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Original Research

Evaluation of Nickel and Chromium Ion Release During Fixed Orthodontic Treatment Using Inductively Coupled Plasma-Mass Spectrometer: An *In Vivo* Study

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Abstract:

Background: Fixed orthodontic appliances with the use of stainless steel brackets and archwires made of nitinol have a corrosive potential in the oral environment. Nickel and chromium ions released from these appliances act as allergens apart from being cytotoxic, mutagenic and carcinogenic in smaller quantities in the range of nanograms. This study was done to evaluate the release of nickel and chromium ions from orthodontic appliances in the oral cavity using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS).

Materials and Methods: Saliva samples from 30 orthodontic patients undergoing treatment with 0.022″ MBT mechanotherapy were collected prior to commencement of treatment, after initial aligning wires and after 10-12 months of treatment. Salivary nickel and chromium ion concentration was measured in parts per billion (ppb) using ICP-MS.

Results: Mean, standard deviation and range were computed for the concentrations of ions obtained. Results analyzed using ANOVA indicated a statistically significant increase of 10.35 ppb in nickel ion concentration and 33.53 ppb in chromium ion concentration after initial alignment. The ionic concentration at the end of 10-12 months of treatment showed a statistically significant increase in of 17.92 ppb for chromium and a statistically insignificant decrease in nickel ion concentration by 1.58 ppb. Pearson's correlation coefficient showed a positive correlation for an increase in nickel concentration after aligning, but not at the end of 10-12 months. A positive correlation was seen for an increase in chromium ion concentration at both time intervals.

Conclusion: Nickel and chromium ion concentration in saliva even though below the recommended daily allowance should not be ignored in light of the new knowledge regarding effects of these ions at the molecular level and the allergic potential. Careful and detailed medical history of allergy is essential. Nickel free alternatives should form an essential part of an orthodontist's inventory.

Key Words: Chromium, inductively coupled plasma-mass spectrometer, nickel, saliva

Introduction

Nickel (Ni) and chromium (Cr) containing alloys are present in great numbers in a wide variety of appliances, auxiliaries, and utilities used in orthodontics and thus become an integral part of almost every routine orthodontic intervention.

The use of various combinations of metal alloys for prolonged durations in orthodontic patients warrants special consideration regarding their biocompatibility. The oral cavity is a complete corrosion cell, with many factors that enhance the biodegradation of orthodontic appliances.[1]

Saliva acts as an electrolyte for electron and ion conduction, and the fluctuation of pH and temperature, the enzymatic and microbial activity, and the various chemicals introduced into the oral cavity through food and drink are all corrosion conductors. The inherent heterogeneity of each metal alloy and its use with other alloys, the microsurface discontinuity, the forces acting on the appliances and the friction between wires and brackets also add to the corrosion process.[2]

Nickel-titanium (NiTi) archwires contain 47-50% nickel and are the richest source of nickel in the intraoral environment of the average orthodontic patient. Recent evidence has attributed carcinogenic, mutagenic, cytotoxic, and allergenic actions to nickel in various forms and compounds.[3]

The particular emphasis recently placed on nickel arises from the abundance of evidence connecting this element with a wide range of pathological conditions. Specifically, nickel amounts as low as 2.5 ng/mL (ppm) have been found to impair the chemotaxis of leukocytes and stimulate neutrophils to become aspherical and move slowly. Most importantly, non-toxic concentrations of nickel may inflict direct DNA base damage and site-specific DNA strand scission (single-strand breaks), whereas an indirect route of nickel-induced DNA alteration involves the inhibition of enzymes known to restore DNA breaks. Finally, nickel ions at non-toxic concentrations may promote microsatellite mutations and inhibit the repair

of nucleotide excisions, thereby contributing to genetic instability.[4]

Nickel is a powerful sensitizer metal and a common allergen. The inflammatory response, from an immunologic standpoint, is considered as type IV hypersensitivity and is manifested as Nickel Allergic Contact Stomatitis (NiACS).[5]

Because most research on the amounts of metal ions released from orthodontic alloys has shown that they fall below the recommended daily dietary intakes of nickel and chromium, this might be a false assurance of safety, since chronic low levels of metal ions can alter cellular metabolism and morphology, and produce inflammation and even DNA instability. In addition, some *in-vivo* studies reported biologic toxicity in orthodontic patients.[6]

In dental applications, fluoride-containing commercial mouthwashes, toothpaste, and prophylactic gels are generally used to avoid dental caries or to reduce dental sensitivity. The fluoride ions degrade the protective titanium dioxide film formed on titanium and titanium alloys. Since the outermost surface of nickel-titanium archwires contains mainly titanium dioxide film with small amounts of nickel oxide, fluoride enhanced corrosion of the nickel-titanium archwire may occur.[7]

Therefore, in the practice of orthodontics it has become imperative to know the exact amount of each ion released and to subsequently provide this information to each patient undergoing therapy.

Materials and Methods

In this study, 30 patients undergoing orthodontic treatment, at the Department of Orthodontics and Dentofacial Orthopaedics, M. R. Ambedkar Dental College and Hospital, Bangalore were included. Patients were included in the study after obtaining ethical clearance from the institution and informed consent from the patients. Equal number of males and females in the age range of 10-25 years formed the study sample. Patients underwent treatment for over 10-12 months with 0.022″ × 0.028″ slot (Victory series, 3M Unitek Dental Products, Monrovia, California, USA) brackets. Molars were banded with 0.022″ × 0.028″ slot buccal tubes (Victory series, 3M Unitek Dental Products, Monrovia, California, USA) welded onto band material. The wires used in a sequential manner were nickel-titanium wires (0.014″, 0.016″), heat activated nickel-titanium wires (0.017″ × 0.025″, 0.019″ × 0.025″) and stainless steel wires (0.017″ × 0.025″, 0.019″ × 0.025″) from 3M Unitek Dental Products, Monrovia, California, USA. Patients were selected based on the absence of any piercings or metal restorations, good health and absence of prolonged use of any medication, absence of any systemic disease and no intraoral or extraoral auxiliary appliances soldered or welded to bands.

The sampling was performed before commencement of fixed mechanotherapy, after completion of alignment and leveling with nickel-titanium wires and 10-12 months after the start of treatment. Sample collection was carried out after rinsing with 15 mL of distilled and deionized water for 30 s. Approximately, 5 mL of saliva was collected from each subject by spitting into a beaker and transferred to an assigned polypropylene container. The samples were labeled from 1-30 and assigned into groups A, B and C to be sent for analysis.

Group A: Pre-treatment saliva sample;

Group B: Saliva sample after aligning archwires;

Group C: Saliva sample after 10-12 months of fixed mechanotherapy.

The samples were kept at −20°C until they were processed to eliminate interference and to reduce the effects of the biological matrix (protein, salt, etc.). Inductively coupled Plasma-Mass Spectrometer (ICP-MS) (Thermo Fisher Scientific make, X Series -2) at Analytical Research and Metallurgical Laboratory Pvt Ltd., Bangalore was used to estimate the ion concentration in the saliva samples. The concentration of both nickel and chromium ions was recorded in parts per billion (ppb).

Statistical analysis

Statistical analysis was performed using RM-ANOVA and correlation was checked with Pearson's correlation coefficient.

Results

The results were computed with 30 pre-treatment samples, 29 samples after initial aligning wires and 28 samples at 10-12 months.

The results of metal ion estimation using ICP-MS indicated a mean concentration of 47.91 ± 34.66 ppb before the commencement of orthodontic treatment. A mean nickel concentration of 58.26 ± 32.28 ppb was found after alignment using superelastic wires. A statistically significant mean nickel release of 10.35 ppb was noted in the initial alignment phase.

The final evaluation of nickel content was done at 10-12 months and 46.33 ± 26.95 ppb of mean nickel concentration was recorded. Contrary to the previous *in-vitro* findings, a mean decrease of 1.58 ppb was found at the end of 10-12 months of orthodontic treatment compared with the pre-treatment values. However, this decrease in nickel concentration was found to be statistically insignificant (Table 1 and Graph 1).

A mean chromium concentration of 69.15 ± 60.48 ppb was noted before treatment. A mean chromium release of 33.53 ppb resulting in a chromium concentration of 102.68 ± 68.12 ppb was found at the end of the alignment.

The final mean chromium concentration of 87.67 ± 63.47 ppb showed a net increase of 17.92 ppb, which was statistically significant (Table 2 and Graph 2).

The net concentration of nickel and release of nickel was found to be less than chromium.

Pearson's correlation coefficient showed moderately positive correlation between all values (Tables 3, 4 and Graphs 3-5) except the change in nickel concentration between pre-treatment level and after 10-12 months of treatment, which showed a weak positive correlation (Table 3 and Graph 6).

Discussion

Metallic orthodontic appliances are usually made up of 18/8 stainless steel (18% chromium and 8% nickel). The resistance of stainless steel to tarnish and corrosion is associated with the passivating effect of chromium.[1]

Some archwires with elastic properties (shape memory alloys) can contain >50% nickel. Release of nickel from metallic orthodontic appliances has been observed in several *in-vitro* studies.[8-10]

There is increasing concern about the biocompatibility of dental materials; this might be due to a real increase in the frequency of allergic reactions to materials or to an increase in awareness of adverse effects from these materials.[11]

A great number of *in-vitro* studies have concluded that orthodontic appliances have a corrosive potential; separately the wires and brackets, and as a simulated appliance in the artificial salivary medium. However, when appliances are placed in the oral cavity, they are mechanically activated to facilitate tooth movement. The movement of the archwire and friction of brackets might lead to further corrosion and might enhance release of metal ions from the orthodontic appliance. On the contrary, no conclusive results have been found from *in-vivo* studies rendering them less useful for clinical application.[12] This void in our knowledge has paved the way for further research on this subject.

This study was conducted to evaluate the release of nickel and chromium from orthodontic appliances during sequential wire change from initial shape memory alloys to subsequent use of stainless steel using ICP-MS.

Previous *in-vivo* studies involved metal ion estimation in saliva,[13,14] serum,[15] urine,[16] oral mucosal cells,[6,17] gingival plaque[18,19] in humans and even non-invasive matrices like hair and invasive matrices (kidney, liver, lungs, aorta and oral mucosa) in animals.[20]

In this study, saliva of patients undergoing orthodontic treatment was used for measurement of metal ions to obtain a direct measurement of ion concentration, which could then be compared with the standard permissible value. Furthermore, several studies have shown that orthodontic appliances release metal ions through electro-galvanic currents, with saliva acting as a medium for continuous erosion over time.[21-23]

The frequency of nickel hypersensitivity has been found to be 10 times more in females than in males. In this study, equal number of boys and girls were included to eliminate any gender bias.[11]

Previous *in-vivo* studies have estimated metal ions at very short intervals. The criticism to these studies mounts from galvanic corrosion studies which have concluded that corrosion increases with the manifestation of fatigue in the appliance. The fatigue in an orthodontic appliance manifests due to continuous loading forces to move the teeth. The fatigue in an orthodontic appliance, thus, increases with time.[23] Hence, the present study was designed to estimate ion release 10-12 months into treatment instead of a short interval.

Patients were advised to use non-fluoridated mouthwash due to the proven effect of fluoride on ion release.[7]

Table 1: Association between nickel values over the study period using repeated measures ANOVA.

Groups	Nickel (mean±SD)	*P* value*
Pre-treatment	48.78±35.75[a]	0.06
Post-alignment	59.19±32.82[a]	
10-12 months	46.33±26.95	

*Denotes repeated measures ANOVA followed by Tukey's *post hoc* test. [a]Denotes interactions within groups

Table 2: Association between chromium values over the study period using repeated measures ANOVA.

Group	Chromium (mean±SD)	*P* value*
Pre-treatment	69.74±62.33[a]	0.001**
Post-alignment	102.68±68.65[b,c]	
10-12 months	87.07±63.47[c]	

*Denotes repeated measures ANOVA followed by Tukey's *post hoc* test. [a,b,c]Denote interactions within groups. **Denotes high statistical significance

Table 3: Correlation between nickel values over the study period was evaluated using pearson's correlation coefficient.

Comparison groups	Pearson's correlation coefficient (*r*)	*P* value	Interpretation
Pre-treatment - post-alignment	0.67	0.00**	Moderately positive correlation
Pre-treatment: 10-12 months	0.34	0.08	Weak positive correlation

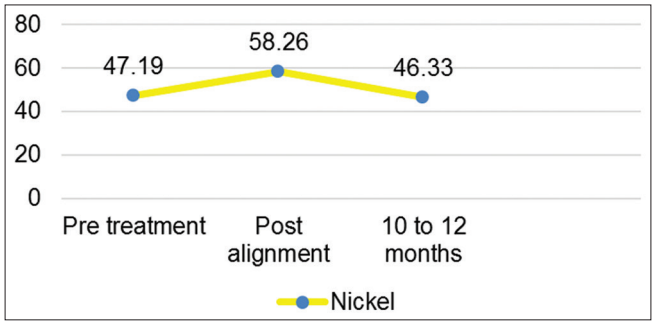
**: *P* value ≤0.05 shows positive correlation

Table 4: Correlation between chromium values over the study period was also evaluated using Pearson's correlation.

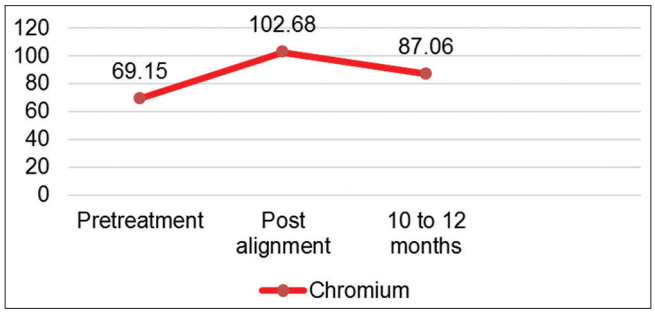
Comparison groups	Pearson's correlation coefficient (r)	*P* value	Interpretation
Pre-treatment - Post-alignment	0.69	0.00**	Moderately positive correlation
Pre-treatment: 10-12 months	0.71	0.00**	Moderately positive correlation

**: *P* value ≤0.05 shows positive correlation

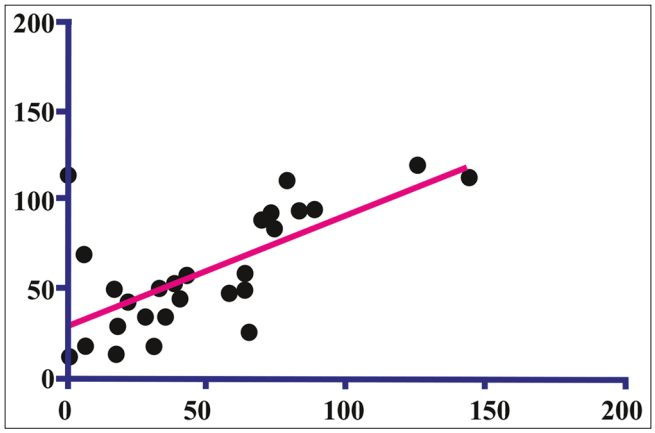
Saliva collected by chewing paraffin or gum has a different organic composition than does unstimulated saliva. In the resting state, about two-third of the volume of whole saliva is produced by the submandibular glands. However, when the salivary glands are stimulated, the parotids can account for at least half the whole saliva volume in the mouth. Thus, stimulation could change the protein composition of saliva. Nickel rapidly combines with proteins. Consequently, a change in protein composition of saliva could also affect nickel concentration. Moreover, the lack of saliva wetting of the oral cavity, including teeth, by stimulated saliva collection limits the exposure of the appliances to salivary flow and possibly induces a false negative result.



Graph 1: Association of nickel concentration over the study period.

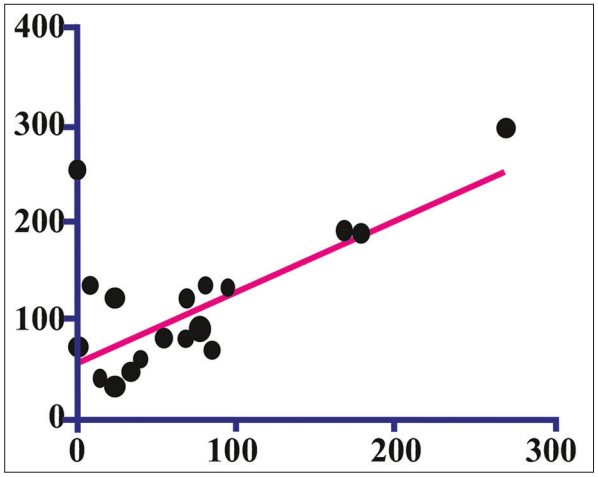


Graph 2: Association of chromium concentration over the study period.

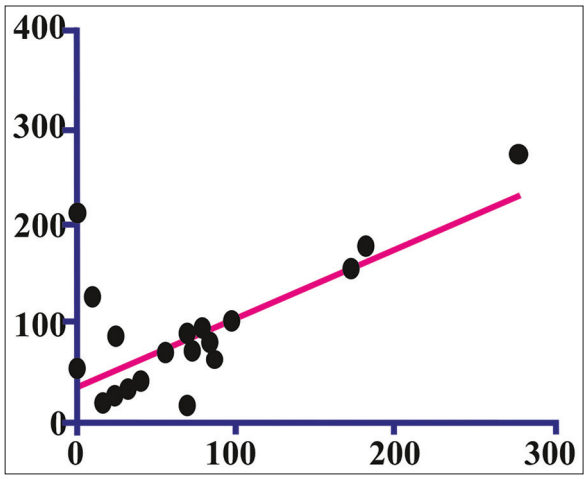


Graph 3: Correlation between pre-treatment nickel concentration and post-alignment nickel concentration.

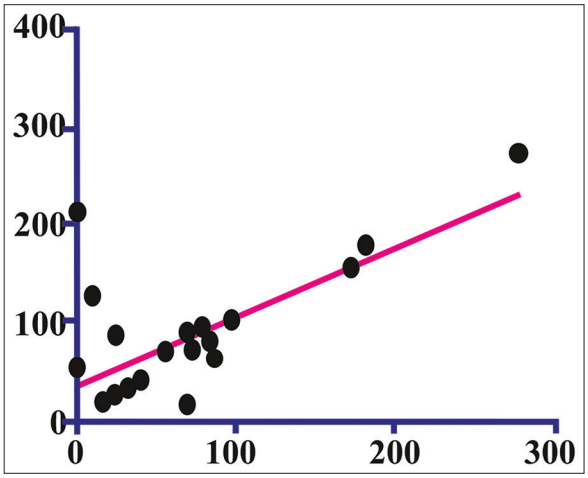
The secretion of saliva is regulated by reflexes involving the autonomic nervous system. The saliva flow rate depends not only on the stimulus, but also on duration and intensity.



Graph 4: Correlation between pre-treatment chromium concentration and post-alignment chromium concentration.



Graph 5: Correlation between pre-treatment chromium concentration and chromium concentration at 10-12 months.



Graph 6: Correlation between pre-treatment nickel concentration and nickel concentration at 10-12 months.

The parasympathetic branch provides the main stimulus for salivation, causing a high flow rate of watery saliva, compared with the sympathetic stimulus, which leads to a lower rate of much more viscous saliva. Emotional state also influences the saliva flow rate; for example, anxiety and depression can cause a dry mouth.[24]

Saliva collection was done by passive drool into a sterile container. Passive drool was preferred over absorbent devices, which can sometimes cause interference during testing. High-quality polypropylene containers which could withstand storage temperature of –20°C were used. Samples were stored in a freezer at 4°C for no longer than 4 h and then frozen at –20°C to prevent any opportunity for bacterial growth until they were processed and diluted with deionized water to eliminate interference and reduce the effects of biological matrix (protein, salt, etc.).[25]

Previous *in-vitro* studies have used Atomic Absorption Spectrometer which has a detection limit of 3 ppb for chromium and 6 ppb for nickel and ICP-Optical Emission Spectrometer (ICP-OES) with a detection limit of 0.2 ppb for chromium and 0.5 ppb for nickel. ICP-MS used in this study has much lower detection limits of 0.0003 for chromium and 0.0002 ppb for nickel.[26]

Evaluation of such low values is in accordance with previous studies, which have concluded that low ion concentration can induce biological effects in cells. Nickel can lead to DNA alterations mainly through base damage and DNA strand scission even at very low concentrations.[27]

Another advantage of using ICP-MS over ICP-OES is the sample size. 0.1-1 g of sample is needed for analysis with ICP-OES, whereas 0.01-0.1 g of sample is adequate for analysis using ICP-MS.[26]

This study showed a wide range of variation in nickel and chromium concentration in saliva, which has also been found in previous studies.[8,10,21,28]

The initial rise in nickel and chromium during the phase of alignment and leveling is consistent with previous studies which report an initial increase in nickel and chromium and subsequent decreased concentrations.[23] The higher concentration over this long period of time than in the previous *in-vitro* studies can be attributed to the dynamic loading of the appliance in the *in-vivo* conditions. Also, long-term immersion of specimens does not result in higher release of metal ions because of saturation of immersion medium.[29]

The clinical behavior might include the corrosion of orthodontic alloys during the intraoral service of appliance and utilities, which have a broad spectrum of corrosion types and many mechanisms. Thus, pitting corrosion has been identified in brackets and wires, on the other hand crevice corrosion occurs in loci exposed to corrosive environments, often through the application of elastomeric ligatures on a bracket, and arises from differences in metal ion or oxygen concentration between the crevice and its vicinity. This factor was eliminated in the present study due to routine use of 0.009″ stainless steel ligation. However, some plaque deposition was found to be inevitable in spite of regular reinforcement of oral hygiene instructions at each visit. The attack can, thus be attributed to the lack of oxygen associated with plaque formation and the byproducts of microbial florae; this depletes the oxygen, disturbing the regeneration of the passive layer of chromium oxides. Also, fretting corrosion develops during sliding of a metallic wire on the slot of the bracket with the underlying mechanism involving the cold welding at the interfaces under pressure; this results in rupturing of the contact points (wear oxidation). In addition, enzymatic activity and microbial attack on material surfaces have been identified in dental applications of materials.[29]

The net concentration of nickel and release of nickel was found to be less than chromium. This finding is consistent with a previous study.[14] This can be attributed to the binding of nickel to salivary proteins, which would render the nickel unavailable in ionic form to be detected by ICP-MS. Previous studies have also found that the precipitated corrosion products contained much higher amounts of chromium than nickel. They also found that nickel was released primarily as soluble compounds, whereas chromium was released primarily as insoluble compounds, which could explain the findings of the present study.[8]

A previous *in-vitro* study[4] reported no evidence of nickel release for nickel-titanium wires. This can be attributed to the densely precipitated titanium oxide layer on the surface of the alloy, which acts as a barrier for the diffusion of nickel on the surface, thereby minimizing it's reactivity with the surrounding environment. The increase in nickel and chromium during the initial alignment phase is contrary to the *in-vitro* behavior of the wire alone. This can be attributed to the presence of other components of the appliance such as the brackets and the soldered trans-palatal arch.

Increased urinary excretion of nickel after insertion of an orthodontic appliance, as noted in a previous study, can also explain the statistically insignificant decrease in salivary nickel concentrations.[16]

The variation of parameters can also be attributed to the manufacturing process, which involves the type of alloy and the characteristics of metals used. Second, environmental factors such as mechanical stress, diet, time of the day, salivary flow rate, health and psychosomatic factors also influence the metal ion release.[30]

The results hold implications for the biocompatibility of orthodontic appliances. The amount of metal released from orthodontic appliances in saliva is significantly below the average dietary intake and did not reach toxic concentrations, which is consistent with the previous studies.[21] However, it can be a false assurance of safety because even non-toxic concentrations could be sufficient to induce biological effects in cells from the oral mucosa.[27]

The subject of hypersensitivity is of prime importance. Although some studies[31] have concluded a decreased frequency of allergy with nickel contact at an early age, nevertheless, hypersensitivity, dermatitis, and asthma have been reported in some patients with orthodontic appliances.[21] Some oral clinical manifestations in orthodontic patients, such as gingival hyperplasia, labial desquamation, angular cheilitis, multiform erythema, and periodontitis, might be associated with an inflammatory response induced by the corrosion of orthodontic appliances and the subsequent release of nickel. This inflammatory response, from an immunologic standpoint, is considered type IV hypersensitivity. It is manifested as NiACS, and its etiology and diagnosis are difficult to establish. It has also been concluded that a previous allergic reaction should be considered a predictive factor of NiACS clinical manifestations and should be noted in the patient's medical history.[5]

Even though allergy in some orthodontic patients has been a documented reaction, the true concern should be possible cytotoxicity or, even more importantly, the genotoxicity of orthodontic appliances. Genotoxicity comprises either mutagenic or carcinogenic process.[32] Possible genotoxic effect can be caused by metal ions which cause DNA damage by decreased DNA migration through crosslinking DNA. Metal ions have an indirect route to DNA breakdown which involves inhibition of enzymes which are known to restore DNA breaks.[4] Persistent DNA damage can lead to mutations. In labile tissue such as the buccal mucosa, the cellular proliferation of a damaged cell might cause many defective cells. Cellular toxicity will also affect the cell's metabolism and in turn, its function and repair capacity[17] and lead to apoptotic cells.[27] All these short-term and long-term risks make it a mandatory practice to elicit an extensive history of allergic reactions and then proceed for the orthodontic treatment with an appropriate choice of appliance.

Titanium alloys are known for their biocompatibility. However, biocompatibility is not guaranteed when titanium brackets are combined with different archwires. Titanium wires and epoxy coated nickel-titanium wires exhibited the least corrosive potential.[21] For patients allergic to nickel, the use of titanium or epoxy coated wires during orthodontic treatment is recommended.

Newer nickel free wires likes nickel-lite (Cobalt chromium alloy), Connecticut New Archwire beta titanium, titanium niobium, timolium titanium, gold plated wires and resin coated wires along with nickel free brackets such as ceramic, plastic, polycarbonate, gold, and nickel-lite hold a promising future.[33]

Hence, in the present scenario it is recommended that orthodontists be alerted but not alarmed of this perpetuating nickel and chromium hazard. This study also urges a need to investigate further, the effect of nano-concentration of ions at the molecular level so that evidence-based practice guidelines can be formulated for safer clinical practice.

Conclusion

The present study was conducted to evaluate the nickel and chromium ion release from fixed orthodontic appliance. Saliva samples from 30 patients were collected before commencement of treatment, after alignment and after 10-12 months of appliance therapy. It was concluded that:

- There is a statistically significant increase in the nickel and chromium ion concentration after the initial aligning phase with an increase of 10.35 ppb in nickel ion concentration and an increase of 33.53 ppb in chromium ion concentration.
- A net increase of 17.92 ppb was found in salivary chromium ion concentration at the end of 10-12 months, which was statistically significant.
- A net decrease of 1.58 ppb was found in the salivary nickel ion concentration at the end of 10-12 months of treatment. This decrease in nickel ion concentration was, however found to be statistically insignificant.
- A positive correlation was found in the initial rise in nickel concentration. However, no correlation was found for the change in nickel ion concentration at the end of 10-12 months.
- A positive correlation was found for the increase in chromium ion concentration after the initial alignment and at the end of 10-12 months.

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