Quantitative Assessment of Serum Nickel and Chromium Levels in Orthodontic Patients: An in vitro Study

1Rohan Rai, 2V Surendra Shetty, 3Rajiv Ahluwalia, 4Sujitha George

ABSTRACT

Objective: The aim of this study was to analyze quantitatively the serum fluid levels of nickel and chromium in orthodontically treated patients due to biodegradation of the appliance over a period of time using an inductively coupled plasma emission spectrometry (ICP).

Materials and methods: This cross-sectional study included 24 patients undergoing Begg’s fixed appliance mechanotherapy between the age group of 12 to 18 years, with 12 males and 12 females consuming mixed diet. Six nonorthodontically treated individual were considered as controls. The experimental group consisted of patients wearing the appliance from 6 months, 12 months, 18 months and 24 months; with each group consisting of 6 samples: Three males and three females.

Results: Results obtained indicated that although nickel level in the serum was significant initially in the samples when compared to the controls, there was a gradual decrease of serum nickel level when the appliance was present for a longer duration. However, serum chromium levels showed no significant changes with time.

Keywords: Inductively coupled plasma emission spectrometry (ICP), Trace elements, Nickel and chromium levels.


INTRODUCTION

Stainless steel finds a wide range of application in orthodontics because of its property to resist tarnish and corrosion; it still cannot withstand the ionic, thermal, microbiologic and enzymatic environment of oral cavity. The austenitic stainless steel commonly used for orthodontic bands and wires consists of 18% chromium and 8% nickel, 71% iron, 0.2% carbon and other metals like titanium, manganese, silicon, sulfur, molybdenum which may subsequently absorbed systemically.

Samir Bishara and Barredt pointed out the possibility of having elevated levels of nickel in the blood. Their study using whole blood could not show conclusive results, as the blood proteins interfered with the serum chromium levels. In order to combat this problem they suggested the use of serum instead of whole blood, as it was impossible to extract the serum component once the blood, was frozen. Therefore the present study was undertaken using inductively coupled plasma emission spectrometry.

MATERIALS AND METHODS

This cross-sectional study included 24 patients undergoing Begg’s fixed appliance mechanotherapy who were considered as experimental samples. Six nonorthodontically treated individual were considered as controls. All the samples were between the age group of 12 to 18 years consisting equal number of males and females, consuming mixed diet.

The experimental groups were divided equally according to duration of appliance wear, as patients wearing the appliance since 6, 12, 18 and 24 months. Each group consisted of six experimental samples, three males and three females.

All the samples, both the experimental and control were healthy individuals with no previous history of allergy to nickel and chromium.

Six milliliters of venous blood was collected from samples using Sarstedt monovette EDTA-K tubes. The serum after clotting was immediately centrifuged at 3000 rpm, 15 to 20 minutes at room temperature (Fig. 1). In the 6 ml of blood taken...
a total amount of 2 ml of serum was obtained per sample. The available serum was divided for separate analysis of nickel and chromium (Fig. 2). All the serum samples were frozen and taken to the laboratory overnight with precaution, for the analysis with inductively coupled plasma—emission spectrometry (ICP) (Figs 3 and 4).

The ICP used for this study is described below:

**Equipment:** Labtam, 8410 Australia was used in this study. High voltage and radio frequency generator was used to ionize argon gas which is in turn produces high temperatures. The various metal ions in solution phase are introduced into plasma whereby they get erected. These elements while returning to normal state emit energy radiations. These energy radiations are separated first using grating, prism, etc. and passed individually through photo multiplier to convert high radiation into electrical current. The resultant electrical current is measured using computer along with other appropriate devices.

**Method of estimation:** For estimating nickel and chromium in blood serum, the samples were subjected to acid digestion to remove organic matter. Nickel and chromium present, if any were brought into solution phase and made up to a known volume. The solution was exposed to plasma having high temperatures with precalibrated data of standard nickel and chromium solution, the quantitative estimation for same elements was carried out.

The excitation wavelength chosen for measurement are as follows:
- Nickel—231.04 nm
- Chromium—267.813 nm
Sample introduction: The nickel and chromium levels in the serum sample is very less. The detection limit of the ICP in our study had a detectable range only up to 10 ppb, it was decided to do the standard addition method to increase the levels of nickel and chromium in the serum samples. The experimental solution was introduced to the ICP and result was obtained by the data generated by a computer.

The result so obtained was calculated by subtracting the standard solution value from the net reading. The value thus obtained showed the net nickel and chromium levels in serum samples (Figs 5 and 6).

RESULTS

Results of Nickel Sample

The normal values for blood level nickel have been reported to be between (2.4 ± 0.5 ppb, 4.8 ± 1.3 ppb, 6.0 ± 1.0 ppb, 30 ± 19 ppb).²

Findings

The data obtained from the Table 1 indicated that of the 24 samples analyzed, eight (33.3%) showed reading below the control values (0.4 ppb), eight (33.3%) measured value at 0.5 ppb which being the lower limit of the controls. Five (20.8%) rate between 0.5 to 0.9 ppb within the normal range, the remaining two (12.6%) showed reading of 0.8 ppb. No significant difference between males and females was noted.

Results of the Chromium Samples

The normal values for blood level chromium are reported to be between (0.371 ppb, 1.4 ± 0 ppb).²

Findings

The values in Table 3 indicate that of the 24 samples analyzed for chromium serum levels, six (25%) measured 0.3 ppb at the lower limit of the control, sixteen (66.6%) showed values between 0.3 to 0.5 ppb (within the control range) and the remaining two (8.3%) showed more than 0.5 ppb, i.e. above the higher limit of the control value. No significant difference among the males and females was seen. Results obtained from Table 4 indicate that there was no significant change in serum chromium levels when time A was compared with B, C and D. Thus, indicating that serum chromium level remain unaltered in patients who are orthodontically treated.

DISCUSSION

Metallic alloys are routinely used in dentistry in a variety of applications including fabrication of prosthetic appliances, orthodontic appliances, and indirect restoration of teeth. The metals most commonly used include gold, nickel, cobalt chromium, tin, aluminum, titanium, iron mercury, etc. These metals have been used successfully in dentistry for a number of years.

Almost all the metallic alloys used routinely in dentistry have a definite composition of their own; usually varying from one another. However, most of the metallic alloy have one thing common among them, the occurrence of trace elements. The so-called ‘trace elements’ derive their name due to their presence in very small amounts relative to the amounts of the main constituents and because of this there are difficulties in recognizing them in low concentration. Earlier these elements were also called as ‘oligometals’. Trace elements also play a vital role as catalysts in various enzymatic reactions in living tissues.² They may also be an important part of proteins.

Nickel is one of the most common causes of allergic dermatitis especially in women. It can lead to nickel dermatitis in the scalp, eyelids, earlobes, lips, necks, etc. Dimethylglyoxime spot test discovered by Fleigl⁴ can be used to determine hypersensitivity to nickel. Similarly chromium can cause chromate dermatitis. Chromates may have a corrosive necrotizing effect on living tissues with the formation of ulcers or ‘chrome-holes’. Chrome ulcers generally occur on exposed areas of the body, chiefly on the hands, forearms and feet. Patch testing with potassium dichromate could be performed.

Various studies have been carried out to establish the levels of nickel and chromium in blood, saliva and urine samples. Kerosou et al⁵ investigated nickel and chromium concentrations in saliva of patients with different types of fixed appliances.
Table 1: Serum levels of nickel in 24 samples are given below (concentrations in parts per billion)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Time 1</th>
<th>Time 2</th>
<th>Time 3</th>
<th>Time 4</th>
<th>Control (x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15.80</td>
<td>0.80</td>
<td>0.48</td>
<td>0.48</td>
<td>0.46</td>
</tr>
<tr>
<td>B</td>
<td>15.84</td>
<td>0.84</td>
<td>0.50</td>
<td>0.50</td>
<td>0.48</td>
</tr>
<tr>
<td>C</td>
<td>15.60</td>
<td>0.60</td>
<td>0.80</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>D</td>
<td>15.60</td>
<td>0.60</td>
<td>0.40</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>E</td>
<td>15.67</td>
<td>0.67</td>
<td>0.50</td>
<td>0.52</td>
<td>0.50</td>
</tr>
<tr>
<td>F</td>
<td>15.53</td>
<td>0.53</td>
<td>0.50</td>
<td>0.50</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Note: Time 1 to 6 months, Time 2 to 12 months, Time 3 to 18 months, Time 4 to 24 months, (A,B,C)—males, (D,E,F)—females

Table 2: Nickel released in different time intervals are compared

<table>
<thead>
<tr>
<th></th>
<th>Time 1 vs Time 2</th>
<th>Time 1 vs Time 3</th>
<th>Time 1 vs Time 4</th>
<th>Time 2 vs Time 3</th>
<th>Time 2 vs Time 4</th>
<th>Time 3 vs Time 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_{0.05} = 2.394$</td>
<td>$t_{0.05} = 2.981$</td>
<td>$t_{0.05} = 3.148$</td>
<td>$t_{0.05} = 0.753$</td>
<td>$t_{0.05} = 0.586$</td>
<td>$t_{0.05} = 0.167$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Significant</td>
<td>Significant</td>
<td>Significant</td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Note: Above values show time 1 when compared to time 2, 3 and 4 indicates significant increase of serum nickel level in the first 6 months of appliance wear

Table 3: Serum levels of chromium in 24 samples are given below (concentration in parts per billion—ppb)

<table>
<thead>
<tr>
<th></th>
<th>Time A</th>
<th>Time B</th>
<th>Time C</th>
<th>Time D</th>
<th>Control (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a¹</td>
<td>15.40</td>
<td>0.40</td>
<td>0.41</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>b¹</td>
<td>15.38</td>
<td>0.38</td>
<td>0.38</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>c¹</td>
<td>15.40</td>
<td>0.40</td>
<td>0.42</td>
<td>0.38</td>
<td>0.41</td>
</tr>
<tr>
<td>d¹</td>
<td>15.39</td>
<td>0.39</td>
<td>0.44</td>
<td>0.44</td>
<td>0.41</td>
</tr>
<tr>
<td>e¹</td>
<td>15.75</td>
<td>0.75</td>
<td>0.44</td>
<td>0.42</td>
<td>0.42</td>
</tr>
<tr>
<td>f¹</td>
<td>15.52</td>
<td>0.52</td>
<td>0.42</td>
<td>0.42</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Time A—6 months, time B—12 months, time C—18 months, time D—24 months, (a¹,b¹,c¹)—males, (d¹,e¹,f¹)—females

Note: The values obtained in Table 4 is by subtracting the chromium content of the standard solution used (15 ppb of chromium)

Further comparison was made between time intervals to analyze, if active chromium absorption took place

Table 4: Chromium released in different time intervals are compared

<table>
<thead>
<tr>
<th></th>
<th>Time A vs Time B</th>
<th>Time A vs Time C</th>
<th>Time A vs Time D</th>
<th>Time B vs Time C</th>
<th>Time B vs Time D</th>
<th>Time C vs Time D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t = 1.281$</td>
<td>$t = 1.537$</td>
<td>$t = 1.537$</td>
<td>$t = 0.256$</td>
<td>$t = 0.256$</td>
<td>$t = 0$</td>
</tr>
<tr>
<td></td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
<td>$p &gt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

The results suggested that nickel and chromium concentrations of saliva were not significantly affected by fixed orthodontic appliances during the first month of treatment which is in agreement with the present study. Hwang measured the metal released from the fixed orthodontic appliances and concluded that there was a decrease in metal released as immersion time increased.

Studies were conducted by Park and Shearer, Samir E Bishara et al and Bhupathi Reddy et al. They studied in vitro the corrosion rates of standard orthodontic appliance consisting of bands, brackets and either stainless steel or nickel-titanium archwires in a prepared artificial saliva. The results indicated that the orthodontic appliance increased nickel level initially between 7 to 14 days and the rate of nickel release leveled off. The chromium release was maximum about the 14th to 16th day and the release leveled off gradually. Although the study used artificial saliva was prepared to produce a realistic picture of the human saliva, it would not produce the natural oral environment for the degradation of the orthodontic appliances.

Samir E Bishara et al further continued their study to check for changes in the blood level of chromium and nickel. They found that there was no significant or consistent increase in nickel blood levels in the initial period of the treatment. However, the levels of chromium could not be determined...
because it required only the serum and not the whole blood. It was thought that chromium is selectively bound to the red blood cells, so further the investigation concluded that studies should use serum.

It was found that the release of nickel in the first 6 months was higher than at rest of the time periods. This could be attributed due to the maximum appliance degradation in the initial period of the treatment. The release of an element like nickel is higher initially as the force increases the degradation of the appliance. At the same time, nickel is more cathodic in nature which may be the cause of initial higher release of nickel than chromium. Although studies by Faccioni7 et al concluded that Nickel and Cobalt do have significant biologic effect on the oral mucosa; studies by Samir E Bishara2 et al showed that Nickel release decreases gradually with degradation of appliance over a period of time. The present study is in agreement with Samir E Bishara2 et al findings. As the deformation deactivates, though the oral environment remains the same, the return of the material more towards the normal stage causes gradual decrease in the release of nickel (Table 2).

CONCLUSION

From the results obtained the following can be concluded:

The results obtained indicate that orthodontic appliances used in their ‘as-received’ condition corrode in the oral environment releasing both nickel and chromium, but in amounts significantly below the average dietary intake. Although an initial rise in serum levels of nickel, it later tapered to the permissible blood level for nickel. Also, insignificant chromium systemic absorption takes place during orthodontic appliance wear.

REFERENCES