Assessing the relationship between Tooth Heavy Metal Deposition and Periodontal Disease in Smokers and Non-Smokers

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ABSTRACT

Objective: Heavy metals threaten life by accumulating in the body via various sources as water, air and foods. Smoking is one of the important factors that causes this problem. Despite there is an abundant number of studies showing the detrimental effects of smoking on periodontal health, the mechanisms that cause these harmful effects is not clearly known yet. The main aim of our study is to discover whether heavy metal deposition on the tooth surface is related to the disease-causing potential of smoking.

Materials and Method: Total of 80 individuals consist of 43 women and 37 men were included in this study. The participants were divided into 4 groups of 20 individuals each according to the results from clinical examination and anamnesis. Plaque index, gingival index, pocket depth, bleeding on probing and clinical attachment level were recorded clinically. The teeth indicated for extraction were collected. Cd, Pb, Ni, Cr and Fe depositions on teeth were measured by ICP-OES device. Kolmogorov-Smirnov, Student T, Mann Whitney U, One way ANOVA, Kruskal Wallis, Ki-Kare, Pearson, and Spearman tests were performed for the statistical analysis.

Results: Smoking increases the accumulation of heavy metals such as Cd, Ni, Cr, and Pb. The Pb level was higher in both the smoking group and the periodontitis group, compared with the control group. Fe levels were found high in the non-smoking healthy group. Cr and Fe levels were found higher in women while Cd level was higher in men. Positive correlations were found between Pb and plaque index, gingival index, pocket depth and bleeding on probing; and also between Ni and plaque index. (p=0.000, p=0.009, p=0.025, p=0.011, p=0.019)

Conclusion: In conclusion, our study explored the connection between heavy metal deposition on tooth surfaces and the disease-causing potential of smoking. Smoking has been identified as a significant factor in the increased accumulation of heavy metals, including Cd, Ni, Cr, and Pb. The higher Pb levels seen in both the smoking and periodontitis groups, compared with the control group, suggest a potential link between Pb accumulation and periodontal health. Additionally, differences based on gender were observed, with women showing higher Cr and Fe levels, while Cd levels were more elevated in men. The positive correlations between Pb and various periodontal indices, along with the correlation between Ni and plaque index, shed light on the potential influence of heavy metal deposition on periodontal health. While our findings enhance our understanding of the interplay between smoking, heavy metal deposition, and oral health, further research is needed to fully comprehend the underlying mechanisms. Such insights could lead to interventions aimed at minimizing the adverse effects of heavy metal accumulation on oral health.

Keywords: Heavy metal, deposition, Icp-oes, Periodontitis, Smoking

INTRODUCTION

According to the classification system accepted at the 2017 World Workshop, periodontitis is a destructive disease with features such as gingival inflammation, clinical attachment loss, alveolar bone loss, periodontal pocket formation, increased mobility, and tooth loss (1). Pathogenic bacteria in the plaque and the toxins produced by them stimulate the host defence and as a result, tissue destruction occurs. In case of progression of the disease, clinical attachment loss, increase in probing depth and alveolar bone destruction occur (2).
Although bacteria and host defence system play a primary role in the onset and progression of periodontal disease, many local, and environmental, systemic and genetic factors are effective in the development, severity and control of the disease. These factors increase the individual's probability of getting the disease (3). Cigarettes contain thousands of harmful chemicals in gaseous and particulate form. The gas phase contains many toxic compounds such as ammonia, carbon monoxide, formaldehyde, hydrogen dioxide, nitrogen oxide. The particle phase contains compounds such as addictive nicotine and carcinogenic tar (4). It has been found that smoking is associated with a wide range of diseases such as lung cancer, mouth, larynx, esophagus, pancreas, uterus and bladder (5). Besides many systemic effects of smoking, it also has serious negative effects on oral health and periodontal diseases (6). It is known that smoking is an effective environmental risk factor in the formation and development of periodontal diseases. When smokers and non-smokers are compared, it has been shown that the prevalence and severity of periodontal disease increase and the disease onsets earlier (6, 7). Smoking suppresses the clinical findings of periodontal inflammation such as gingival bleeding, redness and edema. The increase in bleeding on probing following cessation of smoking suggests that smoking suppresses the inflammatory response (8). Metals with more than 5 g/cm³ density are considered heavy metals. While exposure to heavy metals is increasing day by day due to both the increase in industrialization and the spread of wastes to the environment, it threatens life by causing accumulations in the body through air, water and food. For this reason, studies that examine the toxic and undesirable effects of heavy metals are emphasized. Heavy metals show their effects acutely or chronically (9). Heavy metals, which are in charge of biological events in the body and must be present in certain amounts, are classified as essential and have toxic effects after a certain dose. Non-essential heavy metals show toxic effects even in trace amounts and can cause diseases (10).

Cadmium is usually found together with zinc, copper and lead mines (11). Bone tissue is an important target organ in cadmium toxicity and cadmium stored in bones can impair bone mineralization. Cadmium has various effects on bone tissue. The first effect is that it stimulates the formation of oxidative stress and causes damage to the bone tissue. The second effect is to increase matrix destruction by directly stimulating osteoclasts (12-15).

Lead, which is frequently used in industry, has very harmful properties for living things. Organic and inorganic forms are available. While inorganic lead is found as particles in the atmosphere, organic lead mostly mixes with foodstuffs and drinking water. Therefore, organic lead is more dangerous for human health. (16, 17). Chronic lead accumulation affects bones in two main ways. It has been reported that lead deposition directly in bone cells impairs bone mineralization and reduces bone production by affecting osteoblast functions (18-21).

Nickel is used in iron production, coins, batteries and a wide variety of industries (22). In vitro studies on nickel have shown that high Ni concentrations inhibit bone mineralization by suppressing alkaline phosphatase activity and have a cytotoxic effect that induces cell apoptosis of osteocytes in culture (23, 24).

The use of chromium in the industrial field is wide (25, 26). Indeed, research has demonstrated that osteoblasts absorb chromium through membrane transporters, leading to elevated levels of reactive oxygen species, oxidative stress, and DNA damage. In addition, it was found to decrease ALP activity, mineralization and osteoclast number in cultures.(27-29)

Iron is a very important mineral for human body. Its most important task in the body is to carry oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. Iron also strengthens the immune system and has important roles in electron transfer, cell respiration, cell proliferation and differentiation. It has been suggested that iron plays a role in both enamel development and mechanical properties of enamel (30).

To our knowledge, there are limited studies on heavy metal accumulation in oral tissues of smoking and its effect on periodontal tissues. Within the scope of our study, the presence of heavy metal accumulation due to smoking in periodontal tissues and its possible disease-causing effect will be evaluated.

MATERIAL and METHODS

The study protocol was approved by the Recep Tayyip Erdoğan University Faculty of Dentistry Ethics Committee with protocol number 2019/101. Before the study, the individuals included in the study were informed about the purpose of the study and the application to be made, and the informed consent form was read and signed. A total of 80 individuals, 43 female and 37 male, aged between 18-67 were included in our study. According to clinical examinations and medical anamnesis, individuals were divided into four groups with 20 samples in each group.

GROUP A: Periodontitis, smokers

GROUP B: Periodontally healthy, smokers

GROUP C: Periodontitis, non-smokers

GROUP D: Periodontally healthy, non-smoking individuals

Patients with any amalgam, cast restorations, brackets, and those likely to be exposed to heavy metals due to their occupation were not included in the study. The criteria for including smoking patients required a history of smoking ≥ 10 cigarettes daily over the past 5 years, while non-smokers were defined as individuals who had never smoked throughout their lives. Pack year was calculated by dividing the number of cigarettes smoked daily by 20 and multiplying the result by the number of years in which the cigarette was smoked (31). While evaluating the periodontal health of the individuals participating in the study, 2017 classification criteria of American Academy of Periodontology and the European Federation were used.

Plaque index (PI), gingival index (GI), bleeding on probing (BOP), probable pocket depth (PD) and clinical attachment level (CAS) were measured from patients who applied to the clinic. All clinical measurements were taken by the same investigator using William's probe, from the mesio-buccal/labial, mid-buccal/labial, disto-buccal/labial, mesio-palatinal/lingual, mid-palatinal/lingual, disto-palatatal/lingual surfaces of the teeth.
Collection of Dental Samples

Eighty unfilled permanent tooth specimens from different age and gender groups that had to be extracted for periodontal, orthodontic, etc. reasons were collected in plastic containers with screw caps and stored in formaldehyde until the day of analysis.(32)

Preparation of Samples by Microwave Digestion

Before processing, all containers were soaked in 20% nitric acid for 24 hours and then rinsed with distilled water. Prior to analysis, the tooth samples underwent organic matter removal using a toothbrush. The process of organic matter removal from the teeth involved immersing each sample in a 10% hypochlorous acid solution (25 ml) for 24 hours, followed by rinsing with distilled water. After rinsing, all paintings were left to dry in an oven at 100 °C for 24 hours (32). Afterwards, the dental samples were powdered in a mortar and dried at 85 °C. (33) 0.3 g of the dried dental samples were weighed and placed in microwave digestion system containers. Microwave decomposition was performed by adding 8 ml of HNO₃ (nitric acid) and 2 ml of HCl (hydrochloric acid) on the samples (34). After the process, the solutions were cooled and poured into 50 ml volumetric bottles and filtered with the help of a filter. After filtration, the solutions were made up to 25 ml with ultrapure water.

Determination of Heavy Metal Content of Samples by ICP-OES

ICP-OES is a device used for the quantitative determination of many elements. With this device, successful analyzes can be made even at low concentrations. This method, which is used in the study to measure some heavy metal concentrations (Pb, Cd, Fe, Ni and Cr) in tooth samples decomposed in the microwave combustion system, is based on the principle of atomization of metals in the plasma and measuring the emission of plasma light (35). Samples are aerosolized and sent to the plasma. It is evaluated with the calibration curve prepared beforehand in accordance with the standards of metals (36).

Statistical Analysis: SPSS package program was used for statistical evaluation. The suitability of the data for normal distribution was examined with the Kolmogorov-Smirnov Test. Comparisons of numerical variables between two independent groups were made with Student T test when the normal distribution condition was met and Mann Whitney U test when it was not. Comparisons of numerical variables between more than two independent groups were made with One way ANOVA when the condition of normal distribution was met, and Kruskall Wallis test when it was not.

Correlation coefficient and statistical significance were calculated with Pearson test for the relationships between normally distributed variables, and correlation coefficient and statistical significance were calculated with Spearman test for the relationships between non-normally distributed variables. Correlation coefficients were accepted as r ≥ 0.81-1.0 excellent, 0.61-0.80 very good, 0.41-0.60 good, 0.21-0.40 fair, 0.0-0.20 poor. Statistical significance level was accepted as p<0.05

RESULTS

A total of 80 individuals were included in our study and the gender, mean age and smoking data of our patients are shown in Table 1.

Upon comparing the mean ages among the groups, no statistically significant difference was observed (p=0.248). Gender was not distributed normally in the groups (p=0.035). There was no statistically significant difference between the groups in terms of duration of smoking, daily amount smoked and cigarette pack year (p=0.397). A statistical difference was found in terms of smoking year and cigarette pack year (p<0.001, p=0.001).

Mean values of PI, GI, PD, CAL and BOP is shown in Table 2.

In the comparison of Cd values between groups, it was found that Cd values of group A were statistically significantly higher than those of groups C and D (p<0.05). It was observed that Cd values of group B were statistically higher than those of group D (p=0.001).

When Pb values were compared between the groups, it was found that Pb values of group A, B and C were statistically significantly higher than group D (p<0.001).

In the comparison of Cr values between the groups, it was observed that the Ni values of group A were statistically higher than the other groups (p<0.001). Ni values of Group B were statistically significantly higher than Group C (p<0.001).

During the comparison of Cr values among the groups, it was determined that the Cr values of Group A and D were statistically significantly higher than those of Group B and C (p ≤ 0.001).

When comparing the Fe values among the groups, it was observed that the Fe values of Group D were significantly higher than those of the other groups (p < 0.001).

The correlation between the clinical examination data measured in our study (PI, GI, BOP, PD, CAL) and the heavy metal levels measured in the teeth is shown in Table 4.

Table 1. Descriptive data of the patients included in the study

<table>
<thead>
<tr>
<th>Gender (n=80)</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>43</td>
<td>53.8</td>
</tr>
<tr>
<td>Male</td>
<td>37</td>
<td>46.3</td>
</tr>
<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>47.4 ± 8.7</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>Smoking (n=40)</td>
<td>Mean ± SD</td>
<td>Median</td>
</tr>
<tr>
<td>Duration of smoking (years)</td>
<td>17.1 ± 11.0</td>
<td>15</td>
</tr>
<tr>
<td>Amount of smoking (days)</td>
<td>15.7 ± 6.7</td>
<td>15</td>
</tr>
<tr>
<td>Package year</td>
<td>15.1 ± 15.6</td>
<td>9.55</td>
</tr>
</tbody>
</table>
**Table 2. Clinical Measurements of the Teeth**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PI</th>
<th>GI</th>
<th>BOP</th>
<th>PD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Med. (Min-Max)</td>
</tr>
<tr>
<td>A</td>
<td>2.46 ± 0.61</td>
<td>2.03 ± 0.60</td>
<td>0.78 ± 0.32</td>
<td>4.17 ± 1.50</td>
<td>5.71 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>(1-3)</td>
<td>(1-2.5)</td>
<td>(0.25-1)</td>
<td>(2.7-8.3)</td>
<td>(4.66-7.83)</td>
</tr>
<tr>
<td>B</td>
<td>0.38 ± 0.40</td>
<td>0.25 ± 0.38</td>
<td>0.04 ± 0.09</td>
<td>1.95 ± 0.52</td>
<td>5.66 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>(0.25-1.5)</td>
<td>(0.13-1.5)</td>
<td>(0-0.25)</td>
<td>(1.3-1.6)</td>
<td>(7.6-7.66)</td>
</tr>
<tr>
<td>C</td>
<td>2.29 ± 0.48</td>
<td>2.39 ± 0.50</td>
<td>0.90 ± 0.21</td>
<td>4.10 ± 1.65</td>
<td>5.32 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>(2.13-1.5)</td>
<td>(2.5-2.25)</td>
<td>(1-0.25)</td>
<td>(2.4-7.66)</td>
<td>(7.6-7.66)</td>
</tr>
<tr>
<td>D</td>
<td>0.36 ± 0.36</td>
<td>0.39 ± 0.38</td>
<td>0.11 ± 0.19</td>
<td>1.81 ± 0.40</td>
<td>5.17 ± 0.073</td>
</tr>
<tr>
<td></td>
<td>(0.25-0.1)</td>
<td>(0.25-0.1)</td>
<td>(0-0.50)</td>
<td>(1-2.50)</td>
<td>(7.6-7.66)</td>
</tr>
</tbody>
</table>

**Table 3. Comparison of heavy metals between groups**

<table>
<thead>
<tr>
<th>GRUP</th>
<th>Cd</th>
<th>Pb</th>
<th>Ni</th>
<th>Cr</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>Median (Min-Max)</td>
<td>Median (Min-Max)</td>
<td>Median (Min-Max)</td>
<td>Median (Min-Max)</td>
<td>Median (Min-Max)</td>
</tr>
<tr>
<td>A</td>
<td>0.21 ± 0.22</td>
<td>2.43 ± 2.24</td>
<td>3.92 ± 2.16</td>
<td>3.95 ± 0.65</td>
<td>2.62 ± 1.30</td>
</tr>
<tr>
<td></td>
<td>(0.15-0.49)</td>
<td>(2.48-5.85)</td>
<td>(4.87-10.8-27)</td>
<td>(3.93-2.67-5.09)</td>
<td>(2.97-0.4-4.6)</td>
</tr>
<tr>
<td>B</td>
<td>0.07 ± 0.10</td>
<td>1.86 ± 0.77</td>
<td>0.85 ± 0.34</td>
<td>3.12 ± 0.37</td>
<td>3.32 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>(0-0.39)</td>
<td>(2.04-3.17)</td>
<td>(0.89-1.39)</td>
<td>(3.18-2.43-7.8)</td>
<td>(2.99-1.8-7.4)</td>
</tr>
<tr>
<td>C</td>
<td>0.01±0.01</td>
<td>2.14 ± 0.88</td>
<td>0.42 ± 0.32</td>
<td>3.23 ± 0.52</td>
<td>2.99 ± 0.70</td>
</tr>
<tr>
<td></td>
<td>(0-0.10)</td>
<td>(2.23-3.19)</td>
<td>(0.49-0.99)</td>
<td>(3.09-2.59-4.2)</td>
<td>(2.72-2.26-5.7)</td>
</tr>
<tr>
<td>D</td>
<td>0.00 ± 0.00</td>
<td>0.07 ± 0.31</td>
<td>0.55 ± 0.55</td>
<td>4.08 ± 0.56</td>
<td>5.53 ± 1.68</td>
</tr>
<tr>
<td></td>
<td>(0-0)</td>
<td>(0-1.38)</td>
<td>(0-1.48)</td>
<td>(4.17-3.29-4.83)</td>
<td>(5.41-3.07-9.26)</td>
</tr>
</tbody>
</table>

**Table 4. Correlation between heavy metal levels and clinical measurements**

<table>
<thead>
<tr>
<th></th>
<th>PI</th>
<th>GI</th>
<th>BOP</th>
<th>PD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>r</td>
<td>0.172</td>
<td>-0.016</td>
<td>0.004</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.127</td>
<td>0.886</td>
<td>0.969</td>
<td>0.403</td>
</tr>
<tr>
<td>Lean (Pb)</td>
<td>r</td>
<td>0.383</td>
<td>0.292</td>
<td>0.251</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.000</td>
<td>0.09</td>
<td>0.025</td>
<td>0.011</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>r</td>
<td>0.263</td>
<td>-0.029</td>
<td>0.047</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.019</td>
<td>0.801</td>
<td>0.680</td>
<td>0.079</td>
</tr>
<tr>
<td>Cron (Cr)</td>
<td>r</td>
<td>-0.054</td>
<td>0.029</td>
<td>0.002</td>
<td>-0.131</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.632</td>
<td>0.799</td>
<td>0.989</td>
<td>0.248</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>r</td>
<td>-0.392</td>
<td>-0.424</td>
<td>-0.417</td>
<td>-0.358</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In addition to plaque and the host defense system, it is well-established that various risk factors play a significant role in the onset and progression of periodontal diseases. Smoking, among the risk factors affecting the severity of periodontal disease, is accepted as one of the most important preventable risk factors (37). Despite numerous studies demonstrating the adverse impact of smoking on periodontal health, the precise mechanism through which it inflicts detrimental effects on periodontal tissues remains unclear (38). Numerous studies employ various methods, including inductively coupled plasma-atomic emission spectroscopy...
Nickel exposure causes the formation of free radicals in various tissues in both humans and animals, leading to various modifications in DNA bases, lipid peroxidation, and alteration of calcium and sulfhydryl homeostasis (54). In our study, a statistical comparison was conducted between the nickel (Ni) values of the group consisting of smokers with periodontitis and the other groups, revealing significantly higher levels (p < 0.001). Based on this outcome, we hypothesize that the elevated Ni values accumulated in the teeth might contribute to the development of periodontitis. Moreover, a positive correlation was noted between nickel and pocket depth (PD) in the group of non-smokers with no periodontitis. This relationship could be explained by the fact that nickel's allergenic effects can lead to tissue hyperplasia (55).

Optimal Fe levels are essential prerequisites for periodontal health, and shifts in any direction can produce detrimental effects. In animal studies, it has been found that excess iron accumulation in the body increases TNF-α and IL-6 levels and causes oxidative stress (56). In a study, iron was detected in the ameloblasts of rats, and it was suggested that iron plays a role in both enamel development and mechanical properties of enamel. The presence of iron in odontoblasts has been shown, and it is thought that this may be related to the cofactor role of iron in collagen synthesis (57). In the study of Motta (30), it was suggested that iron serves as a reinforcing and resistant agent to wear and cracking in teeth. Research has demonstrated that smoking hinders the morphological development and mineralization level of hard tissue in the mandibular first molars of young rats (58). In our study, the low iron level in smokers supports these data.

CONCLUSION

This study aimed to investigate the effects of heavy metals and smoking, both significant factors affecting oral health, on periodontal well-being. The examination conducted revealed that smoking enhances the accumulation of heavy metals within oral tissues, potentially exerting adverse impacts on periodontal health. Furthermore, an observed positive correlation between nickel-induced tissue hyperplasia and pocket depth in non-smokers sheds light on an intricate relationship. These findings contribute to a better understanding of the potential effects of heavy metal accumulation and nickel-mediated tissue responses on periodontal health.

In summary, our study delved into the realms of heavy metal accumulation and the impact of smoking on periodontal health. However, further research is warranted to unravel the complexities of this field. Future studies should focus on unravelling the intricate mechanisms and potential interventions pertaining to heavy metal accumulation and smoking's effects on periodontal health.

These findings provide a crucial foundation for future research endeavors aiming to delve deeper into the relationship between periodontal health and heavy metal accumulation.

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Ethical approval: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions.

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