Comparative Evaluation Of Human Saliva For The Presence Of Nickel And Chromium Released From Orthodontic Appliances Due To Radio Frequency Electromagnetic Radiation (Cell Phone Usage) – A Clinical Study

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Abstract

Introduction: Due to the enormous increase in their usage throughout the world, the effect of cell phone radiation on human health has been an area of recent interest. Orthodontic appliances stays in mouth for long durations, hence the release of potentially harmful metal ions like nickel, chromium etc. is of high importance. This study is done to evaluate the amount of Nickel and Chromium ions released from orthodontic braces during initial leveling and aligning and to estimate if any, change in the release of metal ions when exposed to Radio Frequency Electro Magnetic Radiation (Cell phone usage)

Method: Subjects were divided into two groups, the control group patients were instructed not to use the mobile phone for one month. Experimental group patients were allowed to normally use their mobile phones. Saliva samples were evaluated for the presence and amount of metal ions using mass spectroscopy instrument (ICP-MS)

Results: Cell phone usage leads to an increase in the release of nickel and chromium ions into the saliva of patients with fixed orthodontic appliances. There exists a positive correlation between the duration of mobile phone use and the nickel ion release from fixed orthodontic appliances.
Conclusion: There is an increase in release of nickel and Chromium from fixed orthodontic appliances when exposed to radio frequency electromagnetic fields emitted by mobile phones. Use of newer polycrystalline ceramic brackets in patients who tend to use cellphones more, or creating awareness to reduce the cell phone usage while undergoing orthodontic treatment would be better alternatives.

Keywords: Nickel, Chromium, Cell Phone, Toxicity

Introduction
Cell phones have currently become an indispensable part of communication worldwide and have brought drastic changes in our lifestyle by offering advantages of multiple applications and convenience. In the past two decades, the mobile phone subscriptions have grown exponentially worldwide with over 5.6 billion mobile phone subscription. According to TRAI in its telecom statistics report, India has 1,026.37 million active mobile users on 2G, 3G and 4G networks – in 2018. In an era of transit from 4G to 5G it is important to assess the potential harmful effects of radio frequency electromagnetic radiation that surrounds us. We are all living in an envelop of radiation amount of which is directly proportional to radiation hazards on human health, which lacks scientific evidence, thus provoke a research question.2

A typical metallic fixed orthodontic appliance system contains 8-12% Nickel (Ni), 17-22% chromium (Cr) and various proportions of manganese, copper, titanium and iron. Various factors such as salivary conditions, temperature, pH variations, mechanical loads, microbiological and enzymatic activity, physical and chemical properties of food and oral health conditions provide an environment for the corrosion of orthodontic appliances which lead to the release of metal ions into the saliva.

First in line Ni complexes, have been found to be carcinogenic, allergenic and mutating substances even at nontoxic concentrations. It has been also a highlighted metal for hypersensitivity.5,6 Second is chromium, which induces oxidative stress, DNA damage, apoptotic cell death, altered gene expression, increased production of reactive oxygen species (ROS) and lipid peroxidation, enhanced excretion of urinary lipid metabolites, DNA fragmentation and apoptotic cell death in both in vitro and in vivo models as demonstrated in several studies.7

Researches have confirmed that mobile phones cause significant increases in salivary oxidative stress, salivary flow and a decrease in amylase activity 13,14 A positive dose dependent response trend between parotid gland tumor and mobile phone usage has also been found10,11 It has been reported that Mobile phone usage has a time-dependent influence on the concentration of nickel in the saliva of patients with orthodontic appliances.1 Therefore this study was done to evaluate the amount of metal ions released from orthodontic braces due to radio frequency electromagnetic radiations emitted from mobile phones.

Methods
Study design and setting: The interventional follow up study was done in the Department of Orthodontics and Dentofacial Orthopedics, Coorg Institute of Dental Sciences, Coorg, Karnataka

30 subjects who were undergoing orthodontic treatment with fixed appliance mechanotherapy, were selected according to the inclusion and exclusion criteria for the study.

Study population included Patients with permanent dentition aged between 15 and 20 years of both genders.

Study excluded Patients with systemic illness or medication intake, Patients who smoke or consume alcohol, Patients with metallic restorations in mouth or...
patients with poor oral health or open untreated caries lesions or gingival inflammation.

The study procedure was then explained, and an informed consent was taken from the patient &/or the parents. All patients were bonded with Koden Silver series MBT 022 slot stainless steel brackets, had either 0.014 nickel titanium or 0.016 nickel titanium arch wires or transpalatal and lingual arches fabricated in 19-gauge stainless steel wire when deemed necessary. Saliva samples were collected from all the patients immediately before bonding.

Patients were divided into two groups,

**Group A**: Consisted of 15 patients who were undergoing treatment with MBT prescription stainless steel brackets and were asked not to use mobile phones for the next one month. Patients of this group were selected such that they are less likely to use mobile phones, this included school going children of the selected age group and deaf persons who does not own a mobile phone.

**Group B**: Consisted of 15 patients who were undergoing treatment with MBT prescription stainless steel brackets and asked to record the total mobile phone usage time for the next one month as mentioned below

Duration of mobile phone usage was estimated by installing and checking an application called ‘App Usage’ Values were recorded on the day of bonding and after one month, patients were also asked to neither share their mobile phones nor use others phones for calling purposes

At the end of one month the final saliva samples were collected to check the nickel and chromium levels.

Group A samples were considered the control group and Group B samples were considered the experimental group.

The patients were instructed to abstain from eating seafood and canned food, drinking hot tea and coffee and rinsing their mouth with fluoridated products during the study period. They were also advised to use their mobile phones only for making and receiving calls during the study.

**Storage and transport of saliva samples**

All the samples were stored at -20° C and were then transported to the laboratory in a coolant box. Samples were analyzed using inductively coupled plasma-mass spectrometry to measure the amount of metal ions.

**Statistical Analysis**

The data were analyzed using SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp. Released 2013). The descriptive statistics included mean, standard deviation and percentages. The inferential statistics included Independent t test, paired t test and Pearson’s Correlation for the comparisons. The level of significance was set at 0.05 at 95% Confidence Interval.

**Results**

Amount of nickel and chromium ions present in all the samples were assessed, results showed a significant increase in the amount of both nickel and chromium ions between the experimental and control group.
Table 1: Comparison of Mean measured concentrations of nickel and chromium in saliva in control and experimental group before and 1 month after bonding.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard deviation</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nickel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.80000</td>
<td>.632456</td>
<td>-9.000</td>
<td>0.000 (H.S)</td>
</tr>
<tr>
<td>Post</td>
<td>5.00000</td>
<td>.666667</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.10000</td>
<td>1.370320</td>
<td>-10.585</td>
<td>0.000 (H.S)</td>
</tr>
<tr>
<td>Post</td>
<td>6.00000</td>
<td>1.563472</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chromium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>13.40000</td>
<td>4.903513</td>
<td>-4.025</td>
<td>0.003 (H.S)</td>
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<tr>
<td>Post</td>
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<td>4.629615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>11.00000</td>
<td>3.741657</td>
<td>-3.273</td>
<td>0.010 (S)</td>
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<tr>
<td>Post</td>
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<td>4.402020</td>
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</table>

HS- Highly significant
NS-Non Significant
S- Significant

Graph 1: Comparison of Mean measured concentrations of nickel ions (ng/ml) in saliva in control and experimental group before and 1 month after bonding.
Graph 2: Comparison of Mean measured concentrations of chromium ions (ng/ml) in saliva in control and experimental group before and 1 month after bonding.

Mean value of nickel in saliva, in patients who were asked not to use their cell phones, before bonding the brackets was found to be 3.8 ng/ml while Chromium showed a value of 13.4 ng/ml.

At the end of one month the mean value of nickel and chromium in their saliva was found to be 5 ng/ml. and 14.9 ng/ml. Mean value of nickel in saliva in cellphone using group patients, before bonding was found to be 4.1 ng/ml while chromium was found to be 11ng/ml.

The mean value of nickel at the end of one month, for them was found to be 6ng/ml. and chromium 13.4 ng/ml. (Table 1)

Table 2: Comparison of Mean release (difference of Post-Pre) of nickel and chromium between the experimental and control group.

<table>
<thead>
<tr>
<th></th>
<th>Post-pre difference</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel</td>
<td>Control</td>
<td>1.2000</td>
<td>.42164</td>
<td>-3.130</td>
<td>0.006 (H.S)</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>1.9000</td>
<td>.56765</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>Control</td>
<td>1.5000</td>
<td>1.17851</td>
<td>-1.094</td>
<td>0.012 (S)</td>
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<tr>
<td></td>
<td>Experimental</td>
<td>2.4000</td>
<td>2.31900</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean of nickel release at the end of one month was higher than the pre bonding samples and the change was found to be highly significant in both the groups, whereas for chromium the change was highly significant in Group A where as in Group B it was found to be significant (Table 2)

Table 3: Correlation of Nickel and Chromium ions released with cellphone usage in experimental group

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Pearsons correlation coefficient</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Nickel</td>
<td>Time usage</td>
<td>6.3030</td>
<td>1.97273</td>
<td>0.919</td>
</tr>
<tr>
<td></td>
<td>Mean difference of release from pre to post</td>
<td>1.9000</td>
<td>.56765</td>
<td>0.037 (S)</td>
</tr>
</tbody>
</table>
Pearson’s rank correlation test showed that a positive correlation was found between the duration of mobile phone use and the nickel and chromium release. This correlation was statistically significant in one month of this study. (Table 4)

**Discussion**

Fixed orthodontic treatment generally lasts about 2 years, so it is important to estimate any release from the fixed orthodontic appliances as it becomes a chronic exposure. The nickel ions released may be deposited in different tissues at different levels 14, and might serve as a chronic risk factor to physical health and may adversely affect the biological functions of the whole organism. The measure of the rate at which body tissues absorb radiofrequency electromagnetic radiation is called as the Specific Absorption Rate or SAR. The SAR value of a phone depends on the model of the handset. Different countries have placed radiation exposure limits on the maximum level of SAR for modern handsets. In the US, the Federal Communication Commission has set a maximum SAR limit of 1.6 W/kg, while in Europe this limit is 2W/kg. 10 The Government of India has made it mandatory that all new designs of mobile handsets shall comply with the SAR values of 1.6W/kg averaged over 1g of human tissue. 40

The parotid gland, being located 4-10mm deep under the skin at the anterior border of the external ear is more vulnerable to be influenced by exposure to the RFER on the side of the head where the mobile phone is held. 1 There is significant increase in the salivary flow rate, salivary gland volume, salivary oxidative stress, total proteins and albumin and a decrease in the salivary amylase activity with mobile phone use. 10 In the aggressive oral environment flushed with saliva, the salivary ions and nonelectrolytes constantly flow against the wires, brackets and bands. Corrosion process occurs from the progressive dissolution of the surface film of the metallic orthodontic appliances and the release of metal ions directly into the saliva. The galvanic differences between the metal alloys and the physiological fluids in the mouth can also trigger electrochemical reactions which lead to corrosion. 34

The increased salivary flow rate has a diluting effect on the macromolecules and ions in saliva. 21. The concentration of salivary calcium, magnesium and phosphorus were lower in mobile phone users. 10 The greater flow rate, in addition to the lower concentration of ionic components in saliva, causes more nickel and other heavy metals leaching from the orthodontic appliances. 34

Our results show that the mean level of nickel increased from 3.8 ng/ml to 5 ng/ml in the saliva of the patients in group A during the initial one month of leveling and aligning, Confirming increase in Ni and Chromium which is statistically significant. This was a mean percentage increase of 24 % (P = 0.043). Whereas the mean level of chromium increased from 13.4 ng/ml to 14.9 ng/ml in the saliva of the patients in group A. This was a mean percentage increase of 11.89 % (P = 0.049)

In group B patients, the mean level of salivary nickel increased from 4.1 ng/ml to 6 ng/ml , and chromium increased from 11ng/ml to 13.4 ng/ml during the one month when these patients had used mobile phone placed directly against the ear for a mean duration of 6.3 hours. This was a mean percentage increase of 32.69 and 16.79 respectively for nickel and chromium and it was found to be statistically significant. A positive correlation was
found between the duration of mobile phone use and the nickel and chromium release. This correlation was statistically significant in one month of this study. Which supports the results of Saghiri et al\textsuperscript{1} and Nanjannawar et al\textsuperscript{21}.

All the study participants used mobile phones with SAR values below 1.6 W/kg. An increase in temperature reduces the ability of a material to repassivate and thereby makes it vulnerable to corrosion. Temperature fluctuations can also affect the nature of the environment by changing the solubility of a constituent that can affect the corrosion behaviour of the material.\textsuperscript{42}

The patients were hence instructed to avoid taking hot tea and coffee and were asked not to smoke during the study. Corrosion resistance of the arch wires is found to decrease in direct proportion to the increase in the fluoride concentration in the mouth.\textsuperscript{43} The participants were therefore advised to avoid using fluoridated toothpastes and mouthwashes during the study period. Saliva collected by chewing paraffin or gum has a different organic composition compared to Unstimulated saliva. In the resting state, about two-third of the volume of whole saliva is produced by the submandibular glands. When the salivary glands are stimulated, the parotids can account for at least half the whole saliva. Thus, stimulation could change the protein composition of saliva. Since metals rapidly combines with proteins, a change in protein composition of saliva could also affect metal concentration. This might possibly induce a false negative result. Therefore Unstimulated saliva was collected from the participants.

All saliva samples were stored in a freezer at \textdegree{20}\textsuperscript{0} C to prevent any opportunity for bacterial growth until they were processed and subjected to metal ion content analysis in the laboratory using inductively coupled plasma mass spectrometry.

Inductively coupled plasma mass spectrometry can detect metals and several non-metals at concentrations as low as one part in 1015 (part per quadrillion, ppq) on non-interfered low background isotopes. The sample is ionized with inductively coupled plasma and then the ions are separated and quantified using a mass spectrometer. Compared to atomic absorption spectroscopy and inductively coupled plasma atomic emission spectroscopy, which were used in many previous studies, ICP-MS has greater speed, precision, and sensitivity.\textsuperscript{28}

In the present study it was found that the release of nickel and other heavy metals from orthodontic braces increased when exposed to radio frequency electromagnetic radiation.

Also it was found that mobile phone usage has a time dependent influence on the concentration of nickel in the saliva of orthodontic patients.\textsuperscript{1} In today’s world of portable connectivity and multifunctional services, it is impractical and unrealistic to advise orthodontic patients to reduce or refrain from mobile phone use. It is thus imperative to find a way to reduce the effect of mobile phone radiation on orthodontic appliances and the consequent release of nickel ions.

Use of ceramic brackets would be a more realistic alternative as they raise no potential harm of metal release. With the evolution of newer monocrystalline brackets, ceramic brackets can be used as effectively as metal brackets

Conclusion

Results of the study conclude that mobile phone usage leads to an increase in the release of nickel and chromium ions into saliva of patients with fixed orthodontic appliances.

There is a positive correlation between the release of these metal ions with the duration of cellphone usage. As orthodontic appliances stays in patient’s mouth for longer
duration, replacement of metal with the newer monocrystalline ceramic braces would be a better alternative.

But the values of nickel and chromium shows that they are present only in trace amounts. Therefore it is not an alarming situation to use the metal braces for orthodontic treatment.

This interventional study has elucidated the increase in release of nickel and other heavy metal ions from fixed orthodontic appliances when exposed to radio frequency electromagnetic fields emitted by mobile phones.

**Abbreviations**

Ni - Nickel
Cr - Chromium
SAR - Specific Absorption Rate
REFR- Radio Frequency Electromagnetic Radiation

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