Correlation of Salivary Melatonin Levels and Many Periodontal Parameters in Cigarette Smokers with Periodontitis

Alhussein Mohammed Ali* and Ayser Najah Mohammed
Department of Periodontology, College of Dentistry, University of Baghdad, Baghdad, Iraq.
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ABSTRACT

Background: Adults who have periodontitis are more likely to lose teeth, which affects their quality of life and causes masticatory dysfunction. Most people agree that smoking tobacco makes them more likely to get oral mucosal and periodontal diseases and makes the oral microbiome less balanced. Melatonin is a naturally occurring hormone that is released in a variety of organs.

Objectives: To examine the relationship between smoking and salivary melatonin levels in periodontitis patients vs healthy people, and to establish a relationship between melatonin levels and periodontal parameters.

Materials and methods: The study was conducted at the College of Dentistry, University of Baghdad, Baghdad, Iraq. The study period was from February to May 2022. There were a total of 74 male subjects aged 35-55 years, and they were divided into four different groups; Group 1: a control group of non-smokers (n = 12), Group 2: a control group of smokers (n = 12), Group 3: a group of individuals with periodontitis (n = 25), Group 4: a group of individuals with both periodontitis and a record of smoking (n = 25). A whole, unstimulated sample of the participant’s saliva (5 ml) was taken, and this was followed by an evaluation of clinical periodontal variables (probing pocket depth and clinical attachment level). To measure melatonin concentrations, saliva samples were biochemically analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA).

Results: Melatonin concentrations were shown to be lower in groups with periodontitis than in control groups, as well as lower in groups with smoking than in groups without smoking. It was found that there was a strong negative link between periodontal variables and the amount of melatonin in the saliva.

Conclusion: According to this study, a reduced melatonin level was noticed in the salivary fluid of the smoker (healthy and periodontitis) groups in comparison with the non-smoker (healthy and periodontitis) groups. This suggests that active cigarette smoking had a retarding effect on the salivary levels of melatonin.

Keywords: Periodontitis; Saliva; Melatonin; Smoking.

INTRODUCTION

Periodontitis means inflammation and pathological damage to the gums and the parts of the teeth that hold them in place. Periodontal disease (PD) is now understood to be a complex disease, with plaque acting as its initiating cause [1]. Periodontal pathogens must be present, but they alone are not enough to cause periodontitis. Factors like smoking, diabetes, and the ability to evade the immune system, for instance, will change the immune-inflammatory reactions that may result in tissue destruction and, eventually, periodontitis [2].

There is a lot of evidence that smoking is bad for your oral health. Cigarette smoking is one of the main causes of dental problems like periodontitis and oral cancer [3]. Smoking causes a long-lasting inflammatory process that damages the alveolar bone and the periodontal ligament, which can lead to tooth loss [4]. Free radicals are reactive substances, and an excessive amount of them is known to be a significant factor in oxidative damage to biomolecules that leads to degenerative diseases [5]. There has been speculation that cigarette
MT levels in smokers with Periodontitis

MATERIALS AND METHODS

Study design

The study was designed as an observational case-control study. This study was approved by the Ethics Board of the Baghdad University School of Dentistry and corresponded to the Helsinki and Tokyo standards for human research (Reference Number 533 on April 17, 2022). The study was conducted at the College of Dentistry, University of Baghdad, Department of Periodontics, Baghdad, Iraq. The study covered the period from February to May 2022. Seventy-four male subjects between the ages of 35 and 55 years were picked according to inclusion and exclusion criteria (Table 1). All individuals were distributed into four groups; Group 1: a non-smoker healthy (12 subjects), Group 2: smoker healthy (12 subjects), Group 3: non-smoker periodontitis (25 patients), and Group 4: smoker periodontitis (25 patients).

Clinical assessment

The whole mouth was inspected with the University of Michigan O probe, with Dental Williams probe periodontal Color coded marking at 1, 2, 3, 5, 7, 8, 9 and 10 mm (Hu-Friedy, Germany). The examination included all teeth, with the exception of wisdom teeth. Full examinations of clinical periodontal parameters were carried out as follows: Probing pocket depth (PPD), and clinical attachment level (CAL) were assessed at six sites (buccal/labial, lingual/palatal, distobuccal/labial, mesiolingual/palatal, distolingual/palatal) per tooth for each patient. Periodontal health was defined as PPD ≤ 3 mm, and intact periodontium (no gingival recession). Periodontitis groups were defined as interdental CAL equal to or greater than two in nonadjacent teeth or buccal/oral CAL ≥ 3 mm with pocketing > 3 mm detected at ≥ 2 teeth [16]. To avoid contamination, samples were stored in a compact cooling box after collection. The subject’s identification number from the case sheet was written on the tube. Then, the samples were centrifuged at 4000 rpm for 15 minutes, and stored at -20°C in the freezer until analyzed by ELISA [17].

Table 1. Inclusion and exclusion criteria.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Male participants.</td>
<td>• Females.</td>
</tr>
<tr>
<td>• Systemically healthy participants with or without smoking habit.</td>
<td>• Alcohol drinking.</td>
</tr>
<tr>
<td>• Periodontitis participants with or without smoking habit.</td>
<td>• Melatonin and vitamin supplements up to 6 months before the research.</td>
</tr>
<tr>
<td>• Present with at least 20 teeth.</td>
<td>• Patients who have just completed or are receiving significant periodontal treatment, as well as those who have taken an anti-inflammatory or antimicrobial course within the last three months.</td>
</tr>
<tr>
<td>• Cigarette-smokers who have smoked at least five cigarettes each day for at least a year.</td>
<td>• Dual smokers of water pipe and E-cigarette smokers as well as cigarette smoking.</td>
</tr>
<tr>
<td>• Subjects who wish to participate in the study.</td>
<td>• Patients with clear pathology lesions in the mouth, such as oral malignancy.</td>
</tr>
<tr>
<td>• Alcohol drinking.</td>
<td>• Decline to participate.</td>
</tr>
</tbody>
</table>

MT levels in smokers with Periodontitis

Melatonin (MT) (N-acetyl-5-methoxytryptamine) was discovered by Aron Lerner in 1958. It is a small neuroendocrine molecule that plays important roles in both healthy and sick states [7]. This hormone is mostly made in the pineal gland, but it can also be made in the digestive tract, skin, retina, and bone marrow [8]. MT was shown to be present in plants in 1995, and it has since been suggested that it is a bioactive dietary ingredient [9]. MT was also observed in plant waste, including pomegranate peels, bitter orange peels, and leaves [10].

In healthy individuals, MT secretion is at its peak between twelve and two a.m., whereas it is at minimum concentrations throughout the day [11]. Important functions of MT include its ability to promote healthy sleep, reduce oxidative stress, participate significantly in the immune system, possess antimicrobial properties against numerous bacteria and viruses, and affect bone metabolism by influencing both osteoblasts and osteoclasts [12].

The amount and severity of periodontal disease affected the levels of MT in the saliva. People with severe periodontitis had the lowest levels [13]. Balaji and Rao (2020) measured MT concentrations in gingival tissue, plasma, and saliva of smokers with periodontitis and found that gingival and plasma levels of MT were lower in smokers. Still, salivary levels of MT were higher in smokers. To the best of our knowledge, the previous study was the only one that measured melatonin levels associated with cigarette smoking with periodontitis. Thus, the current study aimed to measure MT levels in the saliva of smokers with periodontitis compared to healthy controls and find an association between smoking and MT levels in periodontitis patients. The null hypothesis of this study, there is no correlation between salivary MT levels and smoking cigarettes in patients with periodontitis. According to the alternative hypothesis, there is a correlation between salivary MT levels and smoking cigarettes in patients with periodontitis.
Melatonin measurement

An enzyme-linked immunosorbent assay kit (MyBioSource Systems, CA, USA) was used to detect the MT concentration in the saliva. The above-presented kit reported an assay sensitivity of < 1.56 Pg/ml. The tests were conducted according to the manufacturer’s instructions. The ELISA procedure for this study was the sandwich ELISA technique. A total level of salivary MT was approximated based on its observed concentration spectrophotometrically at a wavelength of 450 ± 10 nm.

Statistical analysis

The results obtained were statistically analyzed using SPSS version 22. For nominal variables, the standard deviation (SD) and mean were used. Additionally, the independent sample t-test, one-way analysis of variance (ANOVA) with the least significant difference test, and Pearson correlation (r); were used for inferential statistics. Regarding the normality distribution of quantitative variables, the Shapiro-Wilk test was used. The statistical difference was considered when the P-value < 0.05.

RESULTS

Regarding the age of the participants, the non-smokers patient group showed the highest mean age (47.120 ± 6.180 years), followed by the smokers patient group (45.000 ± 6.144 years), then the smokers healthy group (43.833 ± 5.921 years), and lastly, the non-smokers healthy group showed the lowest mean age (41.667 ± 5.193 years). These results revealed a statistically non-significant difference among groups (P-value = 0.071). The present study’s findings showed that the smoker patient group had a higher mean value of PPD measurements than the non-smoker patient group, with no-significant differences between groups, on the other hand, the smoker patient group also had a higher mean value of CAL measurements than the non-smoker patient group, and there were notable differences between groups, as demonstrated in Table 2.

MT was found in all of the samples that were analyzed, but the amount of MT was different between samples and groups. The concentrations of MT in saliva decreased notably from healthy to periodontitis individuals. Among the four groups, the non-smokers healthy group had the highest mean value of MT concentration, with a mean and SD of 90.582 ± 21.354, followed by the smokers healthy group with a mean value and SD of 85.435 ± 21.407, the non-smokers patient group with a mean value and SD of 54.790 ± 26.271, and finally the smokers patient group with the lowest mean value and SD of 48.684 ± 27.706. The analysis of the variance test of MT levels for all groups revealed a substantial difference between groups, as indicated in Table 3.

Table 4 shows the least significant difference in MT levels between groups. There were substantial differences between Group 1 and Groups 3 and 4, and there was also a substantial difference between Group 2 and Groups 3, and 4. No statistically significant difference was found between Group 1 and Group 2, as well as no-significant difference between Group 3 and Group 4.

The correlation between MT and periodontal variables among groups revealed a significantly strong negative correlation of MT with clinical parameters, except for the CAL variable in Group 4, the correlation was a no-significant weak negative, as described in Table 5.

DISCUSSION

The study was conducted to determine the MT levels in saliva among groups. In addition, measuring clinical parameters and correlating them with the level of MT. The PPD measurements of the smoker patient group were higher than those of the non-smokers patient group, and the comparison revealed non-statistical significance. This outcome agreed with Meulman et al. (2012) [18], who found that PPD was higher in smokers than in nonsmokers, but disagreed with Ameer and Ali (2015) [19], who found that PPD was higher in nonsmokers than in smokers. This study showed that there were higher CAL in the smoker patient group than in the non-smoker patient group, with highly significant differences. These investigations were consistent with BinShabaib et al. (2019) [20], while these findings disagreed with Ameer and Ali (2015) [19], who found higher measurements of CAL in non-smoker than smoker periodontitis groups. These results may be attributed to the fact that tobacco smoking is associated with an increase in pocket depths, alveolar bone loss, and an increased rate of tooth loss. This makes sense since smoking is a major risk factor for periodontal disease, and the severity of PD is higher in smokers than in nonsmokers [21]. Moreover, smoking is linked to an increase in the production of advanced glycation end products (AGEs) in periodontal tissues, which when they interact with their receptors, augment inflammation [22]. Additionally, the present study found that the highest salivary MT level was in the non-smokers healthy group, while the lowest MT level was in the smoker’s patient group. There were significant differences in salivary MT levels between the healthy and diseased groups. The results of this study were consistent with previous studies [23, 24], which noted that the salivary MT level was significantly lower in patients with periodontitis compared to healthy subjects. The current results were in contrast to those reported by Lodhi et al. (2016) [25], who found that salivary MT levels were higher in patients with periodontitis, followed by those with gingivitis, and lowest in healthy controls. This result may be related to the fact that the etiology of periodontitis is significantly influenced by oxidative stress, it has been shown that MT may effectively scaveng free radicals such as hydroxyl radicals, hydrogen peroxide, and reactive nitrogen species [26]. According to Abdolsamadi et al. (2014) [23], high amounts of free radicals make the body consume more MT, which reduces the MT levels in persons with periodontitis. The decrease in salivary MT levels in smokers compared to non-smoker groups can be explained by the fact that smoking is linked to detrimental effects on periodontium through tissue damage mediated by reactive species derived from a cigarette [27]. MT is likely an effective therapy for minimizing certain adverse consequences of smoking and can be used as a scavenger to counteract the oxidative damage that smoking causes. Moreover, by inhibiting the activation of oxidants and, in turn, stimulating pathways in which antioxidant enzymes are involved, intraperitoneal MT injection decreased oxidative stress damage and mitigated the adverse effects of smoking [28]. This study showed that there was a strong negative correlation between the levels of MT in saliva and periodontal variables. These findings were consistent with those of Srinath et al. (2010) [29], who found that there were strong negative and significant correlations between salivary MT and clinical periodontal parameters. While these results disagreed with Has-
Table 2. Clinical periodontal variables among groups using independent sample t-test *.

<table>
<thead>
<tr>
<th>Periodontal variables</th>
<th>Group 3</th>
<th></th>
<th></th>
<th></th>
<th>Group 4</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>test-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max.</td>
<td>Min.</td>
<td>mean</td>
<td>SD</td>
<td>Max.</td>
<td>Min.</td>
<td>mean</td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>4.00</td>
<td>6.00</td>
<td>4.774</td>
<td>0.622</td>
<td>4.00</td>
<td>6.42</td>
<td>4.803</td>
<td>0.635</td>
<td>0.150</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>1.85</td>
<td>4.00</td>
<td>2.894</td>
<td>0.640</td>
<td>2.57</td>
<td>5.00</td>
<td>3.619</td>
<td>0.705</td>
<td>3.810</td>
<td>&lt; 0.0001†</td>
<td></td>
</tr>
</tbody>
</table>

* No-significant difference P-value > 0.05, † Significant difference P-value < 0.05, SD: Standard deviation, PPD: Probing pocket depth, CAL: clinical attachment level.

Table 3. One-way test analysis of variance test of melatonin (Pg/mL) levels for all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ±SD</th>
<th>Min.</th>
<th>Max.</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group1</td>
<td>90.582</td>
<td>21.355</td>
<td>139.585</td>
<td>11.375</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Group2</td>
<td>85.436</td>
<td>21.408</td>
<td>122.290</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group3</td>
<td>54.791</td>
<td>26.272</td>
<td>117.307</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group4</td>
<td>48.685</td>
<td>19.706</td>
<td>122.966</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference P-value < 0.05.

Table 4. Least significant difference in melatonin (Pg/mL) levels between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Group 2</td>
<td>5.14667</td>
<td>0.621</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>35.79157</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>Group 4</td>
<td>41.89753</td>
<td>0.000*</td>
</tr>
<tr>
<td>Group 2</td>
<td>Group 3</td>
<td>30.64490</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Group 4</td>
<td>36.75086</td>
<td>0.000*</td>
</tr>
<tr>
<td>Group 3</td>
<td>Group 4</td>
<td>6.10596</td>
<td>0.398</td>
</tr>
</tbody>
</table>

* Significant difference P-value < 0.05.

Table 5. Correlation of salivary melatonin (Pg/mL) with clinical periodontal variables in all groups.*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Melatonin r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3</td>
<td>PPD (mm)</td>
<td>-0.854</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td></td>
<td>CAL (mm)</td>
<td>-0.595</td>
<td>0.002†</td>
</tr>
<tr>
<td>Group 4</td>
<td>PPD (mm)</td>
<td>-0.570</td>
<td>0.007†</td>
</tr>
<tr>
<td></td>
<td>CAL (mm)</td>
<td>-0.356</td>
<td>0.080</td>
</tr>
</tbody>
</table>

* r: Pearson correlation, † Significant difference P-value < 0.05.

This study, the condition of periodontitis had a greater impact on MT levels than smoking did. This study provides additional evidence that MT plays an important role in periodontitis pathogenesis; MT can be used as a potential biochemical marker for the detection and progression of periodontitis.

ETHICAL DECLARATIONS

Acknowledgements

None.

Ethics Approval and Consent to Participate

Written approval had been obtained from the Ethics Committee of the College of Dentistry, Baghdad University, Iraq. Study data/information were used for research purposes only. Informed consent from every participant was obtained.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

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Authors’ Contributions

Both authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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