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ORIGINAL ARTICLE



Evaluation of scalp hair nickel and chromium level changes in patients with fixed orthodontic appliance: a one-year follow-up study

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ABSTRACT

Background and objectives: The release of metal ions from orthodontic appliances is part of the dissolution and biomechanical processes of alloys. Nickel (Ni) and chromium (Cr) are the elements commonly used in the manufacture of various components of fixed orthodontic appliances, including bands, brackets and wires. This study was aimed to measure the Ni and Cr ions levels in the scalp hair of patients treated with fixed orthodontic appliances in comparison of the control group.

Materials and methods: The patient group consisted of 24 patients treated with fixed orthodontic appliances for one year, while the control group included 28 healthy individuals without orthodontic appliances. Analysis of the Cr and Ni was performed using atomic absorption spectrophotometer by graphite furnace method. The data were analyzed via student and paired samples t-test and ANOVA repeated measurement test.

Results: After one year, the levels of Ni and Cr in two groups showed significant differences (0.086 ± 0.007 and 0.258 ± 0.009 $\mu\text{g/g}$ for control group and 0.149 ± 0.010 and 0.339 ± 0.013 $\mu\text{g/g}$ for patient group, respectively for Ni and Cr, $p < .001$). ANCOVA test by removing the effects of age, gender and the baseline levels of Ni and Cr showed that changes in these ions in the scalp hair of both groups after one year were statistically significant.

Conclusion: Due to the slightly elevated levels of Ni and Cr ions in the scalp hair of patients treated with fixed orthodontic appliances and considering the cytotoxic and allergic effects of these ions, changing the ingredients in fixed orthodontic appliances is suggested for the future.

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Introduction

The release of metal ions from orthodontic appliances is part of the dissolution and biomechanical processes of alloys [1]. On average, the stainless steel alloys used for orthodontic appliances involves 17 to 22% chromium (Cr) and 5 to 8% nickel (Ni) [2]. Although orthodontic alloys contain anti-corrosion materials, it has been proven that the buccal cavity is prone to corrosion in a dynamic environment; this corrosion can release ions, absorbed by mucosal cells, penetrate into the blood and then affect various body structures like hair [3]. It can also cause allergic and genetic disorders like contact allergies, asthma, hypersensitivity, birth defects and reproductive problems [4–6]. Moreover, the DNA damage and cytotoxic effects of these metals have been shown both *in vitro* and *in vivo* [6].

Over the past two decades, several studies have investigated the release of orthodontic appliance metals causing systemic or intraoral adverse effects. In addition, many *in vivo* studies have been conducted to prove the biocompatibility of orthodontic alloys with Ni-Cr contents in the saliva, blood,

urine and oral mucosal cells of orthodontic patients [6]. In some studies, comparison of the saliva of subjects with and without orthodontic appliances treatment has shown no significant difference in the levels of Ni and Cr [7,8]. However, scalp hair, which is highly affected by these released metals [3] can be considered as an appropriate indicator for measuring the metal content released by orthodontic appliances, due to non-invasive sampling [3]. It is possible to track and measure metal ions in scalp hair [9]. Human scalp hair also has a greater mineral structure, when compared to blood and urine [3]. One report by the United States Atomic Energy and Environmental Organization suggests that human scalp hair is exposed to many metals [3]; however, the scalp hair must be free of organic contaminants to determine the concentrations of these metals.

Considering few research studies in this area and the lack of precise knowledge about the effects of fixed orthodontic appliances on the levels of metal ions in the scalp hair, this study was aimed to measure the Ni and Cr ions levels in the scalp hair of patients treated with fixed orthodontic appliances in comparison to the control group.

Materials and methods

Subject

This case-control study was performed on 52 subjects in two groups (patients and controls) at the Dental School of Babol University of Medical Sciences, Babol, Iran. The patient group consisted of 24 individuals treated with fixed orthodontic appliances for one year, while the control group included 28 healthy individuals without orthodontic appliances. All of the participants were in the age range of 14 to 25 years old and were divided into sub-groups of 14–18 and 19–25 years old. This study began after gaining approval from the ethics committee of Babol University of Medical Sciences. Sampling was done after obtaining a consent signed form from the participants.

Exclusion criteria were: individuals exposed to the Ni and Cr elements, patients with systemic diseases (such as liver disease, kidney disease and cancer), cigarette and alcohol consumer, the presence of any metal restorations such as amalgam fillings or fixed prostheses, or any soldered/extraoral orthodontic appliances and those individuals with dyed hair or allergy problems [10].

Since the release of metals from orthodontic appliances takes some time [11], all of the patients involved in the project had been in contact with fixed brackets in both jaws for one year. In addition, there was no need for tooth extraction in these patients during this time period and all of the permanent teeth had erupted (with the exception of the wisdom teeth/third set of molars). All participants were also free from caries and the fixed orthodontic treatment was performed for all the teeth in the upper and lower jaws.

Uniform treatment protocols

MBT 022 brackets (American Orthodontics, USA) and identical wires (Ortho Technology, USA) were used in all patients for homogenization. The archwires that were used in this study were nickel titanium (NiTi) (Ortho Technology, USA) and stainless steel (SS) archwires (Ortho Technology, USA) in both jaws. The order of the used wires was as follows 0.016" round NiTi, 0.018" round NiTi, 0.019 × 0.025" rectangular NiTi and finally, 0.019 × 0.025" rectangular SS wires. The wire replacement intervals were about 4–6 weeks for each wire, depending on the treatment plan.

Hair sampling

The primary sampling took place immediately before the beginning of the treatment with fixed orthodontic appliances, while the secondary sampling was conducted exactly one year after the treatment. The samples were taken from the back of the head and as close to the hair follicle as possible by stainless steel scissors and then kept in a sealed envelope in darkness until analysis.

Analysis

For analysis of the Cr and Ni levels in hair scalp, dissolution was first carried out on the samples. All chemicals that used

in this study were in analytical grade. The glassware materials that used for this study was soaked in 0.1N HNO₃ for 24 h and washed with distilled water, then with deionized water produced by Direct-Q 3 UV Water Purification System (Millipore, France) and finally stored in laminar flow hood. The hair pollution, debris and proteins were resolved using Triton X-100 (Sigma-Aldrich, Germany) (1:200 dilution for 10 minutes). Then the samples were transferred into a metal-free acetone solution for 10 minutes and eventually washed and cleaned three times with deionized water. The samples were dried in an oven (Fan Azma gostar, Iran) for 6 hours at 80 °C and then 0.5 mg of each sample was immersed in 2.5 ml nitric acid (65%) (Merck KGaA, Darmstadt, Germany) and 1.25 ml hydrogen peroxide (30%) (Sigma-Aldrich, Germany) for 12 hours at room temperature. Finally, the samples were placed in the dryer for 2–3 hours at 80 °C to remove the organic contents. The resulting clear solution was diluted by adding 25 ml of deionized water [3].

After the dissolution process and the preparation of the samples, the levels of Cr and Ni were determined via an atomic absorption spectrophotometer (AAS) (PG-990 with Graphic Furnace Power Supply, China) by graphite furnace method in pyrocoated graphite tubes according to the manufacturer's instructions. Argon was used as purge gas for the graphite furnace. The instrumental parameters that were used for analysis of these two elements are illustrated in Table 1. To this end, wavelengths of 357.9 (band width = 0.4 nm and lamp current = 5.0 mA) and 232 (band width = 0.2 nm and lamp current = 4.0 mA) were used to measure the levels of Cr and Ni, respectively. Various working standards i.e. 0.0, 6.25, 12.5, 25.0 and 50.0 ppb were used for preparing the standard curve of Cr (from Cr 1000 ppm stock, Merck, KGaA, Darmstadt, Germany, HC 114887). Different concentrations of Ni from its 1000 ppm stock (Merck, KGaA, Darmstadt, Germany, HC 127141) were used for plotting the Ni standard curve (0.0, 7.8, 15.6 and 31.25 ppb). The resulted curves had reasonable accuracies (both $r \geq 0.95$). Each sample was tested with three replications. The resulted data were reported as the means ± SE in ppb. For estimation of the analytical precision, one sample in each fifteen samples was analyzed in triplicate and the coefficient of variation (CV) was calculated. The resulted CVs were less than of 10%. The percentage recovery of the analyzed metals was calculated by dividing the amount of metals determined by the amount of added metals and multiplying these values by 100. The metal recovery rates ranged between 85–97%. Precision of the instrument was assessed by analyzing of two digested samples in an independent laboratory and a maximum of 3% differences were observed in the results of both metals.

Table 1. Atomic absorption spectrophotometry settings and furnace program for Cr and Ni analysis.

Step	Temperature (°C) (Cr-Ni)	Ramp (second) (Cr-Ni)	Hold (second) (Cr-Ni)	Argon flow (ml/min) (Cr-Ni)
Drying	70–80	10–5	10–10	300–300
Drying	100–110	10–10	10–15	300–300
Pyrolysis	800–800	10–15	15–10	300–300
Atomization	2100–2000	0–0	3–3	0–0
Clean up	2200–2100	1–1	2–2	300–300

Statistical analysis

The data were analyzed using SPSS V.21 software, via student t-test and a paired samples t-test. ANOVA repeated measurement test was used for analysis of interaction between time and groups. Central tendency and dispersion indexes tables and graphs used for descriptive data. ANCOVA test was used comparing changes in these ions in the scalp hair of both groups after one year by removing the effects of age, gender and the baseline levels of Ni and Cr. A *p* value less than of .05 was considered statistically significant.

Results

This study was carried out on a total of 52 persons, 24 subjects in the case group and 28 healthy individuals in the control group. The raw data of patients are detailed in Table 2. The mean age for the included person was 16.56 ± 2.82 years (17.54 ± 3.04 in the control group and 15.42 ± 2.06 in the case group). The average ages were different between the two groups according to the t-test ($p = .005$).

In the case group, there were seven males and seventeen females; whereas in the control group there were 15 males and 13 females. The difference between the gender distributions in the groups was not statistically significant ($p = .08$). At the beginning of the study, no statistically significant differences in Cr levels were observed between the control and case group ($p = .788$), but the differences for the levels of Ni in scalp hair were statistically significant ($p < .001$). After one year, hair Cr and Ni levels statistically had significant increase in patient group compared to control group (both of them $p < .001$). In addition we observed that the hair Cr levels in the control group, after one year, significantly increased ($p = .004$), but such increase for the Ni levels was not

observed ($p = .938$). At the end of the study in the patient group, both Ni and Cr levels in scalp hair significantly increased ($p < .001$ and $p = .034$; respectively). After one year, Ni and Cr level changes between the two groups was statistically significant ($p < .001$).

The results of the present study demonstrated that the Ni and Cr levels in people treated with fixed orthodontic appliances for one year, significantly increased and interestingly the differences in the Ni content was greater and more obvious when compared to the control group (242% increase in hair Ni content and 11% for Cr) (Table 3 and Figure 1). There was no significant association between the patient's age and any of the ion concentrations at any sessions ($p > .1$). As well, there were no significant differences between the patient's gender with any of the ion measured at any of the intervals ($p > .15$). To correct the effects of age and sex on changes in levels of Ni and Cr, ANCOVA test was used. Results showed that by eliminating the effects of age, gender and baseline

Table 3. Comparison of nickel and chromium levels ($\mu\text{g/g}$ dry hair mass) at baseline and after one year in both groups.

Ions	Time			Changes
	Base line	After one year	<i>p</i> value ^a	
Ni	Control	0.085 ± 0.004	0.086 ± 0.007	.938
	Patient	0.061 ± 0.005	0.149 ± 0.011	<.001
	<i>p</i> value ^b	<.001	<.001	<.001
Cr	Control	0.299 ± 0.011	0.285 ± 0.009	.004
	Patient	0.304 ± 0.011	0.339 ± 0.015	.034
	<i>p</i> value ^b	.788	<.001	<.001

Values in table are Mean $\pm$ SE.

Changes in Cr and Ni shows difference values between baseline and after one year.

^aRepresent *p* value for paired t-test.

^bRepresent *p* value for paired for t-test.

Table 2. Hair scalp level of nickel and chromium ($\mu\text{g/g}$ dry hair mass) in each patient at baseline and after one year.

Patient	Gender	age	Base line				After One Year			
			Nickel		Chromium		Nickel		Chromium	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	F	14	0.055	0.005	0.279	0.012	0.159	0.012	0.307	0.010
2	M	14	0.084	0.006	0.487	0.015	0.151	0.010	0.299	0.010
3	F	14	0.054	0.006	0.340	0.011	0.214	0.010	0.385	0.011
4	M	14	0.044	0.005	0.304	0.012	0.168	0.011	0.362	0.012
5	F	14	0.060	0.005	0.397	0.010	0.164	0.013	0.311	0.013
6	M	15	0.057	0.003	0.340	0.009	0.229	0.010	0.311	0.013
7	F	15	0.074	0.007	0.317	0.015	0.188	0.011	0.311	0.014
8	F	15	0.048	0.006	0.326	0.015	0.121	0.012	0.301	0.013
9	F	15	0.054	0.005	0.361	0.012	0.143	0.009	0.254	0.013
10	F	18	0.056	0.004	0.283	0.011	0.169	0.008	0.260	0.013
11	M	14	0.060	0.001	0.242	0.010	0.141	0.010	0.261	0.011
12	F	14	0.065	0.005	0.322	0.010	0.298	0.010	0.211	0.013
13	F	14	0.140	0.003	0.385	0.009	0.146	0.010	0.353	0.010
14	F	15	0.084	0.008	0.451	0.012	0.132	0.014	0.285	0.014
15	F	16	0.073	0.006	0.271	0.012	0.112	0.012	0.306	0.014
16	M	17	0.041	0.004	0.234	0.011	0.171	0.011	0.320	0.015
17	F	15	0.046	0.005	0.331	0.011	0.135	0.007	0.284	0.012
18	F	15	0.117	0.006	0.369	0.010	0.130	0.007	0.211	0.012
19	F	15	0.083	0.002	0.350	0.010	0.134	0.010	0.337	0.015
20	M	17	0.085	0.005	0.367	0.011	0.096	0.009	0.295	0.016
21	F	14	0.067	0.006	0.229	0.008	0.112	0.007	0.264	0.017
22	F	15	0.038	0.007	0.345	0.010	0.098	0.009	0.353	0.013
23	M	24	0.029	0.005	0.311	0.010	0.044	0.011	0.341	0.012
24	F	20	0.056	0.006	0.416	0.011	0.121	0.009	0.457	0.013

SE: standard error of mean.

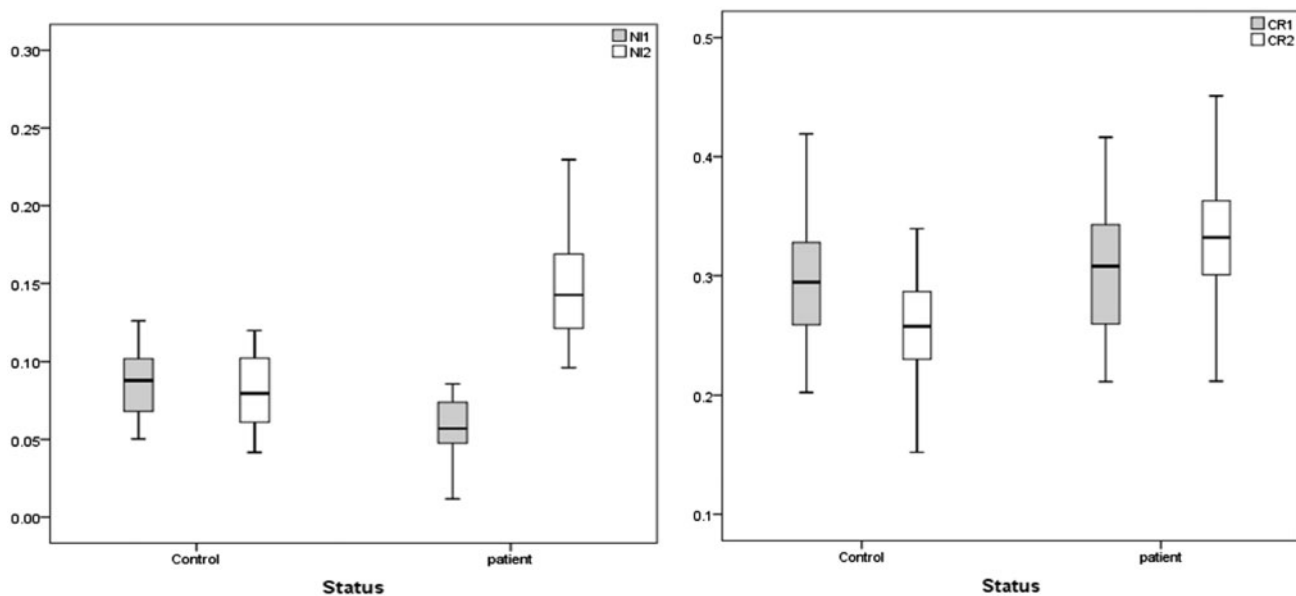


Figure 1. Box plots showing hair nickel and chromium levels at the baseline and after one year in control and patient group ($\mu\text{g/g}$ dry hair mass). Ni1: nickel level at the baseline; Ni2: nickel level after one year; Cr1: Chromium level at the baseline; Cr2: Chromium level after 1 year.

Table 4. ANCOVA test results to eliminate the effects of baseline levels of Ni and Cr, gender and age in two study groups.

	Ni			Cr		
	F	DF	p value	F	DF	p value
Age	1.84	1	.181	1.94	1	.17
Gender	0.3	1	.585	0.037	1	.848
Groups	17.72	1	<.001	16.902	1	<.001
Groups adjusted with gender	0.008	1	.928	0.005	1	.943
Baseline	1.63	1	.208	1.58	1	.215

levels of Ni and Cr, changes in these ions in both groups were statistically significant after one year (Table 4).

Discussion

The results of this study showed that the patients who were treated with fixed orthodontic appliance, their scalp hair Ni and Cr levels increased significantly. The increase in Ni is more than in Cr, when compared to the control group.

There are various studies in which reported that some metals are released from orthodontic appliances to the blood stream and finally to the hair. In a 6-month study in 2014, Amini et al. conducted a similar study to record changes in the levels of Ni and Cr in scalp hair after treatment with fixed orthodontic appliances. They demonstrated that the Ni and Cr levels increased by 381 and 16%, respectively, which was similar to our results [3]. Mikulewicz et al. showed increases in Ni (39%) and Cr (2.5%) in the scalp hair before and after orthodontic treatment, but they did not report a significant difference in the hair Ni and Cr contents [8]. In 2006, Lervini et al. reported that the Ni level of the scalp hair in the group that treated with orthodontic appliances was higher when compared to the control group [12]. Abtahi et al. reported a 4-month investigation of the Ni changes in the scalp hair of patients undergoing fixed orthodontic treatment and reported an increase of 190%, which is similar to the present study [13]. In another study, Martin et al. reported that the

differences between the levels of the Ni and Cr ions in case and control groups were not statistically significant, contrary to the results of this research [14]. Kocadereli et al. reported that treatment by fixed orthodontic appliances do not significantly affect the levels of nickel and chromium in the saliva [15]. Neama et al. did not detect significant differences in the Ni and Cr levels in the saliva of patients with fixed orthodontic appliances [16].

The causes of the differences in these results could be attributed to the type and material of the appliance components, the properties of the used alloys, the exposed surface of the appliance components and environmental factors, including the chemical composition, temperature, pH, volume, microflora and also enzymatic activity of the saliva.

Ni and Cr are elements commonly used in the manufacture of various components of fixed orthodontic appliances, such as bands, brackets and wires [2]. Moreover, the effects of contact with these metals and their compounds have been previously evaluated with regard to health. According to a few studies in this area, the use of fixed orthodontic appliances leads to the release of metal ions via electro-galvanic flow in the saliva as an intermediate environment [17]. The buccal cavity is an environment for the decomposition of metals, especially due to its microbiological and enzymatic properties [1]. Ni, as an allergen, can cause immunological sensitivity in a number of people receiving long-term orthodontics. Cr and cobalt (Co) ions can also cause dermatitis, asthma and adverse biological and cytotoxic effects [1]. The amount of Ni released from fixed orthodontic appliances may be less than the threshold necessary to start an allergic reaction, at least in people who have not shown a previous hypersensitivity to metal [18]; but either way, a small level of free metal may also cause allergies in people who are treated with fixed orthodontic appliances for two to three years, because the symptoms of a Ni allergy may be seen over time.

Studies have also shown that Ni and Cr can have genotoxic effects and a low dose of these ions can cause damage

to the DNA and cell morphology, as well as disrupt the monocytes and endothelial cell activities [19]. Other studies have shown the importance of the side effects of ions released from these appliances [20]. For eg, Amini et al. examined the effects of ions released from fixed orthodontic appliances and concluded that Ni ions are significantly more common in the buccal mucosal cells of patients treated with fixed orthodontics [11]. Faccioni et al. conducted a similar study on the oral mucosa of 85 subjects in case and control groups and reported that the amount of Ni (about 2.52 ng/ml) in the case group was 3.4 times higher than that in the control group (0.73 ng/ml) [5]. Karnam et al. showed that after three months of artificial saliva treatment on the appliances, the Ni content was greater than that of the other ions.

Our study has some limitations. We were not able to analyze the levels of Cr and Ni in the blood and saliva of included persons. Surely more studies with bigger sample size and longer follow up period and sampling from blood and saliva, in addition to hair, can help us for safer application of fixed orthodontic appliances in clinic.

In conclusion due to the slightly elevated levels of Ni and Cr ions in the scalp hair of patients treated with fixed orthodontic appliances and considering the cytotoxic and allergic effects of these ions, changing the ingredients in fixed orthodontic appliances can be considered as a viable solution in the future.

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Disclosure statement

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References

- [1] House K, Sernetz F, Dymock D, et al. Corrosion of orthodontic appliances—should we care? *Am J Orthod Dentofacial Orthop.* 2008;133:584–592.
- [2] Petoumenou E, Arndt M, Keilig L, et al. Nickel concentration in the saliva of patients with nickel-titanium orthodontic appliances. *Am J Orthod Dentofacial Orthop.* 2009;135:59–65.
- [3] Amini F, Mollaei M, Harandi S, et al. Effects of fixed orthodontic treatment on hair nickel and chromium levels: a 6-month prospective preliminary study. *Biol Trace Elem Res.* 2015;164:12–17.
- [4] Ortiz AJ, Fernández E, Vicente A, et al. Metallic ions released from stainless steel, nickel-free, and titanium orthodontic alloys: toxicity and DNA damage. *Am J Orthod Dentofacial Orthop.* 2011;140:115–122.
- [5] Faccioni F, Franceschetti P, Cerpelloni M, et al. In vivo study on metal release from fixed orthodontic appliances and DNA damage in oral mucosa cells. *Am J Orthod Dentofacial Orthop.* 2003;124:687–693.
- [6] Westphalen GH, Menezes LM, Prá D, et al. In vivo determination of genotoxicity induced by metals from orthodontic appliances using micronucleus and comet assays. *Genet Mol Res.* 2008;7:1259–1266.
- [7] Ağaoğlu G, Arun T, İzgi B, et al. Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. *Angle Orthod.* 2001;71:375–379.
- [8] Mikulewicz M, Chojnacka K. Release of metal ions from orthodontic appliances by in vitro studies: a systematic literature review. *Biol Trace Elem Res.* 2011;139:241–256.
- [9] Roushan H, Parsian H, Alijannia A, et al. Hair mercury levels in periodontal patiens in comparison with healthy individuals. *World J Dent.* 2013;5:166–169.
- [10] Eliades T, Trapalis C, Eliades G, et al. Salivary metal levels of orthodontic patients: a novel methodological and analytical approach. *Eur J Orthod.* 2003;25:103–106.
- [11] Amini F, Borzabadi Farahani A, Jafari A, et al. In vivo study of metal content of oral mucosa cells in patients with and without fixed orthodontic appliances. *Orthod Craniofac Res.* 2008;11:51–56.
- [12] Levriani L, Lusvardi G, Gentile D. Nickel ions release in patients with fixed orthodontic appliances. *Minerva Stomatol.* 2006;55:115–121.
- [13] Abtahi M, Jahanbin A, Yaghoubi M, et al. Are more nickel ions accumulated in the hair of fixed orthodontic patients?. *Indian J Dent Res.* 2013;24:298–301.
- [14] Martín-Cameán A, Molina-Villalba I, Jos A, et al. Biomonitorization of chromium, copper, iron, manganese and nickel in scalp hair from orthodontic patients by atomic absorption spectrometry. *Environ Toxicol Pharmacol.* 2014;37:759–771.
- [15] Kocadereli I, Ataç A, Kale S, et al. Salivary nickel and chromium in patients with fixed orthodontic appliances. *Angle Orthod.* 2000;70:431–434.
- [16] Neama ZT. Nickel and chromium ions levels in saliva of patients with fixed orthodontic appliances. *Med J Babylon.* 2014;11:558.
- [17] Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. *Eur J Orthod.* 2006;28:292–297.
- [18] Afridi HI, Kazi TG, Jamali MK, et al. Evaluation of toxic metals in biological samples (scalp hair, blood and urine) of steel mill workers by electrothermal atomic absorption spectrometry. *Toxicol Ind Health.* 2006;22:381–393.
- [19] Natarajan M, Padmanabhan S, Chitharanjan A, et al. Evaluation of the genotoxic effects of fixed appliances on oral mucosal cells and the relationship to nickel and chromium concentrations: an in-vivo study. *Am J Orthod Dentofacial Orthop.* 2011;140:383–388.
- [20] Olmedo P, Pla A, Hernández AF, et al. 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 Chim Acta.* 2010;659:60–67.