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Evaluation of metal ion release from orthodontic brackets by using different mouthwashes – An In-vitro study

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Abstract

Aim and objective: To evaluate the Nickel and chromium metal ion release from orthodontic brackets by using 4 different mouthwashes. To measure the amount of metal ions released from the stainless-steel orthodontic brackets incubated in de-ionised water and four different mouthwashes and to measure the amount of metal ions released from the stainless-steel orthodontic brackets incubated in one herbal mouth rinse and compare the amount of metal ions released in all the mouth rinses with that of de-ionised water.

Material and method: One hundred stainless steel brackets(koden orthodontic brackets) were divided randomly into 5 equal groups and immersed in Oral B (Procter & Gamble, Weybridge, United Kingdom), chlorhexidine (Shahdaru Labratories, Tehran, Iran lisetrine (Poursina Pharmaceutical Laboratories, Tehran, Iran), and Himalaya hiora mouth washes and distilled deionized water and incubated at 37°C for 45 days. Nickel and chromium released from the orthodontic brackets were measured with an inductively coupled plasma spectrometer. For statistical analysis, 1-way analysis of variance (ANOVA) and the post-Hoc tests were used.

Result: One way ANOVA showed that there is a statistically significant difference present in the mean nickel ion and chromium ion release between the various groups with highest release in chlorhexidine and least release in deionized water. Post hoc test used to compare individual group which shows highly significant difference in all groups except between oral-B and listerine which is non-significant.

Conclusion: More nickel and chromium ion release from orthodontics stainless steel brackets by using chlorhexidine mouthwash and least from Himalaya hiora.

Keywords: Himalaya hiora, ANOVA, Biocompatibility.

Introduction

The materials used in orthodontic appliances are metallic alloys and non-metallic materials such as ceramic, composite and polycarbonate. Stainless steel has been the mainly used material in orthodontics since its introduction .in 1932. Orthodontic brackets are made from a variety of materials including stainless-steel alloy which contains approximately 6 to 12% nickel and 15 to 22% chromium.

Oral tissues are exposed to a veritable bombardment of both chemical and physical stimuli, as well as the metabolism of about 30 species of bacteria. The pH of saliva varies from 5.2 to 7.8.

Biocompatibility is one of the prime concerns in dentistry as the materials are invariably used in the oral environment for long periods of time. The metallic alloys used in orthodontics can degrade and release products which can elicit a foreign-body reaction or induce pathologic processes. Regular use of mouthwashes during the orthodontic treatment is recommended to reduce the risk of development of white spots around the orthodontic brackets. Although the prophylactic agent in the mouthwash have been reported to cause corrosion and discolouration.

The leakage of metal ions from fixed orthodontic appliances in the oral cavity may cause local and systemic adverse effects. The major corrosion products are iron, chromium, and nickel for stainless steel alloys. Nickel and chromium have received the most attention because of their reported adverse effects.

Nickel is the most common cause of contact allergy dermatitis in humans. Leaching of these metallic components may be a potential trigger to an allergic reaction. In addition to the allergic issue, carcinogenic, mutagenic, and cytotoxic effects have been assigned to nickel and, to a lesser extent, chromium.

Hence, my dissertation explores this avenue further by studying the effects of mouth rinses (oral-B, Chlorhexidine and Listerine) on the metal ion release from stainless steel brackets and their comparison to metal ion release from herbal mouth rinse (Himalaya hiora)

Material and method

Hundred (AO) stainless steel brackets were divided in five equal groups of twenty each, termed “Group 1”, “Group 2”, “Group 3”, “Group 4”, “Group 5.”

Group 1 was considered as the “control” and will be immersed into de-ionised water. (Fig 1)

Group 2 was immersed in Oral-B moth rinse (Fig 2) (Composition: Alcohol, glycerine, Polysorbate 80, Aroma, Sodium saccharin, Sodium Benzoate, Cetylpyridium Chloride, Benzoic Acid, Cl 42090)

Group 3 was immersed in Hexidine mouth rinse (Fig 2) (Composition: Chlorhexidine Gluconate 0.2%).

Group 4 was immersed in Listerine mouth rinse (Fig 2) (Composition: Benzoic acid, Menthol, Methyl Salicilate, Thymol and Eucalyptol, Poloxamer 407, Cl 42053).

Group 5 was immersed in Himalaya Hiora mouth rinse (Fig 2).

Fig 1:De-ionised mouthwash

Figure 2: Mouthwashes

Figure 3: Sample’s

There are one control group and four mouth rinses group.

Each bracket was incubated in an oven set at a constant temperature of 37°C in “individual 50-mL plastic-capped vials containing 20 mL of 1 mouthwash solution or distilled deionized water for 45 days. The mouth rinses are usually recommended to be used twice a week for about 1 minute.

Figure 4: Incubator

After incubation for 45 days, the immersion solution was tested with an inductively coupled plasma (ICP) spectrometer. Unlike other methods such as atomic emission spectrometry, ICP has the advantage of extracting each ion simultaneously and detecting the metals without the interference of other ions.

Standard stock solutions (100 mg mL-1) of nickel and chromium were prepared by dissolving their appropriate salts in distilled deionized water. More dilute solutions (0.1-10 mg mL-1) of each ion were freshly prepared daily by appropriate dilutions of their stock solutions. To minimize the matrix effect in ICP measurements, the stock solution of each ion was diluted with the appropriate mouthwash. Each solution was analysed nickel and chromium ions. Measurements of pH for each mouthwash and the distilled deionized water were made with a pH meter by using a combined glass electrode.

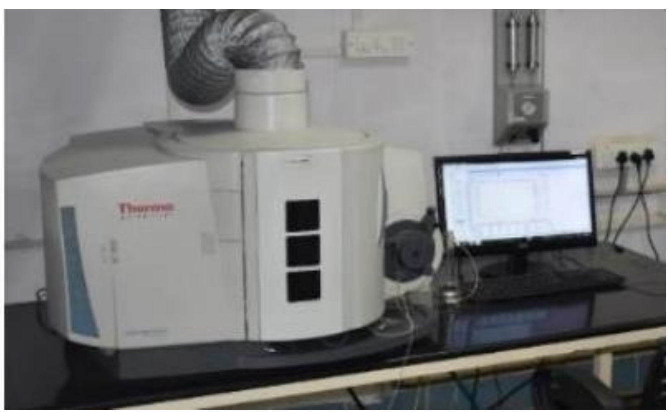


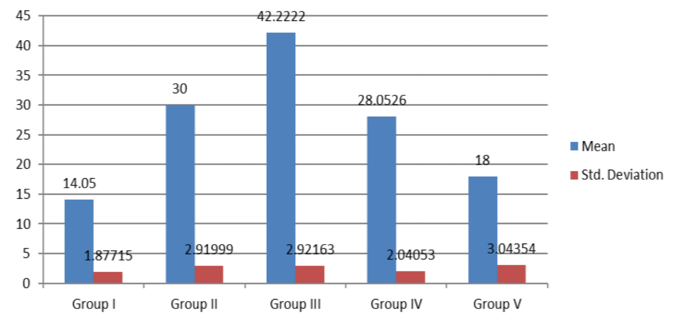
Figure 5: Inductive coupled plasma spectrometry

Statistical Analysis

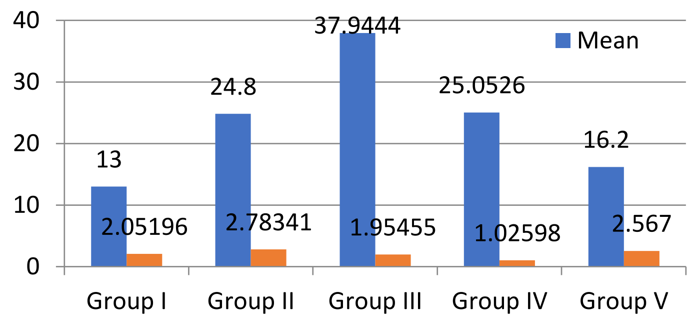
One way analysis of variance (ANOVA) was used to analyze the mean ion concentrations in the 5 groups. Post hoc turkey test were used to compare the mean nickel and chromium ion release of the mouth rinse groups (II,III,IV,V) with the control group (I)

Result

The mean nickel ion release and standard deviation of all the five groups:- 14.05± 1.87 μg/l for group I, 30.00±2.91 μg/l for group II, 42.22±2.92 μg/l for group III, 28.05±2.04 μg/l for group IV and 18.00±3.04 μg/l for group V.



The mean chromium ion release and standard deviation of all the five groups are:- 13.00±2.05 μg/l for group I, 24.80±2.78 μg/l for group II, 37.94±1.95 μg/l for group III, 25.05±1.02 μg/l for group IV and 16.20±2.56 μg/l for group V.



The level of nickel and chromium ion release was in the following order: De-ionised water < Himalaya hiora < Listerine < Oral-B < Chlorhexidine.

One way ANOVA showed that there is a statistically significant difference present in the mean nickel ion release between the various groups with highest release in chlorhexidine mouthwash and least release in deionized water ($P<0.05$, F value -179.46). And there is a statistically significant difference present in the mean chromium ion release between various groups with highest release in chlorhexidine mouthwash and least release in deionized water ($P<0.05$, F value -201.38).

Post Hoc Tukey tests were used compare the groups for the mean nickel and chromium ion release among the various mouth rinse. Comparison of mean nickel and chromium ion release between all groups shows there is a statistically highly significant difference ($p<0.001$). Only comparison between Oral-B and Listerine shows non-significant difference (p ≥0.05) in mean nickel ion release and in mean chromium ion release among the two groups.

Intergroup Comparison between Group I and Group II (Post Hoc Intergroup Comparison)

		Mean	SD	SE	Mean Diff	P value
Nickel	Group I	14.0500	1.87715	.41974	15.95000*	0.001*
	Group II	30.0000	2.91999	.65293		
Chromium	Group I	13.0000	2.05196	.45883	-11.80000*	0.001*
	Group II	24.8000	2.78341	.62239		

Post Hoc Analysis , , *p value ≤0001-Higly Significant

Intergroup Comparison between Group I and Group III

		Mean	SD	SE	Mean Diff	P value
Nickel	Group I	14.0500	1.87715	.41974	-28.17222*	0.001*
	Group III	42.2222	2.92163	.68864		
Chromium	Group I	13.0000	2.05196	.45883	-24.94444*	0.001*
	Group III	37.9444	1.95455	.46069		

Post Hoc Analysis , , *p value ≤0001-Higly Significant

Intergroup Comparison between Group I and Group IV

		Mean	SD	SE	Mean Diff	P value
Nickel	Group I	14.0500	1.87715	.41974	14.00263*	0.001*
	Group IV	28.0526	2.04053	.46813		
Chromium	Group I	13.0000	2.05196	.45883	-12.05263*	0.001*
	Group IV	25.0526	1.02598	.23538		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group I and Group V

		Mean	SD	SE	Mean Diff	P value
Nickel	Group I	14.0500	1.87715	.41974	-3.95000*	0.001*
	Group V	18.0000	3.04354	.68056		
Chromium	Group I	13.0000	2.05196	.45883	-3.20000*	0.001*
	Group V	16.2000	2.56700	.57400		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group II and Group III

		Mean	SD	SE	Mean Diff	P value
Nickel	Group II	30.0000	2.91999	.65293	-12.22222*	0.001*
	Group III	42.2222	2.92163	.68864		

Chromium	Group II	24.8000	2.78341	.62239	-13.14444*	0.001*
	Group III	37.9444	1.95455	.46069		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group II and Group IV

		Mean	SD	SE	Mean Diff	P value
Nickel	Group II	30.0000	2.91999	.65293	1.94737*	0.001*
	Group IV	28.0526	2.04053	.46813		
Chromium	Group II	24.8000	2.78341	.62239	-.25263	0.718 **
	Group IV	25.0526	1.02598	.23538		

Post Hoc Analysis , *p value ≤0001-Higly Significant, **p ≥0.05 –Non-Significant

Intergroup Comparison between Group II and Group V

		Mean	SD	SE	Mean Diff	P value
Nickel	Group II	30.0000	2.91999	.65293	12.00000*	0.001*
	Group V	18.0000	3.04354	.68056		
Chromium	Group II	24.8000	2.78341	.62239	8.60000*	0.001*
	Group V	16.2000	2.56700	.57400		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group III and Group IV

		Mean	SD	SE	Mean Diff	P value
Nickel	Group III	42.2222	2.92163	.68864	14.16959*	0.001*
	Group IV	28.0526	2.04053	.46813		
Chromium	Group III	37.9444	1.95455	.46069	12.89181*	0.001*
	Group IV	25.0526	1.02598	.23538		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group III and Group V

		Mean	SD	SE	Mean Diff	P value
Nickel	Group III	42.2222	2.92163	.68864	24.22222*	0.001*
	Group V	18.0000	3.04354	.68056		
Chromium	Group III	37.9444	1.95455	.46069	21.74444*	0.001*
	Group V	16.2000	2.56700	.57400		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Intergroup Comparison between Group IV and Group V

		Mean	SD	SE	Mean Diff	P value
Nickel	Group III	28.0526	2.04053	.46813	10.05263*	0.001*
	Group V	18.0000	3.04354	.68056		
Chromium	Group III	25.0526	1.02598	.23538	8.85263*	0.001*
	Group V	16.2000	2.56700	.57400		

Post Hoc Analysis , *p value ≤0001-Higly Significant

Discussion

Usually, mouthwash must be used twice a week for about 1 minute. But it is recommended that after mouthwash the patient must not eat, drink, and rinse, so the components of mouthwash are present for a long time, and it is difficult to determine the exact duration of contact between brackets and mouthwashes. We assumed that each time the mouthwash was present for 6 hours in a patient's mouth (24 months, twice a week 5 about 69,000 minutes), so for this study the brackets were immersed in mouthwashes and incubated at 37°C for 45 days (45 days 5 about 64,000 minutes). Also, several studies have demonstrated that the levels of metal release from fixed orthodontic appliances peak at day 7, and that all release is completed within 4 weeks.

Many parameters affect the corrosion of metals in a water environment, including pH level, oxygen content, water temperature, and duration of immersion. For further elucidation of the reasons for ion release in the different solutions, the pH values of the 4 mouth washes and distilled deionized water were measured. The values were 7.5 for distilled deionized water, and 5.5, 5.2, 5.4 and 5.7 for Oral B, chlorhexidine, Listerine and Himalaya Hiora respectively.

The general mechanism for the corrosion and subsequent release of metal ions from stainless steel involves the loss of the passive layer consisting of chromium oxide and chromium hydroxide that forms on contact with oxygen on the surface of stainless steel. Metal is released into the oral cavity with saliva as the medium, and this could be influenced by a high chloride mixture in the saliva or the intake of various foods and drinks with a low ph.

Nickel induces cell death in human lymphocytes through triggering oxidative stress and damage to the mitochondria, one of the important organelle for cell survival. In addition cellular proteolysis induced by $NiCl_2$ can be considered as one of the final stages in $NiCl_2$ cell death mechanism. Lymphocytes death can lead to suppression of immune system that in turn increases the incidence of infectious disease.

The mechanistic cytotoxicity of chromium(VI) is not completely understood, however, a large number of studies demonstrated that chromium(VI) induces oxidative stress, DNA damage, apoptotic cell death and altered gene expression.

In our study, the amount of nickel and chromium released in the control group (Deionized water) were 14.05 µg/l and 13.00 µg/l respectively. This is in contrast to a similar study done by Danaei et al, metal ion release who found the amount of nickel and chromium release in the control group (deionized water) to be 2627.40 µg/l for nickel ion release and 838.10 µg/l for chromium ion release which were higher than the experimental groups. Danaei et al states that distilled water with its pH of 7.5 could not be responsible for its corrosive nature and is unable to state an adequate explanation for its high reactivity and gives the lack of ions in distilled water as a possible reason for its high reactivity which is inconclusive.

Danaei et al who found a higher amount of nickel and chromium ion release when testing with the mouth rinses Oral B (171.50 µg/l), Chlorhexidine (1198.30 µg/l) and Persica (109.70 µg/l). Persica is herbal mouth rinse and in our study, we use Himalaya hiora as herbal mouth rinse. When compared to our study the results are similar, higher amount of nickel ion release found in chlorhexidine and least amount of nickel ion release found in herbal mouthwash. The brackets used in our study were from Koden Orthodontics brackets whereas the brackets in the study by danaei et al were from 3M Unitek.

Barrett et al studied invitro corrosion by simulating an entire arch with full complement of teeth. Bands, brackets and archwire for the entire arch were placed in artificial saliva for 4 weeks and tested for ion release. After 28 days, the nickel ion and chromium ion release was found to be 702 µg/l and 126.9 µg/l for Nitinol archwire and 1262 µg/l and 233.1 µg/l for stainless steel archwire. The higher amount of nickel release when compared to our study could be attributed to the difference in methodology as 10 brackets, 2 bands and an archwire were used for this study. When averaged for a single bracket, these results are similar to our study.

Kerosuo et al studied metal ion release from 3 simulated orthodontic appliances immersed in 0.9% sodium chloride solution. The highest amount of nickel ion release was 44 µg/l and chromium ion release was 4.5 µg/l from the fixed appliance with dynamic condition whereas in static condition it showed a nickel ion release of 17.1 µg/l and chromium ion release of 2.5 µg/l. These can be considered similar to the results in our study. But there is a difference in methodology with simulated orthodontic appliance and sodium chloride being used as the test solution.

When compared to the control group (Deionized water), all the mouth rinses showed a statistically significant increase in the nickel and chromium metal ion release. This can be attributed to the corrosive nature of mouth rinses which can cause the loss of passivating oxide layer of metal alloys and result in leaching of metal ions. This is in agreement with several studies who have higher amount of metal ion release in experimental groups when compared to control groups. However, the control groups in all these studies are a formulation of artificial saliva.

In the present study mouthwashes were used in a static environment, but in the oral environment, more metal ion release could occur due to various factors such as fluidity of saliva in the mouth, effect of diet and also because oxide layers are removed from the bracket by tooth brushing.

In the present study, the mean metal ion release was measured from a single stainless-steel bracket in a vial. More metal ion release can be expected in a clinical scenario where 20 brackets are used.

According to WHO, daily amounts of nickel intake is 200-300µg and chromium intake is 50-200µg, respectively. The health-based value of nickel is 80 µg/L, which is protective of chronic systemic toxicity and average chromium levels in drinking water are 1µg/L. The amount of nickel and chromium ion released in this study were insignificant when compared to daily intake. However, even a small amount of metal ion release can produce sensitivity as the appliance is in place for 2 to 3 years and can cause detrimental effects locally.

The present study indicated that mouth rinses caused an increase in nickel and chromium ion release from stainless steel brackets. Chlorhexidine mouth rinses caused more nickel and chromium ion release when compared to other mouth rinses. A greater amount of metal ion release can be expected from an in-vivo study considering the dynamic oral environment. And herbal mouth rinse causes less nickel and chromium release when compared to other mouth rinses. Thus, it can be concluded that in patients undergoing orthodontic treatment, it is advisable to avoid prolonged application of chlorhexidine in patients who have allergies.

Conclusion

Stainless steel brackets when reacted with chlorhexidine showed release of more nickel and chromium content due to the presence of Chlorhexidine gluconate. It should be reduced because it may cause allergic reactions and carcinogenic effects shows cytotoxicity.

• Nickel and chromium ion release was significantly more in the experimental mouth rinse groups (II, III, IV, V) than in the deionized water control group I.

• Nickel and chromium ion release was significantly higher in the chlorhexidine mouth rinse group (III) when compared to the other mouth rinses groups (II, IV, V).

• Nickel and chromium ion release was significantly less in the Himalaya Hiora mouth rinse group (V) when compared to the other mouth rinses groups (II, III, IV).

However, the metal ion released in all the four mouthwashes and deionized water was within the permissible limit.

Hence, the orthodontic brackets released the most ions in the presence of chlorhexidine mouthwash. It might recommended to avoid prolonged application of chlorhexidine in patients who have allergies.

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