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Ion Release from Orthodontic Brackets in Three Different Mouthwashes and Artificial Saliva: An In Vitro Study.

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Abstract

Introduction*: Stainless steel as material used in orthodontics, since 1932. But it leaches in the presence of saliva by use of mouthwash and toothpastes and creates white spots. This study is done to evaluate and compare the release of various metal ions release from the brackets in respective solutions.*

Methods: *A 120 premolar orthodontic brackets were taken and these brackets were dipped in the individual solution divided into four groups. They were Chlorhexidine, Listerine, Colgate plax and Artificial saliva. A glass beaker was taken for placing solutions and then in each group around 30 brackets were dipped and were kept in incubator at 37 ˚C for around 45 days. Then the amount of ion release from each solution is checked by using atomic absorption spectrophotometer.*

Results: *The results showed that ion release in artificial saliva was significantly higher than 3 mouthwashes. Higher ion release was found with Chlorhexidine as compared to other mouthwashes. There was no significant difference (P.0.05) in nickel, chromium, iron, and copper ion release in the Listerine and Colgate plax mouthwashes. The level of manganese release was significantly different in all 4 groups.*

Conclusion: *Ion release in the Listerine and Colgate plax mouthwashes might be better option than Chlorhexidine for orthodontic patients with stainless steel brackets.*

Keywords*: Mouthwash, Saliva ,Nickel, Chromium, Orthodontic appliances*

I. Introduction

Stainless Steel has been the main stay material in orthodontics, since its introduction in 1932, with a wide range of applications in both fixed and removable appliances. The force per unit activation with stainless steel was greater than gold i.e. high stiffness, excellent work hardening capabilities and low frictional magnitude. This became the standard for the profession. The austenitic stainless steel commonly used for orthodontic brackets, bands and wires contain approximately 18 percent chromium and 8 percent nickel.[1] Intra-oral fixed orthodontic appliances include brackets, bands, ligatures, springs and arch wires that are made of alloys containing nickel, cobalt and chromium in different percentages.

Fixed appliances in orthodontics involve metallic brackets and arch-wires. These brackets are exposed to oral cavity which is hostile in environment where electro-chemical corrosion phenomena can occur. Thus, orthodontic brackets and other auxillary components should be made of highly corrosion resistant metals and metal alloys. In acidic environment and in presence of fluoride ions the corrosion resistance of certain materials can deteriorate. Stainless steel alloy contains 8%-12% nickel and 17%-22% chromium and is used for the metallic parts of orthodontic appliances. Approximately 10% of the population has nickel hypersensitivity and harmful effects of nickel is carcinogenicity, allergenicity and mutating substance.[2]

Regular use of fluoride containing products such as toothpaste and mouthwashes during the orthodontic treatment is recommended to reduce the risk of development of white spots around the orthodontic brackets. Although fluoride ions in the prophylactic agents have been reported to cause corrosion and discolouration, little information is available regarding the effect of different mouthwashes on orthodontic brackets and artificial saliva is used to simulate normal ion release during orthodontic treatment from brackets is also measured. Release of elements can produce discoloration of adjacent soft tissues and allergic reactions in susceptible patient. Factors such as temperature, quantity and quality of saliva, plaque, pH, proteins, physical/chemical properties of solids/liquids food and oral conditions may influence corrosion processes.[3]

Certain ions such as nickel and chromium can result in symptoms of toxicity and allergic reactions. These symptoms can be short-lived and intense or longer lasting and moderate, and some might be resolved, whereas others can become a chronic problem. Since the toxicity of nickel is a concern, and the natural capacity to eliminate nickel exceeds the accumulation capacity, the risks are minimal. However, clinicians should be aware that the release of metal ions might cause a local hypersensitivity reaction at oral soft-tissue sites, such as mild erythema or redness with or without edema. Also, severe gingivitis can be related not only to poor oral hygiene but also hypersensitivity reaction to nickel or chromium ions released from stainless steel. We also need to determine whether these ion releases have clinical significance in sensitizing patients with a history of hypersensitivity.[4]

Saliva is a hypotonic solution containing bioactonate, chloride, potassium, sodium, nitrogenous compounds and proteins1. The pH of saliva varies from 5.2 to 7.8. Metal is released into the oral cavity with saliva as a 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. Also, the characteristics of saliva change according to the patient's health and the time of day. Several studies have investigated whether orthodontic appliances release metal ions, through emission of electro-galvanic currents, with saliva as a medium or through continuous erosion over time. Nickel is the most common cause of metal induced allergic contact dermatitis in human beings and produces more allergic reactions than all the other metals combined. Second in frequency is chromium. The most significant human exposure to nickel and chromium occurs through the diet. The average dietary intake for nickel is 200-300 µg/day and for chromium is 280µg/day.[5]

II. Material And Methods

A 120 premolar orthodontic brackets were taken and these brackets were dipped in the individual solutions divided into four groups. There were four groups such as Chlorhexidine, Listerine, Colgate plax and Artificial saliva.

120 Brackets were randomly divided into 4 equal groups :

Group1= Chlorhexidine mouthwash (30)

Group2= Listerine mouthwash (30)

Group3= Colgate Plax mouthwash (30)

Group4= Artificial saliva (30)

A glass beaker is taken for placing solutions into it and then in each group around 30 brackets were dipped and they are kept in incubator at 37°C for around 45 days. Then the amount of ion release from each solution is checked by using atomic absorption spectrophotometer. All these samples were collected and tested by using atomic absorption spectrophotometer at Nikhil Analytical Laboratory, Sangli.

After incubation for 45 days, the immersion solution was tested with an atomic absorption spectrophotometer. 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. Nikhil Analytical Laboratory ,Sangli using AAS (Atomic Absorption Spectrophotometer) Spectrophotometer is the analytical method of using radiation emission and absorbance phenomena to determine the quantity of a substance in a sample. The spectrophotometer used for this experiment is the set up as a conventional flame atomic absorption spectrophotometer (AAS) for determination of atomic absorption.

III. Results

Mean levels of the ions released in the groups are shown in the Table. The results of the Kolmogorov-Smirnov test showed that, except for chromium, copper and manganese ions, all other ions had normal distributions(Table 1) (Figure 1,2,3 &4). Therefore, a nonparametric test (Kruskal-Wallis) showed that the release of chromium, copper and manganese in the 3 mouthwashes and artificial saliva was significantly different (P=0.001) and Iron and Nickel was found no significant difference between the groups (Table 2)

Also, the Mann-Whitney test with a significance level of (P= 0.008) showed statistically significant difference in chromium, copper and manganese release between Chlorhexidine and Listerine and release of iron was lower in listerine than what was observed for Chlorhexidine(Table 3). Significance level of (P= 0.008) showed statistically significant difference in chromium, copper and manganese release between Chlorhexidine and

Colgate plax and release of copper and manganese ion was lower in Colgate plax than what was observed for Chlorhexidine. Chromium ion release was more in Chlorhexidine group as compared to colgate plax group(P= 0.008) (Table 4). Release of Chromium ion was higher in Chlorhexidine than what was observed for artificial saliva (P= 0.008) and release of manganese ion was lower in Chlorhexidine than what was observed for artificial saliva (P= 0.008) and was found to be statistically significant (Table 5). Release of Copper ion was higher in listerine than what was observed for colgate plax (P= 0.009) and release of manganese ion was lower in listerine than what was observed for colgate plax (P= 0.008) and was found to be statistically significant(Table 6). Significance level of (P= 0.008), (P= 0.009) and (P= 0.023) showed statistically significant difference in chromium, copper and manganese release between Listerine and artificial saliva release of copper and manganese ion was lower in artificial saliva than what was observed for Listerine and chromium ion was higher in artificial saliva as compared to Listerine solution(Table 7). Significance level of (P= 0.008) showed statistically significant difference in chromium, copper and manganese release between Listerine and artificial saliva release of copper and manganese ion was lower in artificial saliva than what was observed for colgate plax and chromium ion was lower in colgate plax as compared to artificial saliva solution(Table 8).

IV. Discussion

Brackets and wires are an integral part of an orthodontic treatment. Stainless steel material earlier were considered as a non harmful material for human body but as the stainless steel material leaches ions within oral cavity it may be considered as harmful nowadays.This study was conducted for comparative assessment of efficacy of different commercially available mouthwashes for patients having fixed orthodontic appliances. For this purpose three different mouthwashes were used such as Chlorhexidine ,Listerine and Colgate plax were used. Artificial saliva was also used.

Chlorhexidine gluconate(0.2%) was tried as an antimicrobial agent in the form of mouthwash . It was marketed for more than 20 yrs as a general disinfectant with the broad antibacterial spectrum against gram +ve and gram –ve pathogens. Chlorhexidine has affinity for bacteria because of interaction between positively charged chlorhexidine molecule and negatively charged groups on bacterial cell wall and permits the agent to penetrate in cytoplasm and cause the death of microorganism.Phenolic compounds Listerine was tried as an antimicrobial agent. Listerine is an essential oil mouthrinse which contains thymol 0.06%, menthol 0.04%, eucalyptol 0.09%, benzoic acid 0.15%, methyl salicylate 0.06%. The mechanism of action of phenol against bacterial cell is extensively complex and probably involve protein denaturation against damaging cell membrane which result in leakage of intracellular component. Due to there low toxicity and high antibacterial activity of phenolic compounds have been incorporated in throat lozenges and mouthrinse used in oral cavity.

Colgate plax mouthwash containing extracts of Salvadora persica, mint, and yarrow with the main ingredients of tannin, flavonoid, calcium, fluoride, chloride, and essence. The mechanism of action of colgate plax due to large amount of fluoride release it may cause damage to the bacterial cell membrane and may cause protein denaturation.

Artificial saliva contain sodium chloride, potassium chloride, sodium sulphate, urea, calcium chloride, distilled water. In the microenvironment of the mouth, the presence of a chloride gradient could contribute to the increased metal degradation observed as one progresses deeper into the crevice between the teeth.

In my present study increase in the level of release of metal ions could be attributed to its corrosive nature and next in chlorhexidine mouthwash. Chlorhexidine mouthwash released greater amounts of metal ions (except manganese) than did the Listerine and Colgate plax mouthwashes.

Chlorhexidine not only cause significant higher amounts of nickel and chromium ions release among the 3 mouthwashes studied, but it also caused no significant higher release of copper than did Listerine(Table). Since the pH values for mouthwashes had no significant difference in the acidity of the 3 mouthwashes, this could be attributed to its corrosiveness of chlorhexidine as compared with the other 2 mouthwashes, this agrees with previous reports about the irrigating effects of chlorhexidine. According to Danaei et al. [6] ,who found more release of nickel and chromium ions when Niti archwires were inserted into chlorhexidine mouthwash which leads to more corrosion of orthodontic archwires.

But the corrosiveness of chlorhexidine is not the sole parameter in the release of all metallic ions from the brackets, because high releases of manganese in Listerine and Colgate plax solutions were observed. The level of manganese release was significantly different in all 4 groups and interestingly, was lowest in chlorhexidine. mouthwash. This might be the result of the fluoride anion in the other 2 mouthwashes (Listerine and Colgate plax) under acidic conditions, the fluoride anion increases the dissolution of manganese. In an acidic environment, corrosion could easily occur even with low fluoride concentrations.[7] There was no significant difference between the nickel and iron ion release abilities of Listerine and Colgate plax.

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. Also, the characteristics of saliva change according to the patient’s health and the time .[8] We used mouthwashes in a static condition, but more metal release could occur because of the fluidity of saliva in the mouth and also because oxide layers are removed by tooth brushing. [9]

In our study, the total amounts of nickel and chromium released during 45 days in artificial saliva were greater, but according to Barrett et al. [10] , this is in accordance with the release of nickel and chromium from deionised water were also similar. They evaluated ion releases from bands and brackets in artificial saliva during a 4-week period by atomic absorption spectrophotometry. The amount of nickel and chromium release in chlorhexidine and artificial saliva during 45 days were more and is not in accordance with the study conducted by Kersuo et al. [11], they studied the ion release from different appliances in sodium chloride under dynamic conditions. . Since they used different appliances (headgear, quadhelix, and fixed appliances), comparisons between studies must be done with due consideration of the problem in measuring surface areas with complex geometry. This might be attributed to differences in study design, measuring methods, solutions, and timing. Also, differences have been found in the metal release between corresponding products of different manufacturers.

However, from our results, it can be concluded that the corrosiveness of the mouthwash, which in turn depends on its chemical structure, is the main factor responsible for the release of metal ions from dental brackets.

V. Conclusion

The results of this investigation led to the following conclusions:

Release of chromium, copper and manganese in all groups showed significantly higher ppm as compared to iron and nickel which found less release.

Chlorhexidine when reacted with stainless steel brackets showed release of more chromium content due to the presence of Chlorhexidine gluconate. It should be reduced because it may cause allergic reactions and carcinogenic effects shows cytotoxicity.Listerine when reacted with stainless steel brackets showed release of more amount of copper content due to the presence of phenolic compounds and the amount should be decreased because it may cause little hypersensitivity reactions such as skin rashes and irritation to eyes.

Colgate plax when reacted with stainless steel brackets showed release of more amount of manganese content due to the presence of fluoride content and may cause irritation to skin and mucous membrane of mouth.

Artificial saliva when reacted with stainless steel brackets showed release of more chromium content due to the presence of chloride content should be reduced because it may cause allergic reactions, carcinogenic effects shows cytotoxicity.

Since the chromium content showed more release of ions among the solvents it should be reduced. Since the stainless steel is the biocompatible material but the reaction of the solvents may cause leaching of the ions upto small extent during orthodontic treatment but if more release occur it may cause cytotoxicity.

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Table 1: Metal ion concentrations (mg/L) in the solutions at 37^0C after 45 days: mean concentration levels and standard deviations

Solutions	Chromium	Copper	Iron	Manganese	Nickel
Chlorhexidine	4.02±0.08	0.92±0.08	0.92±0.08	2.10±0.12	0.92±0.08
Listerine	0.92±0.08	6.14±0.11	0.92±0.08	1.08±0.08	0.92±0.08
Colgate plax	0.92±0.08	3.56±0.08	0.92±0.08	6.18±0.11	0.92±0.08

Artificial saliva	4.98±0.08	0.92±0.08	0.92±0.08	0.92±0.08	0.92±0.08

Table 2: Comparison of Metal ion concentrations (mg/L) in four different solutions

	Group	Mean Rank	Kruskal wallis & p value
Chromium	**Chlorhexidine**	13.00	16.379 **0.001***
	Listerine	5.50	
	Colgate plax	5.50	
	Artificial saliva	18.00	
Copper	**Chlorhexidine**	5.50	16.354 **0.001***
	Listerine	18.00	
	Colgate plax	13.00	
	Artificial saliva	5.50	
Iron	**Chlorhexidine**	10.50	0.00 1.00
	Listerine	10.50	
	Colgate plax	10.50	
	Artificial saliva	10.50	
Manganese	**Chlorhexidine**	13.00	17.529 **0.001***
	Listerine	7.60	
	Colgate plax	18.00	
	Artificial saliva	3.40	
Nickel	**Chlorhexidine**	10.50	0.00 1.00
	Listerine	10.50	
	Colgate plax	10.50	
	Artificial saliva	10.50	

Table 3: Comparison of Metal ion concentrations (mg/L) in Chlorhexidine and Listerine solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Chlorhexidine**	8.00	.000 **.008***
	Listerine	3.00	
Copper	**Chlorhexidine**	3.00	.000 **.008***
	Listerine	8.00	
Iron	**Chlorhexidine**	5.50	12.500 1.000
	Listerine	5.50	
Manganese	**Chlorhexidine**	8.00	.000 **.008***
	Listerine	3.00	
Nickel	**Chlorhexidine**	5.50	12.500 1.000
	Listerine	5.50	

P value<0.05

Table 4: Comparison of Metal ion concentrations (mg/L) in Chlorhexidine and Colgate plax solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Chlorhexidine**	8.00	.000 **.008***
	Colgate Plax	3.00	
Copper	**Chlorhexidine**	3.00	.000 **.008***
	Colgate Plax	8.00	
Iron	**Chlorhexidine**	5.50	12.500 1.00
	Colgate Plax	5.50	
Manganese	**Chlorhexidine**	3.00	.000 **.008***
	Colgate Plax	8.00	
Nickel	**Chlorhexidine**	5.50	12.500 1.00
	Colgate Plax	5.50	

Table 5: Comparison of Metal ion concentrations (mg/L) in Chlorhexidine and Artificial saliva solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Chlorhexidine**	3.00	.000 **.008***
	Artificial saliva	8.00	
Copper	**Chlorhexidine**	5.50	12.500 1.00
	Artificial saliva	5.50	
Iron	**Chlorhexidine**	5.50	12.500 1.00
	Artificial saliva	5.50	
Manganese	**Chlorhexidine**	8.00	.000 **.008***
	Artificial saliva	3.00	
Nickel	**Chlorhexidine**	5.50	12.500 1.00
	Artificial saliva	5.50	

Table 6: Comparison of Metal ion concentrations (mg/L) in Listerine and Colgate plax solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Listerine**	5.50	12.500
	Colgate plax	5.50	1.00
Copper	**Listerine**	8.00	.000
	Colgate plax	3.00	**.009***
Iron	**Listerine**	5.50	12.500
	Colgate plax	5.50	1.00
Manganese	**Listerine**	3.00	.000
	Colgate plax	8.00	**.008***
Nickel	**Listerine**	5.50	12.500
	Colgate plax	5.50	1.00

Table 7: Comparison of Metal ion concentrations (mg/L) in Listerine and Artificial saliva solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Listerine**	3.00	.000
	Artificial saliva	8.00	**.008***
Copper	**Listerine**	8.00	.000
	Artificial saliva	3.00	**.009***
Iron	**Listerine**	5.50	12.500
	Artificial saliva	5.50	1.00
Manganese	**Listerine**	7.60	2.000
	Artificial saliva	3.40	**.023***
Nickel	**Listerine**	5.50	12.500
	Artificial saliva	5.50	1.00

Table 8: Comparison of Metal ion concentrations (mg/L) in Colgate plax and Artificial saliva solutions

	Group	Mean Rank	Mann-Whitney & p value
Chromium	**Colgate plax**	3.00	.000
	Artificial saliva	8.00	**.008***
Copper	**Colgate plax**	8.00	.000
	Artificial saliva	3.00	**.008***
Iron	**Colgate plax**	5.50	12.500
	Artificial saliva	5.50	1.00
Manganese	**Colgate plax**	8.00	.000
	Artificial saliva	3.00	**.008***
Nickel	**Colgate plax**	5.50	12.500
	Artificial saliva	5.50	1.00

List Of Graphs

Graph 1:

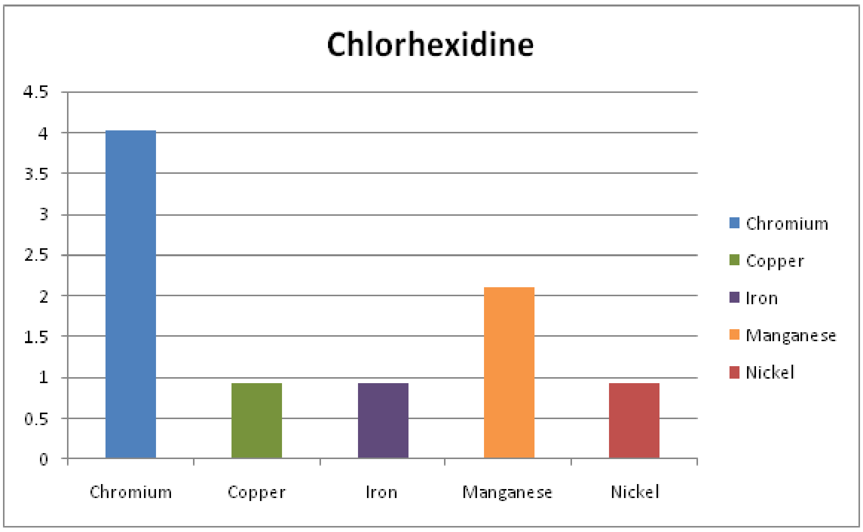

Figure 1 shows mean levels of the ions released in chlorhexidine group showed Chromium release 4.02, copper 0.92, iron 0.92, manganese 2.1 and nickel 0.92 respectively.

Graph 2:

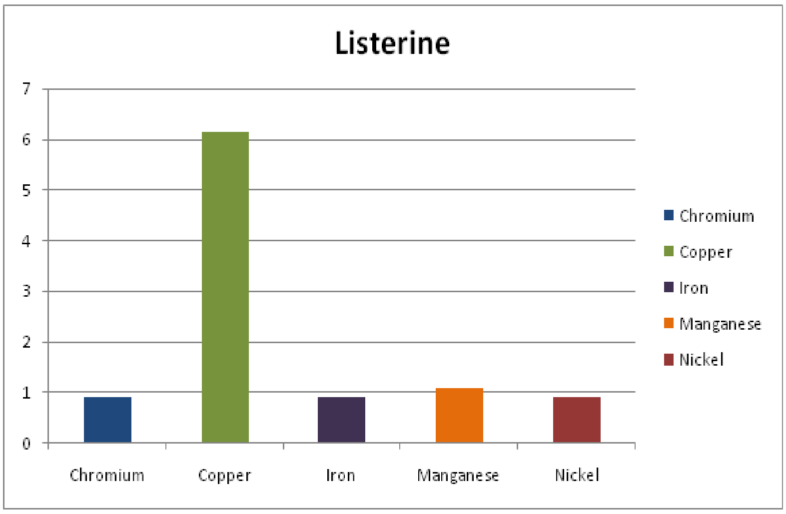

Figure 2 shows mean levels of the ions released in listerine group showed Chromium release 0.92, copper 6.14, iron 0.92, manganese 1.08 and nickel 0.92 respectively.

Graph 3:

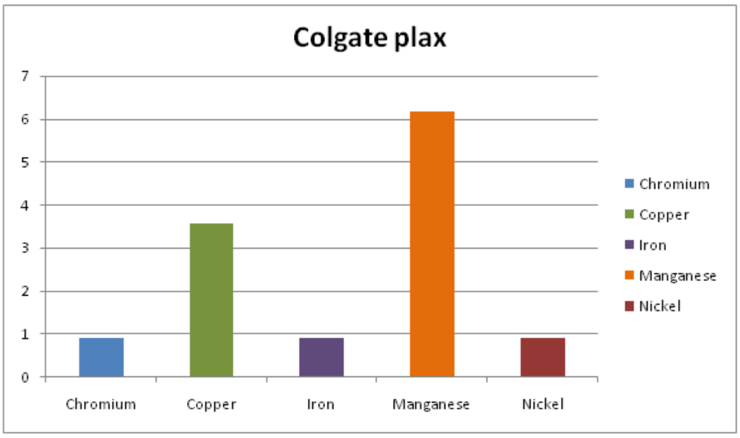

Figure 3 shows mean levels of the ions released in colgate plax group showed Chromium release 0.92, copper 3.56, iron 0.92, manganese 6.18 and nickel 0.92 respectively.

Graph 4:

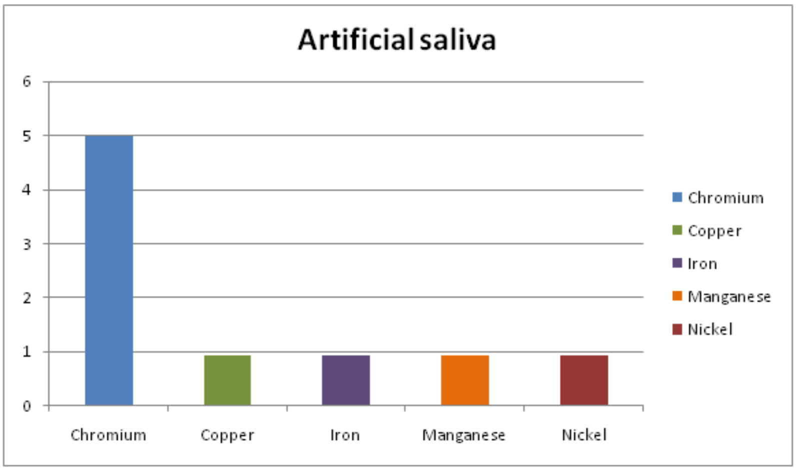

Figure 4 shows mean levels of the ions released in artificial saliva group showed Chromium release 4.98, copper 0.92, iron 0.92, manganese 0.92 and nickel 0.92 respectively.

Figure legends

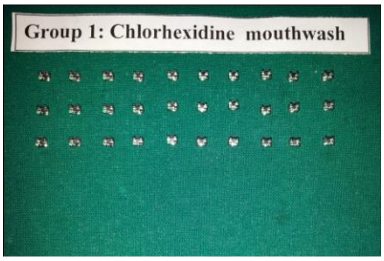

Fig 1:Group 1 contain thirty brackets

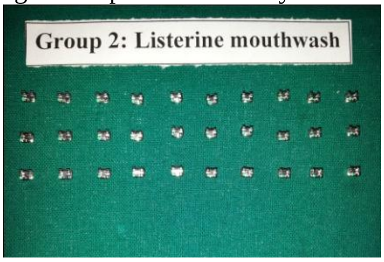

Fig 2:Group 2 contain thirty brackets

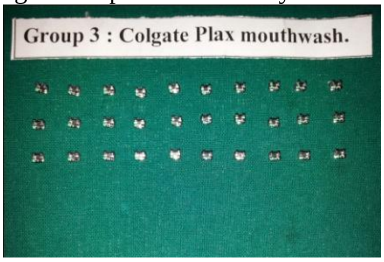

Fig 3:Group 3 contain thirty brackets

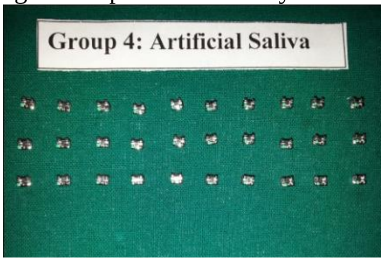

Fig 4:Group 4 contain thirty brackets

Fig 5:Chlorhexidine Mouthwash

Fig 6:Listerine Mouthwash

Fig 7:Colgate plax Mouthwash

Fig 8:Artificial saliva

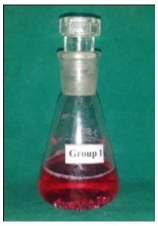

Fig 9:Chlorhexidine Mouthwash containing brackets

Fig 10: Listerine Mouthwash containing brackets

Fig 11: Colgate plax Mouthwash containing brackets

Fig 12: Artificial saliva containing brackets

Fig13: Incubator containing four beakers along with brackets dipped in solutions

Fig14: Atomic Absorption Spectrophotometer