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Comparative Evaluation of Human Saliva for the Presence of Nickel Released from Coated and Uncoated Nickel Titanium Archwires in Orthodontic Patients by Inductively Coupled Plasma –Atomic Emission Spectroscopy (ICP-AES) - A Clinical Study

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ABSTRACT

Aim: To determine the presence of Nickel, released from orthodontic arch wires, in the human saliva by inductively coupled plasma – atomic emission spectroscopy (ICP-AES)

Materials and Method: 39 subjects were equally divided into three groups A, B and C. Groups A and B were bonded with Pre adjusted Edgewise brackets 0.018" slot. Group A received uncoated NiTi arch wires and Group B received coated NiTi arch wires while Group C without orthodontic appliances served as controls. 3ml of salivary samples were collected from each subject one on the end of first month and the other on the end of second month of treatment and was stored at - 20° C. Saliva sampling was done and centrifuged at 7000 rpm for 10 minutes. Presence of Nickel in the given samples was analyzed by Inductively Coupled Plasma – Atomic Emission Spectroscopy.

Results: The mean Nickel levels in the saliva released from Uncoated NiTi wires were significantly higher than coated NiTi wires and control group.

Keywords: Atomic Emission Spectroscopy, Inductively Coupled Plasma, orthodontics.

INTRODUCTION

Different components of orthodontic appliances like arch wires, brackets, bands, stainless steel ligatures and stainless steel components of removable appliances are potential sources of Nickel. Nickel Titanium (NiTi) arc wires contain 47% - 50% Nickel which makes it the richest source of Nickel in intraoral environment of the average orthodontic patient.[1] Nickel is ingested with the intake of foods in the range of 300-600 µg per day. The average daily excretion in urine was reported as 2.5 to 28µg and its presence in human saliva ranges from 0.8 to 4.5 µg/L [5]. Various forms and compounds of Nickel are proved to be carcinogenic, mutagenic, cytotoxic and allergic[2]. Several case reports of Nickel allergy have been reported due to Nickel release in orthodontic patients which is more frequent with Nickel Titanium arch wires. The Nickel allergy manifests as burning sensation, rashes, swelling and painful erythematous lesions in the oral and labial mucosae.[2] Dermal lesions like redness, itching, eczema, fissuring, desquamation, irritation and soreness have been associated with extraoral appliances.[1,2] Occasionally symptoms such as fever, compatible with general allergic reaction were reported. An abnormal percent of Nickel can

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increase the risk of cancer, infectious diseases, and auto immune diseases.

The chief reason for an orthodontist's concern about Nickel is that it is released into saliva.[3,5,6,7,9,10] Several invitro studies also have proved the release of Nickel[4, 11, 12]. The release of Nickel is not necessarily proportional to the alloy's Nickel content.[2] Other factors such as heat treatment and silver soldering can accelerate Nickel release.[4]

Nickel free substitutes[2] are available in brackets*(ceramic, plastic, titanium, precious metal coated- gold, platinum, palladium, Nickel free stainless steel)* ; bands*(gold coated)*; archwires [*Titanium Molybdenum Alloy (TLA), nitride/epoxy-coated NiTi archwires ,α–titanium]*; ligatures*(Teflon coated)*; face bow *(Teflon coated)*; stainless steel components of Hawley's appliance and variations*(plastic or elastic retainers, elastic positioners or acrylic splints, Invisalign technique)*; orthognathic surgery lag screws and plates*(resorbable polylactic-polyglycolic lag screws and plates).* Although both Nitride and Epoxy resin-coated NiTi archwires are claimed to be Nickel free substitutes, a study by Kim and Johnson[14] proved that the nitride coating of NiTi archwire did not affect the corrosion, but epoxy coating was corrosion resistant. This study aims to investigate the same

MATERIALS AND METHODS

The present study was performed in the Departament of Orthodontics and Dentofacial Orthopedics, Rajah Muthiah Dental College and Hospital, Annamalai University. Patients who reported for fixed appliance therapy at the Department of Orthodontics and Dentofacial Orthopedics were chosen for the study. The sample preparation was done in the Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University. The chemical analysis was done in the Department of CAS in Marine Biology, Annamalai University using Inductively Coupled Plasma – Atomic Emission Spectroscopy (ICP-AES)

A total of 39 patients who reported at the Department of Orthodontics and Dentofacial Orthopedics, Rajah Muthiah Dental College and Hospital, Annamalai University were chosen for the study. All the patients ranged between 20 – 25 years of age. The motive of the study was explained to the patients and consent obtained. Patient selection criteria[5] were as follows:

- All patients were in the permanent dentition period
- Patients did not have any intraoral metallic restorations
- Allergy history revealed no known Nickel hypersensitivity
- Patients were not on any chronic medication or illness
- Patients were not medically compromised
- Non extraction cases
- Did not require any palatal, lingual or extraoral appliances (e.g. TPA, Lingual arch, Face bow)

The subjects of the study were equally divided into three groups: Group A, B and C. Subjects of Groups A and B were bonded with Preadjusted Edgewise brackets 0.018" slot (Fig.1). First molars received bands with welded molar tubes. Group A received uncoated NiTi archwires (Fig.3) and the Group B received coated NiTi archwires (Fig.2). The archwires were secured with elastic modules. A third group of thirteen subjects without orthodontic appliances served as controls (Group C)

SAMPLING OF SALIVA

About 3ml of salivary samples were obtained from each subject of Group C. Two samples from each patient of Group A and B was collected one on the end of first month and the other on the end of second month of treatment and was stored at - 20° C. Saliva was collected as follows: the patients were made to rinse thoroughly with mouthful of double distilled water[5,7,9,10]. Unstimulated[5,9,10] salivary samples were collected of approximately 10 ml into acid washed sterile plastic containers. The samples were stored at -20ºC [5,6,7,9] before processing.

PREPARATION OF SAMPLES

The preparation of saliva samples was performed in the Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University. According to the procedure described by Kerosuo and collegues[6]. About 0.3 ml

of saliva was transferred to 5 ml acid washed glass test tubes. For digestion of the organic matter of saliva 0.9 ml of concentrated Hydrochloric Acid was added to each sample (Fig.4). The test tubes were sealed with aluminium foils sheets (Fig.5) and kept in a water bath at 80°C for 8 hours (Fig.6). The temperature of water bath was frequently checked with a thermometer.

CENTRIFUGE

The deproteinized salivary samples (Fig.7) were centrifuged at 7000 rpm for 10 min to settle the particulate matter (Fig.8, 9). The supernatant obtained from all the samples were then transferred to acid washed 3ml plastic containers, stored and then transported in cool packs for elemental analysis. (Fig.10, 11).

ANALYSIS OF SAMPLE

The chemical analysis was done in the Department of CAS in Marine Biology, Annamalai University. Presence of Nickel in the given samples was analyzed by Inductively Coupled Plasma – Atomic Emission Spectroscopy (Fig.12). Calibrated standards of Nickel (10µg / ml) were made from Merck ICP-AES standard solution (Fig.13). Milli-Q water (Blank) was used for dilution. The samples were then introduced into the Inductiely Coupled Plasma-Atomic Emission Spectrophotometer (Fig.14). 20% Nitric acid was used for washing the capillary tubes after analysis of every sample (Fig.15). Quantitative analysis of Nickel was done three times and the mean value was used as the result. Computer-controlled data collection was used to interpret the results (Fig.12). The technique is based on the principle that when liquidized acid treated specimens are exposed to a temperature in the range of 5000-10,000 K, they emit light, which is recorded by multiple photodetectors. All statistical analysis was performed using the SPSS software package. The arithmetic mean and standard deviation were calculated. Two-way ANOVA with one variable as repeated measurement was used to compare the 2 groups A & B with respect to two month period. Statistical significance level was established at $p < 0.05$.

RESULTS

Table 1: Comparison of nickel levels.

	Nickel Levels			
Groups		Mean	SD	Range
A*	Month 1	0.032	0.023	0.002 – 0.070
	Month 2	0.045	0.027	0.012 – 0.083
B†	Month 1	0.016	0.011	0.001 – 0.046
	Month 2	0.027	0.011	0.0120 – 0.044
C♠		0.015	0.0058	0.011– 0.028

INTERPRETATION

GROUP C (CONTROLS)

The average value of Nickel in saliva of the control group of 13 subjects was found to be 0.015mg/L with the standard deviation of 0.0058mg/L. The minimum Nickel value obtained was 0.011mg/L and the maximum was 0.028mg/L. Further, out of the thirteen controls, 9 subjects had Nickel value less than 0.015mg/L (69.2%).

GROUP A:

Month 1:

The average value of Nickel in saliva of Group A subjects in the first month was found to be 0.032 mg/L with the standard deviation of 0.023mg/L. The minimum Nickel value obtained was 0.002 mg/L and the maximum was 0.070mg/L. Further, out of the thirteen subjects, ten had Nickel value greater than 0.015mg/L (76.9%).*This indicates a significant rise in Nickel levels in the first month of group A compared to the control group.*

Month 2:

The average value of Nickel in saliva of Group A subjects in the second month was found to be 0.045mg/L with the standard deviation of

0.027mg/L. The minimum Nickel value obtained was 0.012mg/L and the maximum was 0.083mg/L. Further, out of the thirteen, 12 subjects had Nickel value greater than 0.015mg/L (92.3%) *This indicates a significant rise in Nickel levels in the second month of Group A compared to the control group.*

GROUP B:

Month 1:

The average value of Nickel in saliva of Group B subjects in the first month was found to be 0.016 mg/L with the standard deviation of 0.011mg/L. The minimum Nickel value obtained was 0.001 mg/L and the maximum was 0.046mg/L. Further, out of the thirteen subjects, five had Nickel value greater than 0.015mg/L (38.4%) *The rise in Nickel levels in the first month of Group B is insignificant compared to the control group.*

Month 2:

The average value of Nickel in saliva of Group B subjects in the second month was found to be 0.027mg/L with the standard deviation of 0.011mg/L. The minimum Nickel value obtained was 0.012mg/L and the maximum was 0.044mg/L. Further, out of the thirteen, 10 subjects had Nickel value greater than 0.015mg/L (76.9%) *The rise in Nickel levels in the second month of Group B is significant when compared to the control group.*

Table 2: Comparision between Group A and Group B. (Two way ANOVA with one variable as repeated measurement was used to compare the 2 groups A & B with respect to two month period.)

MONTH	MEAN	SD	MEAN	SD	't'	df	P
1	0.032	0.023	0.016	0.011	2.12	17.8	0.048
2	0.045	0.027	0.027	0.011	2.17	16.0	0.045

INFERENCE:

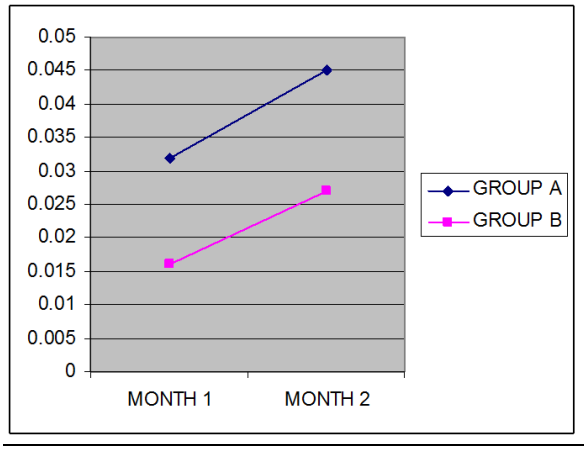
The ANOVA test results indicate that there is a significant difference existing between Group A and Group B. For Group A in general, more Nickel is expressed compared to Group B.

Table 3: Time Comparision.

SOURCE	F	Si8
GROUP	5.218	0.031
TIME	19.818	< 0.001
TIME × GROUP	0.217	0.646 (NS)

INFERENCE

Time comparisons were done irrespective of the group and are found to be significant at P < 0.001. This infers that in second month more Nickel was expressed compared to the first month. The interaction effect between the time and group was found to be non significant. This infers that the change of Nickel expression is similar in both the groups between the two months.



Graph 1: Comparison between group A and group B

DISCUSSION

Nickel Titanium (NiTi) archwires have a unique property of shape memory and superelasticity. This makes their use inevitable in the initial stages of orthodontic fixed appliance therapy. But reports on Nickel hypersensitivity in orthodontic patients during the use of NiTi archwires[16,17] raised concerns on its biologic compatibility. NiTi archwires have a good corrosion resistance due to the presence of Titanium that forms titanium oxide (TiO_2) on exposure to water [2]. T.Eliades *et al* [15] in their surface characterization study of retrieved NiTi archwires, showed the irreversible formation of the precipitates and the development of microcrystalline NaCl, KCl, and Ca-P deposits that can substantially alter the surface composition and topography of the wire. These

alterations may profoundly modify the reactivity of the wire surface with undetermined effects on the corrosion resistance and Nickel dissolution.

A study by Kim and Johnson[14] on the corrosion of orthodontic archwires showed significant corrosion resistance of Epoxy resin-coated NiTi archwires. Microscopic examination of the epoxy resin- coated NiTi wires subjected to potentiostatic anodic polarization revealed no detectable differences in the surface morphology. In addition, the corrosion potential of the wire studied in an electrochemical corrosion cell showed higher breakdown potential making it less susceptible to corrosion. The most clinically relevant methods involve the estimation of Nickel in biologic fluids like saliva, serum and urine[2]. Saliva was used for Nickel analysis as it is the first diluting pool of Nickel. Urine and serum metal concentrations however are dependant on the excretory rate of Nickel, which is a highly individualized parameter and species specific[2].

In the present study, sample collection was restricted to the first two months of orthodontic therapy because it compares two different NiTi archwires which is usually used in the first phase of Preadjusted Edgewise therapy for alignment.

Unstimulated salivary samples were used as stimulation through chewing on a piece of paraffin wax or gum may restrict the collection of saliva to that secreted almost directly from gland[10]. The lack of saliva wetting of the oral cavity including teeth might limit the exposure of appliances to salivary flow which can induce a false negative result. Rinsing the mouth with deionized and distilled water prior to sample collection was followed in the current study based on the findings of T. Eliades *et al* [10] who assessed the effect of rinsing in altering the release potential as rinsing might mask the salivary levels of metals since it dilutes the metal content. It was concluded that no statistically significant differences were found among groups with respect to metal concentrations following rinsing. In the present study the salivary samples were stored at -20° C as Nickel concentrations of saliva are stable for a period of six months in this temperature[7]. Sample preparations are done chiefly to digest the organic matter of the saliva to avoid their interferences during elemental analysis. The preparation of the saliva samples was performed according to the procedure [in] which concentrated Hydrochloric acid was used for organic digestion followed by heating. The reliability of the procedure and ready availability of the components made us to adopt this method for sample preparation.

Following the biochemical preparation procedures, samples are centrifuged at 7000 rpm for 10 minutes, which effectively settled the particulate matter. The centrifuged samples were then subjected for Nickel analysis through Inductively Coupled Plasma - Atomic Emission Spectroscopy. Of the many methods of elemental analysis like Atomic Absorption Spectrophotometry, Atomic Emission Spectroscopy, and Mass Spectroscopy etc, ICP-AES has an advantage of providing information about a wide spectrum of elements in the same sample as opposed to Atomic Absorption[10].

The values of Nickel in the salivary samples obtained in Group B subjects who had Epoxy resin coated NiTi wires were on an average of 0.016 mg/L in the end of first month after appliance insertion. This value was close to the mean Nickel salivary value of control group (0.015mg/L) who did not receive any appliance. However, Nickel levels raised considerably in the second month samples of patients with Epoxy resin coated wires and the mean was 0.027 mg/L. This might have been due to reasons like surface abrasion of the archwire during tooth brushing, replacement of modules by the operator or fictional binding with the brackets etc. When compared to the salivary mean Nickel levels in Group A who received uncoated NiTi wire which was about 0.032mg/L in the first month and 0.045mg/L in the second month, Group B subjects with Epoxy resin coated wires showed a statistically significant less amount of Nickel levels in the saliva. The present study showed significant increase in salivary Nickel level [0.032 mg/L (Group A)] in the first month.

SUMMARY AND CONCLUSION

Nickel hypersensivity had been a concern among Orthodontists and Orthodontic patients with both extraoral and intraoral appliances. Although Nickel is a component in brackets and stainless steel wires, its percentage is relatively very high in NiTi

archwires. Though the composition is not directly related to the corrosion of the wire, case reports[16,17] on Nickel hypersensivity in orthodontic patients during the use of NiTi archwires raised concerns regarding biologic safety. The present study used uncoated and epoxy-resin coated NiTi arch wires. Epoxy resin coated NiTi archwire was claimed as a Nickel free substitute[2] and its corrosion resistance potential has been proved[14]. However, a clinical study to demonstrate its biologic behaviour in the intraoral environment in orthodontic patients was not spotted in the literature. This study has attempted to analyze its Nickel releasing property and has shown satisfactory results. The following results were observed in the present study:

- The mean Nickel levels in the saliva released from Uncoated NiTi wires were significantly higher than the salivary Nickel levels in the Control group (1st & 2nd month)
- The mean Nickel levels in saliva release from Coated NiTi wires were statistically insignificant in the first month when compared to the Control group. However, there was a statistically significant increase in the Nickel level in the second month.
- The mean salivary Nickel levels in the Coated NiTi wire group was significantly lesser than those found in the Uncoated wires. However, the Nickel levels were higher in the second month of both the groups compared to the first month.

Therefore it can be concluded that the reduced Nickel release from the Epoxy-resin coated NiTi archwire makes it a safe option to be used with patients with Nickel hypersensitivity.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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