Evaluation of Nonsurgical Treatment Effects on Salivary Melatonin Level in Periodontal Disease: A Radioimmunoassay Study

Leila Golpasand Hagh, Hojatollah Yousefimanesh, Fatemeh Mohammadi, Akram Ahangarpour

ABSTRACT

Introduction: Periodontitis is a chronic inflammatory infection in which tissue destruction is associated with an imbalance between oxidant-antioxidant systems. Melatonin is an antioxidant biomarker that has an antioxidant action. Nonsurgical treatment is one periodontal treatment method that affects etiologic factors. This study investigated salivary melatonin concentration and its changes following nonsurgical treatment in periodontal disease.

Materials and methods: This study was a case control one where the study groups included 45 patients with periodontal disease and 15 healthy subjects selected as controls. Unstimulated saliva was collected before and after scaling and root planing. Sample was evaluated by radioimmunoassay (RIA) method. In order to analyze the data t-test, ANOVA was used. The p < 0.05 was considered significant.

Results: Melatonin was detected in study group before nonsurgical treatment. Salivary melatonin levels in the severe periodontitis group were significantly lower than the other groups (p < 0.05). The melatonin changes were statistically significant between the groups of patients after intervention. The significant correlation between salivary melatonin with the clinical parameter was observed (p < 0.05).

Conclusion: Nonsurgical treatments improve clinical parameters. Salivary melatonin level has correlation with changes in clinical parameters. The melatonin production after nonsurgical treatment had correlation with severity of disease.

Keywords: Dental scaling, Melatonin, Saliva, Radioimmunoassay, Periodontal diseases.


Source of support: Nil
Conflict of interest: None declared

INTRODUCTION

Periodontitis is a chronic inflammatory infection in which the surrounding and supporting tissues of the teeth are involved. In this disease, alveolar bone, periodontal ligament and gingival are damaged and, if not treated properly, it will lead to loss of teeth. Overgrowth of Gram-negative bacteria in dental plaque is the beginning of periodontal disease process. Innate and acquired immune response neutralizes bacterial invasion. At the same time, periodontal tissue destruction is caused by the secretion of inflammatory cytokines, preosteoclastic factors and matrix metalloproteinase that attempted to limit this invasion. Infiltration of polymorphonuclear leukocytes is a key finding in periodontal disease and free radicals created by these phagocytic cells and bacteria in the inflamed area can lead to significant damage to tissue and immune system response. Furthermore, periodontal disease is associated with an imbalance between oxidant-antioxidant systems. Melatonin is an antioxidant and, in some of the animal studies, potential anti-inflammatory and anticancer role were approved. This cytokine is an indolamine and primarily is secreted by the pineal gland, in a circadian rhythm. However, other parts of the body can produce it too. Physiological roles of this hormone, such as controlling the circadian rhythm of body temperature and activation of immune response is demonstrated. Pattern of serum and saliva melatonin secretion are parallel together, but salivary melatonin levels are not fully reflected through serum levels. Submandibular glands, as a local secondary source, secrete melatonin. Melatonin had an antioxidant action and directly destroyed free radicals and stimulate antioxidant enzymes and increases its efficiency. The properties of metallic bonding, highly lipophilic and its properties to penetrate cell membranes and other cell components of melatonin are also able to inhibit the growth of Gram-negative bacteria. In chronic periodontitis, melatonin levels can change in response to oxidative stress and bacterial invasion. One of the periodontal treatments is scaling and root planing process during which, bacteria biofilm is destroyed and the bacterial load will be decreased and thus the rate of bacteria and cytokines and free radicals will be reduced. Some studies have described the relationship between melatonin and mouth disease, but few studies exist on the effect of periodontal treatment on melatonin levels. Since melatonin has strong antioxidant properties, free radical’s destruction and inhibition of bone resorption and stimulates bone formation, it may have an inhibitory effect on periodontal disease. Therefore, this study investigated salivary melatonin concentration and its changes following nonsurgical treatment of periodontal disease and evaluated the relationship of these changes with periodontal disease.

MATERIALS AND METHODS

This study is a case control study whose subjects were recruited from patients referred to the faculty of Ahvaz...
Dentistry. The study groups included 15 patients with severe generalized periodontitis (SGP), 15 patients with moderate generalized periodontitis (MGP), 15 patients with gingivitis (G) and 15 healthy subjects selected as controls (C). After completing the consent form, volunteers were enrolled into the study. Patients were matched for age and sex with each other.

**Patient Selection**

Patients were classified according to the Periodontology Society of America that is described in the following topics.\(^{25}\)

- Patients had bone loss at more than 30% mouth sites and their clinical attachment loss (CAL) was ≥5 mm classified as suffering from SGP.
- Patients had bone loss at more than 30% mouth sites and their CAL 3 to 4 mm classified as MGP.
- Patients had inflammation without bone loss classified plaque induce gingivitis.
- Control group included patients who had no symptoms of bone loss, inflammation and their periodontal probing depth (PPD) was less than 3 mm.

Complete medical and dental history was recorded for all patients. The following criteria were considered as exclusion criteria:

- Periodontal treatment or antibiotics within the 3 months later, the presence of any systemic disease, such as diabetes, asthma or cancer, working the night shift, dry mouth, salivary gland disease, having less than 20 teeth, drugs affecting melatonin secretion (e.g. sleep medications or immunosuppressive), full or partial denture, pregnancy, lactation, smoking, acute infection, impaired immune systems, history of radiotherapy or chemotherapy.
- CAL, plaque index (PI), bleeding on probing (BOP) and PPD were recorded for each patient. The Williams probe (23 W, Hu-Friedy, Chicago, IL, USA) was used for all measurements.

**Saliva Collection**

Participants were asked to avoid eating, drinking (except water), chewing gum, brushing and using mouthwash for 90 minutes before the study. We asked them to spit 3 to 4 ml of unstimulated saliva into sterile pipe. In order to study the blind, each tube contained a special code. The tubes transferred to the Department of Physiology were centrifuged for 10 minutes at 300 rpm in order to separate particles. After centrifugation, the upper clear layer (supernatant) was separated by sampler and vacate into the microtube. Samples were put into freezer at a temperature of −70°C.

All participants received oral hygiene instruction included teeth brushing, and dental floss application before nonsurgical treatment. After collection of saliva, nonsurgical treatment included scaling and root planing was carried out by means of ultrasonic device (Dentin N.L.90, Esfahan, Farazmehr, Iran). In some patients, treatment was carried out with local anesthesia and hand instrument. The control group had not received any treatment except oral hygiene instruction.

The study groups were asked to come back one month later. After 1 month, clinical parameter ranges and unstimulated saliva was collected again. After complete sampling, the frozen samples returned to liquid then saliva melatonin levels by radioimmunoassay (RIA) (melatonin direct radioimmunoassay, Germany, IBL) was measured. To avoid the effects of circadian, rhythm sampling was done between hours 9 and 11 AM.

**STATISTICAL ANALYSIS**

Collected data were analyzed using SPSS software version 16 (SPSS, Inc, Chicago, IL, USA). The data distribution was evaluated using the Kolmogorov-Smirnov test and had normal distribution (p = 0.9). In order to analyze the data t-test, an ANOVA was used. p < 0.05 was considered significant.

**RESULTS**

The mean age and sex distribution of 60 patients participating in this study is described in Table 1. Analysis results showed that age and gender were not significantly different between groups (p = 0.85).

The data analyzed showed that the effect of age and gender on salivary melatonin levels was not significantly different (p = 0.95); therefore, the age and gender were not effective on salivary melatonin in all groups.

**Melatonin Levels Comparison before and after Treatment**

The data analysis showed that pretreatment melatonin level between groups (severe periodontitis, moderate periodontitis, gingivitis and the control group) was detected (Table 2).

Salivary melatonin level in the severe periodontitis group was significantly lower than the other groups. Salivary melatonin level in the moderate periodontitis group was significantly lower than the control and gingivitis groups. Control group had maximum salivary melatonin levels (p < 0.05).

After the intervention, ANOVA showed that melatonin was statistically significant between the groups of patients in comparison before intervention (p < 0.05).
Table 1: Demographic data of case (gingivitis, periodontitis) and control (healthy patients)

<table>
<thead>
<tr>
<th>Group of study</th>
<th>Age mean (SD)</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>SGP</td>
<td>32.5 (5.05)</td>
<td>8</td>
</tr>
<tr>
<td>MGP</td>
<td>30.61 (5.25)</td>
<td>7</td>
</tr>
<tr>
<td>G</td>
<td>38.3 (5.35)</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>36.7 (5.62)</td>
<td>8</td>
</tr>
</tbody>
</table>

SGP: Severe generalized periodontitis; MGP: Moderate generalized periodontitis; G: Gingivitis; SD: Standard deviation

Table 2: Melatonin levels distribution before and after nonsurgical treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before nonsurgical treatment</th>
<th>After nonsurgical treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Confidence interval 95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Upper bound</td>
<td>Lower bound</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>SGP</td>
<td>1.504 (0.401)</td>
<td>1.72</td>
<td>1.28</td>
</tr>
<tr>
<td>MGP</td>
<td>2.074 (0.255)</td>
<td>221</td>
<td>1.93</td>
</tr>
<tr>
<td>G</td>
<td>3.222 (0.319)</td>
<td>3.39</td>
<td>3.13</td>
</tr>
<tr>
<td>Control</td>
<td>3.950 (0.287)</td>
<td>3.79</td>
<td>3.11</td>
</tr>
</tbody>
</table>

Table 3: The clinical parameter changes following nonsurgical treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Clinical parameter</th>
<th>Before nonsurgical treatment</th>
<th>After nonsurgical treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGP</td>
<td>BOP</td>
<td>86.8 ± 7.39</td>
<td>14.2 ± 0.99</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>5.15 ± 0.41</td>
<td>2.8 ± 0.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>94.33 ± 4.02</td>
<td>15.93 ± 5.52</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>3.57 ± 0.6</td>
<td>2.18 ± 0.47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MGP</td>
<td>BOP</td>
<td>71.4 ± 6.79</td>
<td>11.26 ± 0.59</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>4 ± 0.48</td>
<td>2/26 ± 0.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>75.6 ± 8.87</td>
<td>11.26 ± 3.92</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>2.14 ± 0.36</td>
<td>1.3 ± 0.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>G</td>
<td>BOP</td>
<td>59.13 ± 14.3</td>
<td>4.4 ± 0.48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>2 ± 0.4</td>
<td>1.34 ± 0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>42.53 ± 8.47</td>
<td>8.13 ± 2.26</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Control</td>
<td>BOP</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>PPD</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>PI</td>
<td>12.86 ± 5.22</td>
<td>6.6 ± 2.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CAL</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

After the nonsurgical treatment of severe periodontitis, the amount of melatonin was significantly lower than the other groups. Melatonin level in moderate periodontitis as compared to the gingivitis and control group was significantly lower (p < 0.05). According to Table 2, the melatonin level in gingivitis group was not significantly different from the control group (p = 0.12).

The nonsurgical treatment significantly increased salivary melatonin levels in severe and moderate periodontitis and gingivitis group (p < 0.05). In the control group, significant
changes in melatonin levels were not observed during the study (p = 0.32).

**Clinical Parameter Comparison**

**Bleeding on Probing**

Before nonsurgical treatment, the mean BOP in severe periodontitis group, moderate periodontitis and gingivitis was 86.8, 71.40, 59.13% respectively (Table 3).

BOP values showed significant difference between the study groups before and after nonsurgical treatment. In other words, nonsurgical treatment causes significant reduction in the rate of BOP in study groups (p < 0.05).

Direct relationship was observed between disease severity and changes in BOP. When disease severity was much greater, more changes occurred after nonsurgical treatment.

**Periodontal Probing Depth**

According to Table 3, the average PPD before nonsurgical treatment in severe periodontitis, moderate periodontitis and gingivitis was 5.15, 4.0, 2 mm respectively. ANOVA showed a significant difference between the groups and average PPD after nonsurgical treatment in the study group showed a significant decrease (p < 0.05).

**Plaque Index**

Pretreatment PI in severe periodontitis, moderate periodontitis, gingivitis and control was 94.33, 75.60, 42.53 and 5.22 respectively. Nonsurgical treatment causes significant reduction in the PI at study groups (p < 0.05). Table 3 showed that the difference between groups is statistically significant and has a direct relationship with the severity of disease (p < 0.05).

**Clinical Attachment Loss**

Before treatment, the mean CAL in severe and moderate periodontitis was 3.57, 2.14 respectively. According to Table 3, the CAL after nonsurgical treatment significantly reduced in the treatment group (p < 0.05).

**Salivary Melatonin Levels and Clinical Parameters Comparison after Treatment**

This study shows that the clinical parameters had negative correlation with melatonin level. After nonsurgical treatment,
the clinical parameter will be better (numerical reduction) and vice versa melatonin levels rise. Therefore, clinical improvement after nonsurgical treatment will increase melatonin levels (Figs 1A to D).

DISCUSSION

In this study, salivary melatonin levels before and after nonsurgical treatment of periodontal disease were compared with each other. It was found that melatonin level in periodontal patients decreased and, following nonsurgical treatment, its rate increases. Melatonin changes after nonsurgical treatment in periodontal group occurred more than the control group.

Evaluation of clinical parameters showed that nonsurgical treatment was able to improve these indicators and the BOP, CAL, PPD, PI decreased dramatically.

Saliva samples were used in our study because patient and clinician were comfortable. In previous studies, it has been stated that the saliva sampling method is relatively practical and reliable in clinical studies and will not be harmful to patients. Chronic periodontitis is the most common periodontal disease and one of etiologies is the impaired balance between oxidant and antioxidant system. The melatonin is one of the biomarkers and has antioxidant and antifree radical effects. The study results showed that salivary melatonin level changes were inversely related to disease severity. This means that the secretion of these hormones can reduce in the periodontal destruction. Our study results are consistent with previous studies that had been conducted by Cutando et al. They indicated two roles for melatonin in their study:

a. Eliminate free radicals and act as an antioxidant (such as vitamin C, E and coenzyme Q)

b. Effect of melatonin cell protection in inflammatory conditions.

In this study, among periodontal indices, community periodontal index (CPI) was used. The results of this study showed that melatonin reduced from the CPI1 to CPI2 and this finding is consistent with a recent study. The level of the melatonin increased from CPI3 to CPI4, which is contrary to our study. The main reason for the controversies between studies is that in CPI index, PPD is evaluated instead of using the CAL. Increased PPD can also occur due to false pocket (gingival enlargement) or gingival abscess. Spread of disease in a mouth can also be due to factor, such as diabetes often associated with abscess, which was not controlled in this study. These changes wrongly explain extent and increase in CPI, while the severity of the disease may be as gingivitis. This tissue changes could disrupt the interpretation of the results. The increased levels of melatonin from CPI3 to CPI4 could be due to changes in periodontal pockets without spreading disease.

In the Srinath et al study, it was concluded that GCF and plasma melatonin levels in chronic periodontitis decrease compared with gingivitis and control patients that are consistent to our study. These studies showed that melatonin level reduced in direct proportion to the severity of the disease and confirmed the decrease protective role of melatonin in periodontitis and severe periodontal disease.

Like our study, Bertti et al also concluded that individuals with chronic periodontitis and aggressive had lower salivary melatonin levels compared with control patients. Hormone levels in saliva were increased after nonsurgical treatment, but did not reach the levels of control subjects. Following nonsurgical treatment, the volume of locally destructive factors may remain in the periodontal pockets that delay improvement of microscopic feature. This reason may be due to this aspect that clinical changes are seen earlier than microscopic changes.

According to the present study, the nonsurgical treatment affects salivary melatonin levels and increase up to 50% levels of the hormone in patients with severe periodontitis and moderate periodontitis. Following nonsurgical treatment, the periodontal lesion has not been fully resolved and for complete treatment, we may need to perform surgical approach for osseous defect correction and facilitate patient plaque control. The melatonin levels in patients with gingivitis, increased approximately 15% to levels very close to those in healthy subjects.

In Bertti et al study, clinical parameters include PPD, CAL, BOP were evaluated before and after nonsurgical treatment. In addition, our recent study evaluated changes in the PI.

PPD changes, following nonsurgical treatment, are not due to establishment of new attachment but this is due to readaptation of junctional epithelium at the bottom of periodontal pocket. Following resolve of inflammation at the junctional epithelium, PPD decreases. Sites that heal on probing tend to have significantly more inflammation than nonbleeding sites. BOP reducing also can be seen after nonsurgical treatment that showed reduced inflammation and increased resistance to tissues penetration of periodontal probe. However, studies have shown that epithelial adaptation could be resisted as true connective tissue adhesive against diseases. The absence of BOP has been shown to be a marker of tissue health. Different method may be the controversy reason, because we used RIA and other study used enzyme-linked immunosorbent assay (ELISA) method that had low sensitivity than RIA.
The significant correlation between salivary melatonin with the clinical parameter emphasizes its protective role in oral cavity. Nonsurgical treatment creates useful changes in periodontal pocket and its ecosystem. These changes opened the way for protective factors including melatonin, whose beneficial role was seen in this study.

ACKNOWLEDGMENTS
Ahvaz Jundishapur University of Medical Sciences provided financial support for this study. All of the authors wish to thank the Research and Ethic Committee of Ahvaz Jundishapur University.

REFERENCES
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