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Evaluations of Saliva for Nickel release from Conventional Stainless Steel and Nickel free Orthodontic brackets- An Atomic Absorption Spectrophotometric (AAS) study

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Abstract

Introduction: Nickel, which is a potent allergen, is used as a key metallurgical ingredient for manufacturing orthodontic brackets. The study aims at extracting nickel levels in patient's saliva at different time interval.

Methods: A total of twenty patients, ten bonded with conventional 3M Stainless steel brackets (group A) and another ten with nickel free brackets(group B). Group B was further divided into two groups of five each, based on different manufacturing companies viz Classic orthodontic and d-tech orthodontics. Three samples of stimulated saliva were collected from each patient at the following intervals: before placement of appliance, one hour after placement and a week after the appliance is being placed. The sample were then analysed with an atomic absorption spectrophotometer for amount of nickel release.

Results: Study revealed that the nickel release in 3M stainless brackets in first hour of the bracket placement was very high compared to pre- treatment nickel level. Gradually the release was found to decrease when it was measured after one week. In the second group comprising nickel free brackets by Classic orthodontics the release of nickel gradually increased from the time the appliance was placed in the oral cavity over a period of one week. Third group having d- tech nickel free orthodontic brackets the release in nickel was found to be significantly on higher side after one hour of the appliance placement which eventually decreased over a period of one week.

Conclusions: The final outcome of the present study revealed that manufacturing companies and extended time interval has a profound effect on nickel release from simulated fixed orthodontic appliance. Nickel free brackets used in the study though claim to be absolutely nickel free but some amount of nickel was found in them also.

Keywords: Nickel, Atomic Absorption Spectrophotometer

Introduction

The biocompatibility of dental alloy has been investigated over the past twenty year. However, studies on these issues have given rise to questions without answers confirming the need to learn more about the biocompatibility of these materials. Since this process has not been fully explained, orthodontist may be confused in the selection of biologically safe appliance for their patients.**(1)** A large variety of metallic alloys are routinely used in dentistry. Gold was used in orthodontics for fabrication of the accessories until the 1930s and 1940s. In 1929, stainless steel was used for the first time to replace gold. Orthodontic bands, brackets and wires are universally made of austenitic steel containing approximately 8-12% nickel and 17-22% chromium.**(2)** Nickel in particular is the most common cause of contact dermatitis in women.**(2,3)** In addition to the allergic issues, carcinogenic, mutagenic and cytotoxic effects have been assigned to nickel and to lesser extent chromium.**(4)** The literature shows that nickel is potentially allergic and capable of provoking type IV delayed hypersensitivity reactions that presents signs in the oral cavity including gingival overgrowth, angular cheilitis and labial desquamation.(5) In vitro experiments on cultured human gingival fibroblast showed that ions released from implanted nickel-chromium alloys can cause altered cellular functions.(6) At present, orthodontic bands, brackets and arch wires are primarily made of stainless steel, containing 18% chromium and 8% nickel. Nickel titanium (NiTi) arch wires contain more than 50% nickel. Orthodontic alloys emit electro galvanic currents with saliva as the medium, leading to a release of metal ions. This discharge of nickel ions might be potentially hazardous for the patients undergoing orthodontic treatment.(7,8) It has been reported that 4.5% to 28.5% of the population has nickel hypersentivity, with a higher prevalence in females(9,10) and the frequency of sensitivity increases in patient who have sustained prosthodontics or orthodontic treatments, especially when nickel-containing alloys are used. This study was conducted with the aim to evaluate the nickel release from conventional stainless steel and nickel free brackets and between the nickel free brackets of different manufacturers at different time intervals.

Materials and Method

The study was conducted in the Department of Orthodontics and Dentofacial Orthopaedics of Darshan Dental College and Hospital Udaipur, India and a total of twenty patients were included in the study.

Group A comprised of ten patients bonded with 3M conventional stainless steel brackets (Fig. 1).
Group B comprised of 10 patients bonded with nickel free brackets. Group B was further divided into two groups; group B1 in which the patients were bonded with nickel free brackets from classic orthodontics (Fig. 2) and the group B2 in which d-tech nickel free brackets were used (Fig. 3).

Patients with all the teeth till second premolar of all four quadrants intact and without any metal restorations were included in the study as the metal restorations tend to bias the results. No wire was placed in brackets. Patients consent was taken prior to the start of the study and ethical clearance was obtained from the ethical review board of the College.

Sampling of the saliva: Three samples of stimulated saliva were collected from each orthodontic patient under study at the following intervals: before insertion of the fixed appliance, one hour after placement of the appliance and seven days after the insertion of the appliance. The patients initially were made to rinse their mouths thoroughly with a mouthful of distilled, deionized water. After mouth rinsing, the patient used a piece of paraffin as a chewing gum for stimulation of the salivary secretion. The patient collected approximately 10- 15 ml of saliva into an acid washed plastic test tube.[11] The samples were then carried in an ice box within the period of 90 minutes to the laboratory in the vicinity for the nickel analysis.

Salivary preparation and analysis: Nickel concentration of saliva is stable for six months when stored at -20 ^{0}C.[2,11] Extraction methods can be used for the isolation and purification of elements from biological materials.[2] The use of an Atomic absorption spectrophotometer permits the analysis of metal in biological samples without any separation of metals in biological matrix.[2] For the processing, 0.5ml of saliva was transferred to a smaller plastic test tube, which was pretested for not releasing nickel.[2] For the digestion of the organic matter in the saliva, 0.15ml of concentrated hydrochloric acid (suprapur) was added to each sample.[11] The test tube were closed and kept at 80^{0}C for 8 hours.[11] Later the volumetric flask was used to dilute each of the samples to 10ml^2. The sample were then analysed with an Atomic absorption spectrophotometer **(GF-AAS; Shimadzu Corp, Kyoto, Japan, AA-6610G/GFA)** (Fig. 4) and the nickel concentrations present were calculated as microgram per millilitre. The error associated with this method of analysis was less than 1%. In atomic absorption spectrophotometer there is one light source that emits outs a light of single wavelength. There are different filaments accordingly to detect different metals as in this study nickel filament has been used. The light produced by the combustion of the sample was detected by a monochromatic detector on the other side which detects the light of only one wavelength. Just adjacent to the detector is a readout monitor that converts the light into an electrical signals or calibration graphs.

Instrument specifications:
Instrumental parameters:
Wavelength (nm) = 232.0
Band width (nm) = 0.5
Background correction, D2 lamp
Thermal program:
Drying at 150 - 250^0 C for 30 seconds.
Ashing at 1200^{0}C for 13 seconds.
Atomization at 2500^{0}C for 3 seconds.
Data obtained was entered into SPSS version 19.0 for the analysis. Paired t-test was used for intra-group comparison and Independent t-test was used for inter-group comparison.

Fig. 1: 3M Unitek stainless steel brackets

Fig. 2: Classic nickel free brackets

Fig. 3: d-tech nickel free brackets

Fig. 4: Atomic absorption spectrophotometer

Results

The nickel level in patient's saliva for all three types of brackets are revealed in Table 1. Statistical analysis was performed for the three groups (I, II and III) independently and in combination for analysis of ion release according to the study periods (t_1, t_2 and t_3). The results of the **paired student's t test** revealed statistically significant differences ($P < 0.05$) in the amount of nickel release in saliva during different study periods.

The nickel release in stainless brackets in first hour of the bracket placement was very high considering pre-treatment nickel level as a control group. Gradually the release in nickel was found to decrease when it was measured after one week as shown in Table 2. This probably is due to the unfinished matt surface of the bracket that the initial level was so high. Formation of a biofilm over the bracket surface can also be attributed as one of the reasons for decrease in the nickel level in saliva over a period of one week. In second group comprising of the nickel free brackets by Classic orthodontics the release of nickel gradually increased from the time the appliance was placed in the oral cavity over a period of one week. The results in table II depicts that there was significant increase in nickel content in one week time intervals when compared to pre- treatment nickel content of saliva. Table 2 depicts that in the third group having d- tech nickel free orthodontic brackets the release in nickel was found to be significantly on higher side after one hour of the appliance placement. The nickel content of saliva was found to decrease eventually over a period of one week. The results show that in stainless steel group the release of nickel was significantly higher than the rest two groups. Stainless group was followed by classic orthodontic group and among the three groups the least amount of release during an interval of one hour was seen in d- tech Company. Table 3 shows the intergroup comparison between the two nickel free groups using unpaired sample t-test. When compared for their nickel release it has been found that they were statistically different only in the interval of one hour, classic being little higher than d-tech in nickel release. Difference at one week interval was insignificant.

Table 1: Amount of nickel release in various brackets (µg/L)

3M Unitek conventional stainless steel brackets			
S. No	**Pre- Treatment**	**One hour**	**One week**
1.	5.921	14.652	4.923
2.	4.857	16.217	5.432
3.	5.053	15.321	5.672
4.	6.355	16.027	6.823
5.	4.899	15.982	5.954
6.	7.029	14.721	8.547
7.	6.895	16.021	7.247
8.	7.127	16.982	7.981
9.	5.413	16.921	7.017
10.	6.488	17.015	5.989
d-tech nickel free brackets			
1.	7.019	6.892	7.929
2.	6.927	7.321	7.012
`3.	6.895	7.021	8.021
4.	5.842	6.453	7.923
5.	7.812	8.432	8.217
Classic orthodontics nickel free brackets			
1.	6.723	8.029	8.434
2.	6.652	7.925	8.314
3.	7.925	8.761	8.694
4.	5.928	7.012	9.812
5.	6.218	7.843	8.673

Table 2: Intra-group comparison for various groups using Paired Samples t-test

3M unitek conventional stainless steel group				
	t value	**df**	**P value**	**Inference**
Pre-treatment- One hour	-25.798	9	0.000	HS
Pre-treatment- One week	-2.159	9	0.059	S
One hour- One week	22.102	9	0.000	HS
Classic nickel free brackets				

Pre-treatment- One hour	-9.807	4	0.061	NS
Pre treatment- One week	-4.025	4	0.053	S
One hour- One week	-1.758	4	0.154	NS
d- tech nickel free brackets				
Pre treatment- One hour	-2.248	4	0.055	S
Pre treatment- One week	-2.686	4	0.088	NS
One hour- One week	-1.655	4	0.173	NS

Table 3: Inter-group comparison for various groups using Unpaired samples t-test

Interval	Group	Mean	Standard Deviation	P Value
Diff 1	Classic	1.242	0.283	0.001 (HS)
	d-tech	0.364	0.259	
Diff2	Classic	2.09	1.164	0.102 (NS)
	d-tech	0.981	0.766	
Diff 3	Classic	0.853	1.085	0.635 (NS)
	d-tech	0.556	0.783	

Diff 1= Pre- treatment and 1 hour
Diff 2= Pre- treatment and 1 week
Diff 3= 1 hour and 1 week

Discussion

The present study was aimed at evaluating the nickel ion concentration in saliva of patient's undergoing fixed orthodontic treatment by conventional stainless steel and nickel free brackets. The study also aimed at analysing the pattern of nickel ion release at various time intervals by various brackets. The clinical study was done over a period of one week with the prime objective to benefit the patients who are nickel sensitive. A lot more researches have done and documented in major journals and books pertaining the release of nickel in conventional stainless steel brackets but as far nickel free brackets which the manufacturers claim to be nickel free, nothing substantial has been done so far. When measuring the nickel release from various stainless steel materials into synthetic sweat, blood and urine, scientists observed that the surface finish of the materials significantly affected the nickel release. From the polished materials, the nickel release into each of the test fluids was generally very low. In the case of stainless steel plates with a matt or mirrored finish, the release of nickel appeared increase.[15] The North American Contact Dermatitis group of 13 dermatologists in USA and Canada collects de-identified data on patch tested patients and publishes the epidemiological data every 2 years. They have established that since 1992, nickel has been the most frequent positive allergen in the group of 65 allergens. They also have added that the nickel sensitivity has increased over the past decade by about 4% and the individuals under the age of 18 years had significant higher rates of nickel sensitivity compared to older patients and woman also had significantly higher rate of allergy than men. According to the group, the prevalence of nickel allergy in North America ranges from 14% - 18%.[14] Similar study was done by European Surveillance System on Contact allergies (ESSCA). They found the positive patch test in 17.3% of European population with highest prevalence in Italians (31%) and the least in Dutch population (8.1%). No study so far has been documented having done on Indian population pertaining prevalence of nickel allergy.[14] With this sound knowledge of the nickel as an allergen, selection of the orthodontic bracket on the basis of the alloy and manufacturing process may be fundamental for biocompatibility.[12] Studies have reported that the characteristics of alloy and the manufacturing process are the main factors influencing the corrosion of brackets.[12] This study emphasizes on the detection of the nickel release in patient's saliva with three different manufacturers of the brackets. The reason for having nickel free brackets in the study to check the claims made by the two manufacturers and to ascertain that the nickel released by these brackets are well within the non- sensitization level. The nickel released by three different companies were recorded at three different time intervals; pre- treatment, one hour after placement of the appliance and one week after placing the appliance. No wire was placed in the oral cavity over this period of one week as there were chances of initial wire which is generally conflict the results.

Results of our study revealed that the maximum nickel release among the three test groups was seen in stainless steel group. These results are in agreement with the study done by Maria Francesca, Vittorio Cacciafesta *et al*.,[13] in which they concluded that recycled brackets released maximum amount nickel followed by conventional stainless steel brackets and the least by nickel free brackets. In this study group the maximum release of nickel was seen within the first hour of the appliance delivery which gradually decreased over a period of one week. This result of our study is in agreement with study done by Rodrigo Matos, Luciana mace do *et al*., in which they also found the release of nickel to be maximum immediately after placing the appliance in the oral cavity

Nickel release in classic nickel free brackets showed a gradual increase in nickel concentration in saliva .Pair one comparing pre- treatment and one hour nickel concentration revealed significant results which clearly indicate that some amount nickel is being liberated by this group of nickel free brackets. The one hour differential results when compared with the one hour differential results of stainless steel brackets showed that the results are insignificant indicating that despite the fact the nickel is being released by these brackets but it's in an insignificant amount when compared to stainless steel brackets.

Nickel released by d- tech nickel free brackets showed a similar kind of pattern as shown by stainless steel brackets. The release of nickel ions was maximum at an interval of one hour and gradually decreased. The pair one comparing pre- treatment and one hour nickel ion concentration revealed significant p value. The one hour differential results of d-tech and stainless steel when compared were insignificant depicting that less amount of nickel been released by d- tech nickel free.

The standard for diagnosis of nickel allergy contact dermatitis is Patch test. Patch testing involves placing nickel sulphate (2.5% or 5%) in petrolatum in direct contact with skin for 48 hours and then examining the skin for a local reaction 72 to 96 hours later. Positive reaction exhibits erythema, induration and papules. Strong positive reactions may even results in localised vesiculobullous reactions.[14]

Conclusion

The final outcome of the present study revealed that manufacturing companies and extended time interval has a profound effect on nickel release from simulated fixed orthodontic appliance. Maximum release in routinely used stainless steel brackets was seen at an interval of one hour. So the orthodontist will come to know about the patient being hypersensitive in the first hour of appliance delivery. Nickel free brackets manufacturing used in the study though claim to be absolutely nickel free but some amount of nickel was found in them also. Further, the study carried the scope to explore the difference in nickel release for new and recycled brackets, for NiTi and stainless steel wire and it also had scope to evaluate the release of other metals like lead, chromium and iron. With the availability of new technique sensitive instruments like inductively coupled plasma atomic emission spectrometer (ICP-AES), precise measuring of the metals has become possible. It's a known fact that the periodontium both hard and soft tissue show significant changes all through the orthodontic treatment. But whether the clinical changes are normal or paranormal is not an easy thing to judge by a periodontist. So the most common presentation of nickel contact dermatitis which is the presence of a well demarcated, erythematous, popular eczematous plaque at the site of nickel contact should be well known by both orthodontist as well as periodontist. Allergy avoidance is the most effective strategy to nickel contact dermatitis. Generally quality controlled stainless steel, titanium; sterling silver and platinum are safe in patients with nickel allergy.[15] Patients should be educated about the availability of a nickel detection kit [dimethylglyoxime (DMG)]. DMG on a cotton swab turns pink if a metal object contains detectable nickel.[15]

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