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Research Article
Nickel Variation in Biofilm, Saliva and Buccal Mucosa During Orthodontic Treatment

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Abstract

Background: Nickel alloys (Ni) are broadly used in the fabrication of orthodontic adjuncts, they become an integral part of almost every orthodontic interventions. It is suggested that nickel is a toxic metal and in some cases is capable of producing carcinogenic effects, therefore, it is important to measure its levels in individuals with orthodontic appliances. **Objective:** To determine the levels of nickel in the oral cavity through samples of saliva, biofilm and the oral mucosa before and during 6 months of the orthodontic treatment. **Methodology:** Quasi-experimental study, the size of the sample was stablished according to historical trend with 270 samples taken from 30 subjects to which orthodontic appliances were placed. In order to determine the nickel concentration, the samples were analyzed in a thermo scientific Atomic Absorption Spectrophotometer (AAS) iCE 3000 series with graphite furnace in three different moments and time use of the orthodontic appliances in the mouth. **Results:** When comparing the nickel concentration in 3 times and taking into account the type of sample, significant differences were found in the biofilm, no differences were found according to the commercial house and the type of sample that had the greatest nickel concentration in the 3 times was the biofilm. The nickel levels in the oral cavity change after the placement of the orthodontic appliances, those changes being more significant in the saliva and biofilm samples, the biofilm sample is the one with the greater nickel concentrations before and after the placement of the orthodontic appliances. **Conclusion:** The nickel levels showed a significant increase in the subjects of the sample. Especially in biofilm sample in relation to time of installation of the brackets appliances in the mouth.

Key words: Nickel, spectrophotometry, orthodoncy, orthodontic wires, AAS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nickel usage in orthodontic appliances is wide and varied, it is used in different materials such as braces, bands, tubes and arches[1,2]. These orthodontic appliances are used permanently in the treatment with fixed orthodoncy and its permanency in the oral cavity can release metal ions into the buccal tissue[3]. Many studies are being conducted on nickel's capacity of producing allergic and toxic reactions[4] and carcinogenic effects. The clinical manifestations in the oral cavity may start with oral lesions such as desquamation[5], erythema multiform, gingivitis and gingival enlargement[6].

Nickel release is related to the surface of the braces[7], the time of exposure to the environment, moisture conditions and ions of the saliva of the mouth[8]. The oral environment due to its enzymatic, thermic, microbiologic and chemical characteristics, turn it into a propitious environment for metal degradation, therefore, patients who have orthodontic appliances made with nickel alloys are exposed to corrosion processes which release Ni ions[8].

The objective of the present study was to determine the variations in the Ni levels that are presented during the first 6 months with fixed orthodontic appliances and the usage of nickel-titanium (Ni-Ti) through the analysis of samples of saliva, biofilm and oral mucosa.

MATERIALS AND METHODS

A quasi-experimental study was conducted. Two hundred and seventy samples taken from 30 individuals were included in the study, the size of the sample was obtained through historical trend[1,2]. The participants voluntarily accepted being a part of the study by signing an informed consent and were selected taking into account the following criteria: Subjects treated in the Orthodontics Postgraduate of the University of Cartagena which had conventional fixed orthodontic appliances (Gemini Unitek™ 3M braces, Abzil agile 3M braces and Master American Orthodontics). Subjects with amalgam fillings, metal inlays, dental implants, piercings in the oral cavity, fixed or removable prostheses with metal, subjects who worked or lived near industrial areas, subjects with metal implants in their body, smokers or those with prior orthodontic treatments were not taken into account in this investigation.

Sampling: Saliva samples were taken in the morning before food consumption. Prior to sampling the subjects rinsed their mouths with 5 mL of distilled and deionized water, then they would keep their mouths closed for 5 min without salivary stimulation, after this time a sample of approximately 1.5 mL of saliva was taken in a sterile 1.5 mL polypropylene test tube for PCR. The biofilm samples were collected with a regular TCP® micro-applicator, scrapings of the right upper first molar were taken into a sterile 1.5 mL polypropylene test tube for PCR. The oral mucosa samples were performed through a regular conic Interplast® cito-brush doing ten laps clockwise in the surface of the cheek and taken into a sterile 1.5 mL polypropylene test tube for PCR.

Ni quantification: The samples were analyzed in an atomic absorption spectrometer (Thermo scientific), Atomic Absorption Spectrometer (AAS) iCE 3000 series with Graphite Furnace, UK), which was previously calibrated with three standard nickel nitrate solutions of 0.5, 1.0 and 2.0 mg L^{-1} and a wavelength of 232 nm, these three standards served as a reference to know the concentrations of the evaluated samples in this study. The detection limits were -10 and -40% and it determined the concentration units in ppm. For this, the SOLAAR Data Station version 11.03 (Thermo scientic iCE 3000 series AA spectrometers, UK) software was used, it stablished the nickel concentration in milligram per liter from the observed absorbance in each sample and taking into account the standard calibration curve. All samples were assessed in triplicate.

Statistical analysis: For the analysis and interpretation of the data, the statistical package SPSS version 20 (IBM) was used, initially descriptive statistic measures were applied (central tendency, dispersion, absolute and relative frequencies). To evaluate the comparison of the measurements a paired student's t-test for two groups, one way ANOVA for more than two groups and Tukey's post-test were used to stablish intergroup differences, all with values of probability accepted as significant $p<0.05$. The data presented a normal distribution according to the Kolmogorov-Smirnov test.

As bioethical considerations of the study, the ethical considerations raised in the Resolution 008430 of 1993 in Colombia, Title II Chapter 1, where it is stablished that every investigation where the human being is a subject of study, it should prevail the respect for its dignity and the protection of its rights and well-being. Also, anonymity was guaranteed to the participant population and the use of information only with academic purposes. Equally, it is declared that the bioethical principles stated in Helsinki's declaration for the handling of data obtained from human beings was followed.

RESULTS

In total, 270 samples from 30 participants, which 33.3% where males and 66.7% were females, these presented an age average of 23.7 (SD = 7.7) years, the most frequent type of brace was the Abzil agile (3M) 36.7% and the orthodontic technique with the greatest frequency was Ricketts with a 36.7% followed by the standard with a 33.3% (Table 1).

The samples were taken in three moments, T0: Before starting the orthodontic treatment, T1: 1 week after starting the orthodontic treatment and T2: 6 months after starting the orthodontic treatment, obtaining from each participant samples of saliva, biofilm and oral mucosa. In T0: saliva (mean: 0.0022), biofilm (mean: 0.0033) and oral mucosa (mean: 0.0049). In T1: saliva (mean: 0.0026), biofilm (mean: 0.0037) and oral mucosa (mean: 0.0058). In T2 saliva (mean: 0.0030), biofilm (mean: 0.0031) and oral mucosa (mean: 0.0069).

When comparing the nickel concentrations in the 3 times and taking into account the type of sample, significant differences were found in the biofilm between T0 (mean: 0.0048) and T1 (mean: 0.0058), T0-T1 difference: 0.000807 (p = 0.01) and for saliva between T0 (mean: 0.0022) and T2 (mean: 0.0030), T0-T2 difference: 0.000817 (p = 0.005) (Table 2).

When relating the nickel concentrations in each one of the sample types, in the 3 times of following and taking into account the brand of the braces, no statistically significant differences were found (Table 3).

When comparing the nickel concentrations between saliva, biofilm and oral mucosa, statistically significant differences were found in the 3 times (p = 0.00), where the concentrations were the greatest in the biofilm in T0 (mean: 0.00494), (SD: 0.00159), in T1 (mean: 0.00575), (SD: 0.00166) and in T2 (mean: 0.00692), (SD: 0.00609) (Table 4).

Table 1: Demographics of the study subjects and distribution of orthodontic appliances according to manufacturer

Parameters	Mean	SD
Age	23.67	7.73
Sex	n = 30	%
Male	10	33.3
Female	20	66.7
Type of brackets		
American orthodontics	10	33.3
Abzil agile (3M)	11	36.7
Gemini (Unitek 3M)	9	30
Orthodontic technique		
Ricketts	11	36.7
Standard	10	33.3
MBT	9	30

Table 2: Nickel concentrations in saliva, oral mucosa and biofilm in 3 times of measurement

	T0	T1			T2		
Parameters (ppm)	Mean (SD)	Mean (SD)	T0-T1	p-value¥	Mean (SD)	T0-T2	p-value¥
Saliva	2.213 (0.9387)	2.627 (1.303)	0.41	0.129	3.03 (1.1216)	0.82	0.005*
Oral mucosa	3.327 (1.4022)	3.683 (1.7013)	0.36	0.189	3.143 (1.55)	0.18	0.602
Biofilm	4.943 (1.5865)	5.75 (1.6636)	0.81	0.01*	6.917 (6.0872)	1.97	0.093

¥: Paired student's t test. All of the Mean, Standard Deviation (SD) and difference values were multiplied by 1000

Table 3: Nickel concentrations in saliva, oral mucosa and biofilm according to the brand of the brackets

	American orthodontics		Abzil agile (3M)		Gemini (Unitek 3M)		
Parameters (ppm)	Mean	SD	Mean	SD	Mean	SD	p-value¥
Saliva (T0-T1-T2)	2.503	1.083	2.848	1.266	2.481	1.133	0.383
Oral mucosa (T0-T1-T2)	2.990	0.827	3.673	1.796	3.470	1.803	0.516
Biofilm (T0-T1-T2)	6.520	6.124	5.618	1.914	5.456	1.550	0.209

¥: One way ANOVA. All of the Mean, Standard Deviation (SD) and difference values were multiplied by 1000

Table 4: Levels of nickel concentrations in samples of saliva, biofilm and oral mucosa

	Saliva		Biofilm		Oral mucosa		
Parameters	Mean	SD	Mean	SD	Mean	SD	p-value¥
T0	2.213	0.939	4.943	1.587	3.327	1.402	0.00*
T1	2.627	1.303	5.750	1.664	3.683	1.701	0.00*
T2	3.030	1.122	6.917	6.087	3.143	1.550	0.00*
			Post hoc				
Difference	Saliva-biofilm	p-value£	Saliva-mucosa	p-value£	Biofilm-mucosa		p-value£
T0	2.73	0.00*	1.11	0.005*	1.617		0.00*
T1	3.12	0.00*	1.06	0.028*	2.067		0.00*
T2	3.89	0.00*	0.11	0.992	3.773		0.00*

¥: One way ANOVA. All of the Mean, Standard Deviation (SD) and difference values were multiplied by 1000, £: Tukey's *post hoc* HSD test, *p<0.05

When comparing between the types of samples for T1, differences between saliva and biofilm were found (p = 0.00), biofilm and oral mucosa (p = 0.00) and saliva and oral mucosa (p = 0.028).

In T2 when comparing between the types of samples, differences were found only in the nickel concentrations between saliva and biofilm (p = 0.00) and biofilm and oral mucosa (p = 0.00).

DISCUSSION

Despite the limitations of the present study, among those the lack of evaluation of factors such as the participants' diets, the use of standardized techniques and procedures allow us to obtain pertinent and trustworthy results, taking into account that orthodoncy is one of the dental specialties with the greatest demand nowadays and that its techniques use appliances composed by metals such as nickel, which intervenes in biological processes like cellular viability and proliferation as stablished by D'Anto *et al.*[9] in this sense, authors such as Hafez *et al.*[10] affirm that some metallic ions from the orthodontic appliances possibly have genotoxic, cytotoxic and carcinogenic potential, with decreases cellular viability and induces damages in the DNA.

The results suggest that there is an increase in the concentration of the nickel levels in saliva and biofilm after the placement of the orthodontic appliances and that these vary depending of the time tracking, which matches with the reported by Ousehal and Lazrak[11] who evaluated the nickel levels in saliva through atomic absorption spectrophotometry before the placement of the orthodontic appliances, immediately after the placement and 8 weeks after the placement, which demonstrated that a significant increase in the nickel levels right after the insertion of the Ni-Ti arch existed but it was not significant 8 weeks later. Arcila *et al.*[3] evaluated the nickel concentrations in saliva, biofilm and gums in patients with orthodontic appliances and gingival enlargement, using the same analytic techniques, suggest that the nickel concentrations in saliva are affected notoriously by the presence of fixed orthodontic appliances, also that this concentration varies with time.

Yassaei *et al.*[12] evaluated the nickel concentrations in saliva in 4 times, before the orthodontic treatment, 20 days after, 3 and 6 months after, concluding that no significant differences exist in the nickel amount between each one of the evaluated times. It is contrasted with the obtained results in the present study due to the significant differences found in saliva and biofilm.

Talic *et al.*[13] evaluated the nickel concentrations in saliva of individuals with an without orthodoncy in different times of use of the orthodontic appliances during 32 months, they concluded that even though an increase of the levels in patients with orthodoncy after starting the treatment exists, they stablished that these levels are not toxic and they do not change significantly during time suggesting that the duration of the orthodontic treatment does not affect the nickel levels in saliva. The confrontation with the current results implies that a real and significant change in the nickel levels does occur, especially the levels collected in saliva and biofilm. Even though similarity exists in the impossibility to stablish that the detected levels have the capacity of being considered toxic from a general point of view, although, in the oral cavity gingival enlargements can be detected.

In relation with the nickel accumulation in the biofilm, the results suggest that in this sample the greatest amount of nickel in the three evaluated times was observed by Fors and Persson[14] found that the nickel accumulation is greater in biofilm samples, contrary to that, Arcila *et al.*[3] reported that the majority of the biofilm samples had a nickel level under the detection limits, however, these differences could be generates due to the collecting and analysis techniques used by the different authors.

Regarding the nickel concentrations in the mucosa, no significant differences were found in time, even though a slight increase in the concentration between the first and second measurements existed, which suggests that even though the nickel concentrations in mucosa increase after the orthodontic treatment, some other factors that possibly influence these concentrations exist. Opposite to this, Amini *et al.*[15] evaluated the nickel content in oral mucosa cells from patients with and without fixed orthodontic appliances using atomic absorption spectrophotometry, concluding that the nickel content in the oral mucosa samples was significantly higher in the patients with orthodoncy in comparison to the control groups.

Faccioni *et al.*[16] reported relatively higher nickel levels in oral mucosa of patients under orthodontic therapies, being 3-4 times higher than in the individuals without orthodontic appliances, it should be highlighted that these results are not completely comparable for in the present study a group of patients without orthodontic appliances was not included, however, it is precise to mention and highlight the need to perform more studies about this matter to help proportion better evidence on how the nickel levels from the orthodontic appliances may affect the mucosa of the oral cavity.

To synthetize, the nickel concentrations in the saliva and biofilm after the placement of the orthodontic appliances can change independently from the brand of the braces, whereby it is necessary to evaluate the biocompatibility of all the materials used in orthodoncy to guarantee the safety of the patients, in addition to identify which one is the best type of testing and handling of the sample that allows to monitor the nickel levels during the orthodontic treatment.

CONCLUSION

The nickel levels in the oral cavity vary after the placement of the orthodontic appliances, being these changes more significant in the saliva and biofilm samples, highlighting that the biofilm samples are the ones that contain the greater nickel concentrations before and after the placement of the orthodontic appliances.

It is suggested that no differences exist in the nickel concentrations according to the manufacturer or brand of the analyzed braces, however, these levels do not overcome the physiologically permitted levels.

SIGNIFICANT STATEMENTS

Nickel alloys (Ni) are broadly used in the fabrication of orthodontic adjuncts. Nickel is a toxic metal and in some cases is capable of producing carcinogenic effects. We determine the levels of nickel in the oral cavity through samples of saliva, biofilm and the oral mucosa before and during 6 months of the orthodontic treatment. The Ni concentrations in biofilm, saliva and buccal mucosa increase during measurement times. These findings indicated that nickel levels in the oral cavity vary after the placement of the orthodontic appliances and producing allergic and toxic reactions and carcinogenic effects.

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