <sub>4</sub>	-0.295	-0.331 – -0.258	0.000*
		T <sub>8</sub>	-0.485	-0.52 – -0.044	0.000*
		T <sub>16</sub>	-0.831	-0.866 – -0.790	0.000*
	T <sub>4</sub>	T <sub>8</sub>	-1.904	-0.227 – -0.153	0.000*
		T <sub>16</sub>	-0.536	-0.569 – -0.502	0.000*
	T <sub>8</sub>	T <sub>16</sub>	-0.345	-0.390 – -0.301	0.000*
GCF	T <sub>0</sub>	T <sub>4</sub>	-5.630	-5.947 – -5.312	0.000*
		T <sub>8</sub>	-6.929	-7.492 – -6.356	0.000*
		T <sub>16</sub>	-7.077	-7.578 – -6.575	0.000*
	T <sub>4</sub>	T <sub>8</sub>	-1.299	-1.801 – -0.796	0.000*
		T <sub>16</sub>	-1.447	-1.834 – -1.059	0.000*
	T <sub>8</sub>	T <sub>16</sub>	-0.148	-0.386 – 0.097	0.194

\*Paired sample t-test:  $p < 0.05$ : significant; T0 - mean of the level before treatment; T4 - mean of the level at the end of the fourth month; T8 - mean of the level at the end of the eighth month; T16 - mean of the level at the end of the sixteenth month.

Table 3 presents a multi-comparison test of chromium ion level at different orthodontic treatment period using paired sample t-test. There are statistically significant differences in almost all observations of chromium ions found in hair ( $p < 0.05$ ) except for observations T0 to T8 with a value of 0.933 ( $p > 0.05$ ) and T0 to T16 with a value of 0.067 ( $p > 0.05$ ) which means there are no differences between them. In GCF all observations were found to have a statistically significant value ( $p < 0.05$ ).

**Table 3. Multi-comparison test of chromium ion levels at different period of orthodontic treatment.**

Chromium Level	Group Compared		Mean Difference	95% CI (min – max)	p-value
Hair	T <sub>0</sub>	T <sub>4</sub>	-0.026	-0.037 – -0.015	0.001*
		T <sub>8</sub>	-0.000	-0.021 – -0.020	0.933
		T <sub>16</sub>	-0.204	-0.227 – -0.018	0.000*
	T <sub>4</sub>	T <sub>8</sub>	0.025	-0.002 – -0.053	0.067
		T <sub>16</sub>	-0.178	-0.195 – -0.161	0.000*
	T <sub>8</sub>	T <sub>16</sub>	-0.204	-0.239 – -0.168	0.000*



GCF	T <sub>0</sub>	T <sub>4</sub>	-4.960	-5.216 – -4.704	0.000*
		T <sub>8</sub>	-3.600	-3.984 – -3.215	0.000*
		T <sub>16</sub>	-7.959	-8.279 – -7.638	0.000*
	T <sub>4</sub>	T <sub>8</sub>	1.360	1.056 – 1.664	0.000*
		T <sub>16</sub>	0.654	-3.466 – -2.530	0.000*
		T <sub>8</sub>	-4.359	-5.018 – -3.699	0.000*

\*Paired sample t-test;  $p < 0.05$ : significant; T<sub>0</sub> - mean of the level before treatment; T<sub>4</sub> - mean of the level at the end of the fourth month; T<sub>8</sub> - mean of the level at the end of the eighth month; T<sub>16</sub> - mean of the level at the end of the sixteenth month.

### Scanning Electron Microscopy Images

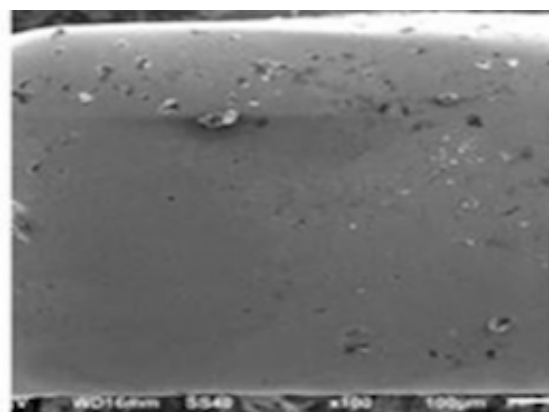
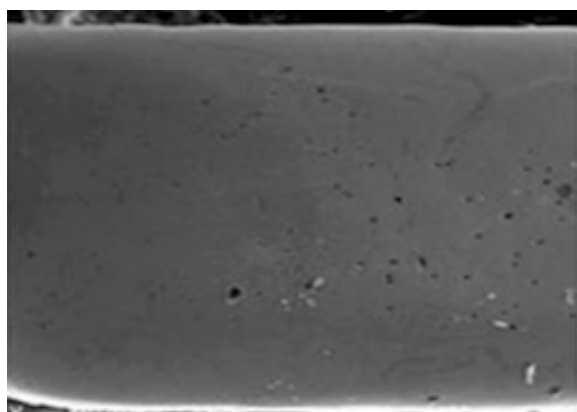


Figure 1. Bracket surfaces from control group (before treatment). No sign of corrosion. Visible only machining defects as scattered dots. SEM image 100 x.

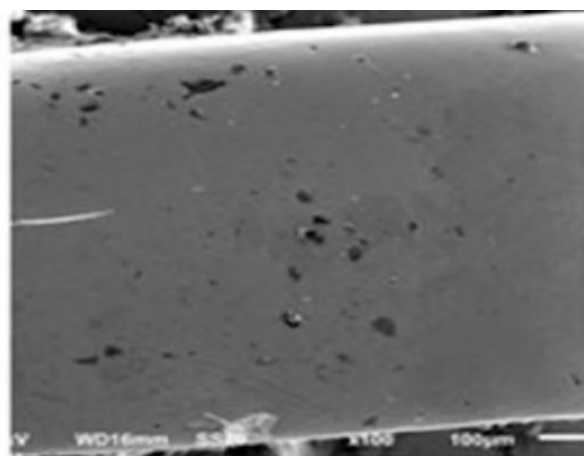
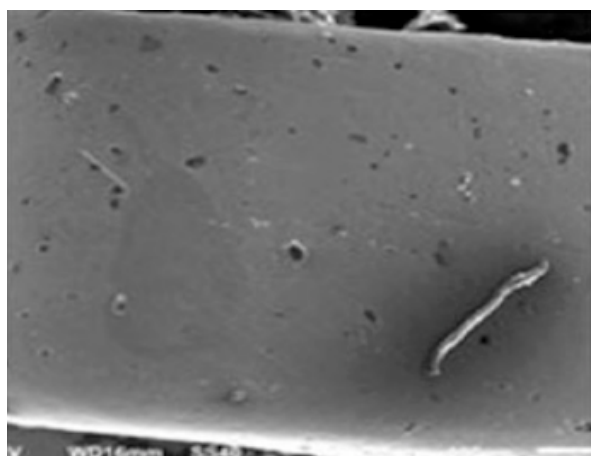


Figure 2. Bracket surfaces after 4 months of use. Discernible small pits of corrosion. SEM image 100 x

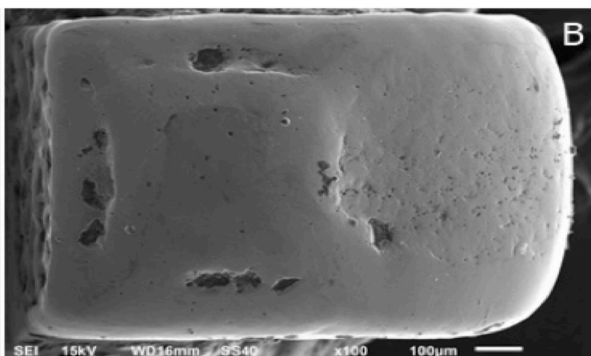
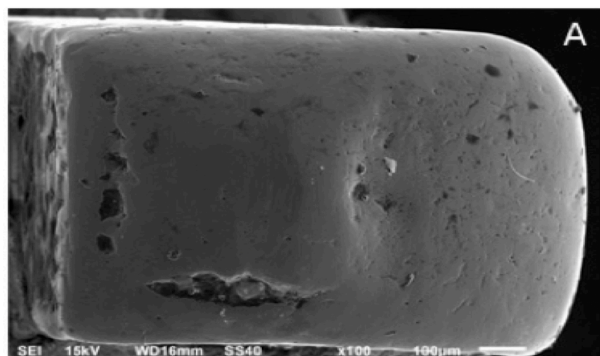
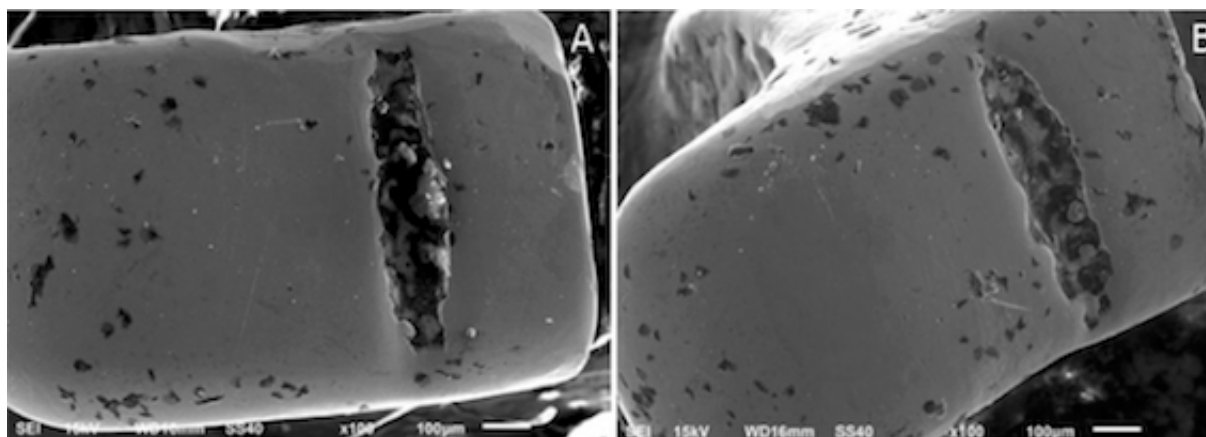


Figure 3. Bracket surfaces after 8 months of use. Discernible more numerous cavities of corrosion. SEM image 100 x.



**Figure 4. Bracket surfaces after 16 months of use. Visible deep and long cavities as sign of corrosion. SEM image 100 x.**

## Discussion

There are several ways to measure nickel and chromium ion levels in orthodontic patients both in vivo and in vitro, for example through saliva, serum, urine, hair, and GCF. The metal ion levels obtained were not similar. The reason might be explained as follows: at the time in the oral cavity, the process of releasing metal ions from orthodontic appliances is influenced by several factors such as temperature, salivary pH, galvanic currents, fluoride-containing toothpaste and mouthwashes [22] a variety of food, soft drinks, bacteria, plaque deposits etc., so the amount of ion released also varies. In the body, many factors also influence the ions to travel such as the metabolic rate, the bond with certain proteins in the blood, the speed of disposal through the kidneys and so on so that it is not clearly identified how much nickel and chromium ions are absorbed by the organism.

The results of this study (Table 1) showed that nickel in hair and GCF levels increased from before treatment (T0) to the fourth month (T4), increased again until the eighth month (T8) and sixteenth month (T16). The differences in nickel levels based on the treatment time were all significant except for T8 to T16 in GCF (Table 2). Chromium ion levels increased both in hair and GCF from T0 to T4 but decreased from T4 to T8, then increased again from T8 to T16 (Table 1). The increase of chromium was in line with a prospective study after 6 months of orthodontic treatment [23]. The decrease of chromium level might be due to the dietary habits of the participants. The increases were statistically significant except for T0 to T8 and T0 to T16 in hair. For chromium levels in GCF all increased statistically (Table 3). The results of this study were parallel with previous studies in the GCF [18,21] and in hair [19].

Averages nickel and chromium levels at all observations in GCF were higher than those in hair (Table 1). It can be explained that the GCF is close to the orthodontic appliance, which can release the metal ions to the saliva. Although in this study we have tried to avoid contamination between GCF and saliva, there is a possibility that saliva and GCF have contact each other before sampling. In addition, the ions travel in the body pass through a long journey and experiencing an elimination process and obstacles before reaching the hair.

The corrosion of orthodontic materials is an important clinical issue. Any metal alloy used in the mouth should have a requirement that they must not produce corrosion products that will be harmful to the body. Metallic part of the orthodontic appliance has a tendency to corrode [1]. We evaluated the corrosion of bracket surfaces through released ions measurement and microscopic assessment.

After 16 months of undergoing orthodontic treatment, the ionic levels in the hair and GCF increased 3-7 times, which is actually not proportional to the daily intake which is around 5 mg/L for nickel and less than 1 mg/L for chromium. But even the slightest addition of metal ions in hair and GCF must be watched out because it can trigger a contact dermatitis. A patient might become allergic in the future even though s/he is not allergic to nickel or chromium today.

Microscopic evaluation under SEM showed that before treatment brackets had no sign of corrosion (Figure 1 A). After 4 months of treatment, there was a visible sign of corrosion as shallow cavities but not many (Figure 1B). For ionic assessment, after 4 months, nickel and chromium levels in hair and GCF increased (Table 1). After 8 months of treatment, the bracket surfaces had more numerous cavities of corrosion (Figure 2). It was supported by the release of nickel and chromium in hair and GCF. After 16 months of treatment, it was shown that bracket surfaces had deep and long cavities as a sign of corrosion. It was parallel with the ions release in hair and GCF. An in vitro study has been done by immersing brackets in a saline solution. After 60 days, EDX analysis showed a decrease in iron and chromium ions, which were statistically significant [16].

The limitation of the study was difficulty in controlling dietary habits and oral hygiene of the patients.

## Conclusion

After 16 months of treatment, compared with baseline, the hair nickel level was increased by 7.7 times; while for chromium was by 3.3 times. Gingival crevicular fluid nickel level was increased by 3.1 times and chromium level was by 5.3 times. The degree of bracket corrosion might be caused by the released of nickel and chromium levels.

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**Conflict of Interest:** The authors declare no conflicts of interest.

## References

- [1] Chaturvedi TP, Upadhyay SN. An overview of orthodontic material degradation in oral cavity. Indian J Dent Res; 21(2):275-84. <https://doi.org/10.4103/0970-9290.66648>
- [2] Dos Santos AA, Pithon MM, Carlo FG, Carlo HL, de Lima BA, Dos Passos TA, Lacerda-Santos R. Effect of time and pH on physical-chemical properties of orthodontic brackets and wires. Angle Orthod 2015; 85(2):298-304. <https://doi.org/10.2319/032914-234.1>
- [3] Castro SM, Poces MJ, Lopes JD, Vasconcelos M, Pollmann MCF. Orthodontic wires and its corrosion. The specific case of stainless steel and beta-titanium. J Dent Sci 2015; 10(1):1-7. <https://doi.org/10.1016/j.jds.2014.07.002>



- [4] Matos de Souza R, Macedo de Menezes L. Nickel, chromium and iron levels in the saliva of patients with simulated fixed orthodontic appliances. *Angle Orthod* 2008; 78(2):245-50. <https://doi.org/10.2319/111806-466.1>
- [5] Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. *Eur J Orthod* 2006; 28(3):292-7. <https://doi.org/10.1093/ejo/cji091>
- [6] 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.
- [7] Bengileil MS, Orfi JM, Abdelgader I. Evaluation of salivary nickel level during orthodontic treatment. *J Clinic Toxicol* 2013; 3:5. <https://doi.org/10.4172/2161-0495.S1.010>
- [8] 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(5):375-9.
- [9] Rai RV, Shetty S, Ahluwalia R, George S. Quantitative assesment of serum nickel and chromium levels in orthodontic patients: An in vivo study. *J Indian Orthod Soc* 2011; 45(4):251-5. <https://doi.org/10.5005/jp-journals-10021-1045>
- [10] 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; 150(1-3):31-6. <https://doi.org/10.1007/s12011-012-9478-6>
- [11] Menezes LM, Quintão CA, Bolognese AM. Urinary excretion levels of nickel in orthodontic patients. *Am J Orthod Dentofac Orthop* 2007; 131(5):635-8. <https://doi.org/10.1016/j.ajodo.2005.07.022>
- [12] Eliades T. Orthodontic materials research and applications: part 2. Current status and projected future development in materials and biocompatibility. *Am J Ortho Dentofacial Orthop* 2007; 131(2):253-64. <https://doi.org/10.1016/j.ajodo.2005.12.029>
- [13] Maijer R, Smith DC. Biodegradation of the orthodontic bracket system. *Am J Orthod Dentofac Orthop* 1986; 90(3):195-8. [https://doi.org/10.1016/0889-5406\(86\)90065-X](https://doi.org/10.1016/0889-5406(86)90065-X)
- [14] Brandão GAM, Simas RM, Almeida LM, Silva JM, Meneghim MC, Pereira AC, et al. Evaluation of ionic degradation and slot corrosion of metallic brackets by the action of different dentifrices. *Dental Press J Orthod* 2013; 18(1):86-93. <https://doi.org/10.1590/S2176-94512013000100019>
- [15] Schiff N, Boinet M, Morgon L, Lissac, Dalard F, Grosogeat B. Galvanic corrosion between orthodontic wires and brackets in fluoride mouthwashes. *Eur J Orthod* 2006; 28(3):298-304. <https://doi.org/10.1093/ejo/cji102>
- [16] Menezes LM, De Souza RM, Dolci GS, Dedavid BA. Analysis of biodegradation of orthodontic brackets using scanning electron microscopy. *Dental Press J Orthod* 2010; 15(3):48-51. <https://doi.org/10.1590/S2176-94512010000300006>
- [17] Maia LH, Filho HL, Araújo MV, Ruellas AC, Araújo MT. Incorporation of metal and color alteration of enamel in the presence of orthodontic appliances. *Angle Orthod* 2012; 82(5):889-93. <https://doi.org/10.2319/092111.599.1>
- [18] Amini F, Shariati M, Sobouti F, Rakhshan 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(5):666-72. <https://doi.org/10.1016/j.ajodo.2015.10.023>
- [19] Mikulewicz M, Choynacka K, Zielinska A, Michalak I. Exposure to metals from orthodontic appliances by hair mineral analysis. *Environ Toxicol Pharmacol* 2011; 32(1):10-6. <https://doi.org/10.1016/j.etap.2011.02.009>
- [20] Isik F, Sayinsu K, Arun T, Unlüçerçi Y. Bone marker level in gingival crevicular fluid during orthodontic intrusive tooth movement: A preliminary study. *J Contemp Dent Pract* 2005; 6(2):27-35.
- [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. <https://doi.org/10.9790/0853-1601061417>
- [22] Rafeeq RA, Saleem AI, Nissan LM. Ions release from fixed orthodontic appliance in two different mouthwash. *J Baghdad Coll Dent* 2014; 26(4):152-5.
- [23] Amini F, Mollaei M, Harandi S, Rakhshan V. Effect of fixed orthodontic treatment on hair nickel and chromium levels: A 6-month of prospective preliminary study. *Biol Trace Elem Res* 2015; 164(1):12-7. <https://doi.org/10.1007/s12011-014-0188-0>