Evaluation of Salivary and Serum Concentration of Nickel and Chromium Ions in Orthodontic Patients and Their Possible Influence on Hepatic Enzymes: An in vivo Study

1Anuj Satija, 2Maninder Singh Sidhu, 3Seema Grover, 4Vikas Malik, 5Puneet Yadav, 6Rohan Diwakar

ABSTRACT

Objectives: The aims of present study were to evaluate nickel and chromium ions concentrations in salivary and serum samples from patients treated with fixed orthodontic appliances and their possible influences on hepatic enzymes.

Materials and methods: Saliva and blood samples were collected from 36 patients ranging in age from 12 to 24 years. Three samples of saliva and two samples of blood were obtained. First saliva and blood samples were collected before inserting fixed appliances. Second saliva samples were collected at 1 week, third saliva sample and second blood sample after 4 weeks of appliance insertion. Serum was prepared by centrifuging blood samples at 3000 rpm for 10 minutes. Spectrophotometric determinations were carried out using electrothermal atomic absorption spectrophotometry.

Results and conclusion: In serum, significant increase in Ni and Cr ion concentration occurred in samples collected after 4 weeks. In saliva samples, nickel and chromium reached their highest levels in first week. Mean liver function enzymes SGOT and SGPT were also significantly increased in 4 weeks. Fixed orthodontic appliances release measurable amount of nickel and chromium when placed in mouth, but this increase does not reach toxic levels for nickel and chromium in saliva and serum to cause harmful effects in human beings.

Keywords: Nickel, Chromium, Fixed orthodontics, Hepatic enzymes.


Source of support: Nil

Conflict of interest: None

Received on: 11/7/14

Accepted after revision: 6/8/14

INTRODUCTION

During the last decade, there has been an increased interest among orthodontic professionals regarding side effects associated with use of biomaterials, especially metallic materials, such as gold, stainless steel, cobalt-chromium, nickel-titanium and beta-titanium. Nickel-titanium alloys were introduced for use as orthodontic wires in 1970s which contain 47 to 50% Ni and thus are the richest source of nickel among all alloys.

Oral environment is particularly ideal for the biodegradation of metals because of its ionic, thermal, microbiologic and enzymatic properties. In oral environment, biodegradation of metals usually occurs by electrothermal breakdown. The association of different metals in oral environment, where saliva is the connecting medium may produce electrogalvanic currents that produce discharge of ions and metallic components. Corrosion of alloys in the oral environment may decrease the strength of metallic appliances. Discoloration on the underlying tooth surface during orthodontic treatment has also been regarded as one of the consequences of crevice corrosion of the bracket bases. Nickel and chromium have dermatological, toxicological and possibly mutagenic effects. It is estimated that 4.5 to 28.5% of population have hypersensitivity to nickel with higher prevalence in females. Chromium allergy is estimated at 10% in male subjects and 3% in female subjects.

Nickel hypersensitivity symptoms include rash, allergy, lung disorder, ear infection and tinnitus. Nickel is most common cause of metal induced allergic contact dermatitis in man and second in frequency is chromium. In addition to the allergic issue, carcinogenic, mutagenic and cytotoxic effects have been assigned to nickel and to a lesser extent chromium. The most significant method for measurement of nickel release before and after onset of orthodontic treatment is salivary analysis, since it is the first diluents of the human body and allows long period of analyses. Also most of the redox reactions occur in liver and nickel is a redox active metal and is able to impair cellular defence mechanisms against peroxidation reactions.

This is the first in vivo study relating salivary and serum levels of nickel and chromium to hepatic enzymes. It has been reported in literature increased serum level of nickel...
have been found to affect lungs, kidney, liver and heart.\textsuperscript{10} Concentrations of serum nickel have been found to decrease in hepatic cirrhosis as a consequence of hypoalbuminemia.\textsuperscript{11} Hence, present study was conducted to measure nickel and chromium ion concentrations in serum and saliva before and after insertion of orthodontic appliance and also to evaluate changes in salivary nickel and chromium ions concentration during orthodontic treatment and their possible influences the hepatic enzyme levels. The null hypothesis was that changes in salivary and serum nickel and chromium will not affect hepatic enzymes levels.

MATERIALS AND METHODS

This study was conducted at the Department of Orthodontics, SGT Dental College and Hospital, Gurgaon (India). The sample size consisted of 36 subjects (21 females and 15 males), who were to commence their fixed orthodontic treatment and had no history of having received any prior orthodontic appliance. The mean age of the sample was 17.5 years (14-24 years).

An informed consent was obtained from all patients who participated in the study, and ethical clearance was obtained. The main criteria for subject inclusion were that subjects should not be suffering from any systemic disease and should not have habits of smoking, red wine intake, excess of tea, coffee or betelnut, tobacco chewing and no amalgam restorations in oral cavity.

Preadjusted edgewise appliance (ORMCO 0.022" MBT prescription) was used in all cases. All patients received 0.016" NiTi wire as an initial wire for alignment and leveling, which was tied into the bracket slot with stainless steel ligatures. None of the patients had palatal or lingual appliances welded to the bands or extraoral orthodontic appliances.

Three samples of saliva were collected from each orthodontic patient. First sample before insertion of the fixed appliance which served as a baseline/reference level for salivary nickel and chromium content, second after 1 week, and third after 4 weeks of insertion of appliance.

For the collection of saliva, patients initially rinsed the mouth thoroughly with a mouthful of deionized and distilled water. The patients, after an initial swallow were instructed to collect approximately 10 ml of saliva with mouth closed and released into the plastic test tube. In this way, approximately 10 ml of saliva was collected into an acid-washed (concentrated HNO\textsubscript{3}) plastic test tube. After collection, the samples were stored in ice box at –20°C in dry ice until they were processed.

Two samples of blood were collected from each orthodontic patient that is before insertion of fixed appliance which served as a baseline/reference level for serum nickel and chromium content and 4 weeks after insertion of the appliance. Blood was obtained from antecubital fossa of arm and centrifuged at 3000 rpm for 10 minutes in order to prepare serum.

Preparation and the Samples of Saliva and Blood

For processing, 0.5 ml of saliva/serum was transferred to smaller plastic test tubes (Cryo Tubes, Polylabs, New Delhi) using a micro-pipette (Biochemical Ltd, Takson, India) which were pretested for not releasing nickel. For digestion of organic matter in saliva/serum, 0.15 ml of concentrated HNO\textsubscript{3} was added to each sample. The tubes were then closed and kept at 80°C for 8 hours in hot air oven. The saliva samples were centrifuged at 3000 rpm for 2 minutes to settle particulate matter. The serum samples were centrifuged at 3000 rpm for 10 minutes to settle particulate matter (Fig. 1).

The chemical analyses for nickel and chromium were done by electrothermal atomic absorption spectrometry (model AAS5 EA, Analytik Jena Ltd, Germany), at the Vimta Labs Ltd, Life Sciences Facility, Hyderabad, India (Fig. 2). Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. The technique makes use of wavelengths of light specifically absorbed by an element. They correspond to energies needed to promote electrons from one energy level to higher energy level. The analytic lines used were 357.9 nm for chromium and 232.0 nm for nickel.

Calibration Curve for Nickel and Chromium (Fig. 3)

A calibration curve was used to determine the unknown concentration of an element (Ni, Cr) in a solution. A sample of known concentration of 20, 40, 60, 80, 100 ppm (obtained
Methodology and Determination of Concentration of Ni and Cr in Saliva and Serum Samples

Twenty microliter of sample was injected directly into graphite tube (Analytik Jena Ltd, Germany) from an automated micropipette (Biochemical Ltd, India). The tube was heated electrically by passing a current of 5.0 milliamperes (mAmp) through it in a pre-programed series of steps which included 15 seconds at 120°C to evaporate the solvent, 6 seconds at 950°C to drive off any volatile organic material and change the sample to ash, and with a fast heating rate to 2100°C for 5 seconds to vaporize and atomize the elements (Graph 1). Finally, tube was heated to higher temperature of 2300°C to make it clean and keep it ready for next sample.

Then a beam of electromagnetic radiation from a hollow cathode lamp (specific for Ni—232.0 nm and Cr—357.9 nm) (Fig. 4) was passed through the vaporized sample. Some of the radiation was absorbed by the metal atoms in the sample. The standard amount absorbed by metal atoms was compared with the calibration curve and this enables calculation of metal (Ni and Cr) concentration in unknown sample by using Beer-Lambert law:

\[
\text{Concentration (µg/l)} = \frac{\text{Absorbance (recorded from AAS)}}{\text{Slope (calculated from calibration curve)}}
\]

by diluting 1000 ppm standard solution from MERCK India) were subjected to and absorbance (Abs) values were plotted vs concentration levels. The calibration curve showed the concentration against the amount of radiation absorbed. If a linear calibration curve was obtained, the slope of calibration could be obtained by using following equation:

\[
A = mc \text{ (Beer-Lambert law)}
\]

Absorbance = slope \times concentration

**Graph 1:** A normal calibration graph or calibration curve of nickel and chromium

**Fig. 2:** Electrothermal atomic absorption spectrometry (model AAS5 EA, Analytik Jena Ltd, Germany), at the Vimta Labs Ltd, Life Sciences Facility, Hyderabad

**Fig. 4:** Xenon lamp as a continuous radiation source

**Fig. 3:** The working of atomic absorption spectrometer
RESULTS

In this study, 36 subjects, mean age of 17.5 years, who were to commence their fixed orthodontic treatment and had no history of having received any prior orthodontic appliance, fixed or removable were selected. The sample distribution was divided into two groups.

Table 1 shows that mean salivary nickel and chromium concentrations were increased in all patients after 1 week compared to baseline and 4 weeks levels. Hence, leaching of nickel and chromium ions from orthodontic wire, brackets and bands were maximum at 1 week when compared to baseline and 4 weeks levels. Also serum levels of nickel and chromium concentrations were increased in 4 weeks interval. Table 2 shows paired t-test for salivary nickel and chromium concentrations. Here, p-value in all three combination was <0.001, hence there was highly significant increase in observation taken at pretreatment and at 1 week, also deviation from pretreatment to 4 week was highly significant but this increase was not as high as at 1 week.

The normal range for serum nickel have been reported to be between 6.587 and 10.912 ppb. The normal range for serum chromium are reported to be between 6.082 and 10.990 ppb.

<table>
<thead>
<tr>
<th>Table 1: Salivary and serum Ni and Cr concentrations (ppb) in individual subjects at different time periods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salivary Ni</strong></td>
</tr>
<tr>
<td>Pre</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Std. deviation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Paired t-test for salivary nickel and chromium (ppb) concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 36</strong></td>
</tr>
<tr>
<td>Salivary Ni</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Salivary Cr</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001: Highly significant

<table>
<thead>
<tr>
<th>Table 3: Paired t-test for serum nickel and chromium (ppb) concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 36</strong></td>
</tr>
<tr>
<td>Serum nickel</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Serum chromium</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*p < 0.001: Highly significant

<table>
<thead>
<tr>
<th>Table 4: Liver function enzyme levels (U/L) in individual subjects after 4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SGOT</strong></td>
</tr>
<tr>
<td>pre(U/L)</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>Std. deviation</td>
</tr>
</tbody>
</table>
Table 3 shows paired t-test for serum nickel and chromium concentrations. The mean value for serum nickel ions for baseline level was 8.265 ppb and for serum nickel 4 weeks levels was 9.948 ppb. The mean value for serum chromium ions for baseline level was 6.477 ppb and at 4 weeks levels was 9.975 ppb. The p-value for baseline and 4 weeks was <0.001 which shows statistical significance difference.

The normal range of SGOT concentrations varies from 18 to 45 U/L and normal range of SGPT concentrations varies from 28 to 49 U/L.

Table 4 shows that SGOT and SGPT levels were increased in all patients at 4 weeks interval compared to baseline group, but they remain within normal range. The p-value for both at baseline and 4 weeks was <0.001 which was statistical significant.

The normal range of alkaline phosphatase varies from 208 to 278 U/L. Alkaline phosphatase levels at 4 weeks were increased in all patients compared to baseline group, but they remained within normal range.

The p-value at baseline and 4 weeks was <0.001 which was statistically significant.

Table 5 shows paired t-test for SGOT, SGPT and alkaline phosphatase concentrations. Here p-value was <0.001, hence there was highly significant increase in observation taken at pretreatment to 4 weeks interval.

**DISCUSSION**

A wide variety of appliances and auxiliaries used in orthodontics contain nickel and chromium containing alloys, and thus they have become an integral part of almost every routine orthodontic intervention.\(^3\) The potential health effects from exposure to these compounds have been scrutinized for more than 100 years, and sensitizing patients to these metals have been a concern.\(^12\)

Nickel has threshold concentration of approximately 30 ppm which may be sufficient to elicit a cytotoxic response.\(^13\) Nickel has been found to induce DNA alterations mainly through base damage and DNA strand scission (single strand breaks), which are site specific. The mechanism of action of mutations caused by nickel is due to inhibition of several enzymes known to restore DNA breaks promoting microsatellite mutations and increasing total genomic methylation, thereby contributing to genetic instability.\(^14\) Nickel toxicity has been reported in literature very well to the extent of death in few cases and postmortem report showed nickel level as µg/g wet weight 7.5 in blood, 47 in urine, 27 in liver, 60 in bile respectively.\(^15\)

In present study, saliva samples were obtained from orthodontic patients in order to analyze the release in the dynamic oral cavity environment. The main advantage of current *in vivo* study as compared to the *in vitro* studies was that the concentrations of salivary nickel and chromium were recorded in the natural oral environment of the patient, where the actual side effects of increased metal concentrations were going to take place.

The present study when compared with study by Gjerdet et al\(^16\), who conducted a study on nickel and iron in saliva of patients with fixed orthodontic appliances found no significant differences in absolute masses of nickel or iron in samples taken in saliva before, and 3 weeks after insertion of fixed appliances. But for samples taken immediately after 1 week, there was a significant increase in both concentrations and masses of nickel and iron. In the present study, there was a significant difference found between the pretreatment samples and the samples taken 1 and 4 weeks.

Kerosuo et al\(^17\) reported nickel release of 16.7 µg from a simulated fixed appliance and 7.5 µg from headgear during 7 days under static conditions. Grimsdottir et al\(^18\) conducted an *in vitro* study and analyzed the release of nickel from different types of metal appliances immersed in physiologic saline and found that amount of metal release was highest from facebows followed by molar bands, brackets and archwires (NiTi).

Petoumenou et al\(^19\) concluded that samples taken immediately after placement of bands, brackets and NiTi archwires showed slight but significant increases in nickel concentration of 78 and 56 µg per liter respectively, compared with pretreatment value of 34 µg per liter. Nickel
leaching occurred after placement of bands, brackets and NiTi archwires, associated with an increase of nickel ion concentration in patient’s saliva and this effect decreased within 10 weeks.

The range of serum nickel concentrations increased mainly from 8.265 to 9.948 ppb 4 weeks after insertion of fixed appliances (Table 3). There was a significant difference (p < 0.001) found between the no appliance samples and the samples taken 4 weeks after bonding and banding tested by the students t-test.

The range of serum chromium concentration increased mainly from 6.477 to 9.975 ppb 4 weeks after insertion of fixed appliances (Table 3). There was a significant difference (p < 0.001) found between the pretreatment samples and the samples taken 4 weeks after bonding and banding tested by the students t-test.

Nickel can be taken into the body by eating food, drinking water, or breathing air. Average dietary intake of nickel in adult persons is 165 µg/day but may reach 900 µg/day in diets rich in cocoa, oatmeal, nuts and soya products.20,21 Only 1 to 5% of ingested nickel is absorbed in the body; remainder is excreted in the feces.

About 5% of the amount ingested is absorbed into bloodstream through intestines, while 20 to 35% of inhaled nickel is absorbed through lungs. Total 68% of nickel reaching in blood is rapidly metabolized in liver and rest is excreted in urine, while 2% remains in kidneys with very short biological half-life of 0.2 days (about 5 hours).22 The remaining 30% is evenly distributed to all remaining tissues of body, including the kidneys, liver and clears with a biological half-life of more than 3 years (1,200 days). Measurements of nickel concentrations in plasma from retired nickel refinery workers demonstrated that nickel was retained for several years after termination of nickel exposures.23

Literature supports direct correlation of nickel concentrations in plasma and urine.24-26 Also concentrations of serum nickel are decreased in hepatic cirrhosis, as a consequence of marked hypoalbuminemia. Hepatic toxicity, manifested by microvesicular steatosis, transient diminution of hepatic glutathione concentration, increased activity of serum alkaline phosphatase, develops in nickel chloride treated rats.

Also it has been reported in literature that release of nickel in the first 6 months was higher than at rest of time periods. This could be attributed to maximum appliance degradation in the initial period of treatment. At the same time, nickel is more cathodic in nature which may be the cause of initial release of nickel and chromium. This is in correlation with the present study findings. As the deformation deactivates, though the oral environment remains same, the return of the material more towards normal stage causes gradual decrease in the release of nickel.27

Urine and serum are the body fluids with widest utility as specimens for nickel analysis in biological monitoring programs. Hence, serum was used in the present study to find out possible systemic effects on liver by observing changes in SGOT, SGPT and alkaline phosphatase levels.

Hence, we aimed to find out correlation between salivary and serum levels and further serum sample was used to find out changes in SGOT, SGPT and alkaline phosphatase levels to rule out any hepatic changes.

The results of the present study also showed that there was a statistical difference in SGOT, SGPT and alkaline phosphatase concentrations before and 4 weeks after insertion of different fixed orthodontic appliances, but they remain within normal level.

Natarajan28 evaluated genotoxic effects of fixed appliances on oral mucosal cells and the relationship to nickel and chromium concentrations which was an in vivo study. The author concluded that nickel and chromium alloys of orthodontic appliances emit metal ions in sufficient quantities to induce localized genotoxic effects, but these changes revert on removal of appliances.

Hafez29 described cytotoxicity, genotoxicity, and metal release in patients with fixed orthodontic appliances, which was a longitudinal in vivo study. The author concluded that fixed orthodontic appliances decreased cellular viability, induced DNA damage, and increased the nickel and chromium contents of the buccal mucosa cells. Compared to control group, these changes were not evident at 6 months, possibly indicating tolerance for or repair of the cells and the DNA.

There are very few studies in the literature showing systemic influences of Ni and Ti in orthodontic patients. Hence, future research should be carried for a longer period of time to study the effects of corrosion processes and mechanical phenomena, such as wear and fatigue on release of nickel and chromium in oral cavity. A larger sample size should also be used to eliminate the variability of results.

**CONCLUSION**

Following conclusions were drawn from the results of present study:

- The results indicate that orthodontic appliances corrode in oral environment and release nickel and chromium. Although, there is initial rise in salivary and serum nickel and chromium levels in 1 week but significantly tapered to permissible blood level in 4 weeks. Hence, this should
be taken care of in patients having allergy to nickel and chromium.

Also, there was significant rise in hepatic enzymes level from baseline to 4 weeks but it was within normal range.

REFERENCES


