

Relationship between Salivary Melatonin Level and Periodontal Diseases

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ABSTRACT

Statement of the problem: Melatonin has powerful antioxidant effects, has an immunomodulatory role, stimulates the synthesis of type I collagen fibers, and promotes bone formation. Melatonin is also secreted in the saliva, although its role in the mouth is not known well.

Purpose: The aim of this study was to examine the correlation between salivary melatonin level and periodontal diseases.

Materials and methods: Fifty subjects with a mean age of 40.44 ± 6.38 years were equally divided into 5 groups: 10 healthy subjects, 10 subjects with gingivitis, 10 subjects with localized moderate chronic periodontitis, 10 subjects with generalized moderate chronic periodontitis and 10 subjects with generalized severe chronic periodontitis. Saliva samples were collected from all the subjects and melatonin levels were determined using an enzyme-linked immunosorbent assay. Two-way and one-way ANOVA and Tukey test were used to analyze relationships between the variables.

Results: Healthy subjects had significantly higher salivary melatonin level (5.29 ± 0.50 pg/mL) compared to patients with gingivitis (4.35 ± 0.30 pg/mL) ($P < 0.001$). The difference between salivary melatonin level in patients with gingivitis and periodontitis was significant ($P < 0.001$). Level of melatonin in patients with generalized severe chronic periodontitis (3.39 ± 0.10 pg/mL) was significantly lower than that in other groups ($P < 0.01$).

Conclusion: This study showed that salivary melatonin level in patients with periodontal diseases is lower than that in healthy subjects, and it has a negative correlation with the severity of disease, suggesting that melatonin might have a protective role against periodontal diseases, although further research is required to validate this hypothesis.

Keywords: Melatonin, gingivitis, periodontitis, saliva.

INTRODUCTION

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Melatonin (MT) is a neuroendocrine hormone secreted mainly by the pineal gland in the brain.⁽¹⁾ However, recent studies have shown that MT is synthesized not only in the brain but

also in various body parts, including retina, ovary, Harderian gland, placenta, kidneys, respiratory tract and, finally, in the GIT, where MT has been found to be generated in EE cells in about 500 times larger amounts compared to the pineal gland. This gland produces melatonin in a circadian manner, synchronizing a number of biologic processes in a day-night rhythm.⁽²⁾ MT is produced with a circadian rhythm characterized by elevated blood levels during the night.⁽³⁾ In healthy individuals, maximal secretion of MT occurs between midnight and 2:00 a.m., whereas the minimal production occurs during the day.⁽⁴⁾ Melatonin levels can be determined by repeated measurements of plasma or saliva melatonin or urine sulfatoxy-melatonin.⁽⁵⁾ Melatonin is also secreted in the saliva, although its role in the mouth is not known well. The most important effect of MT seems to result from its potent antioxidant, immune-modulatory, protective and anti-neoplastic properties. It stimulates synthesis of type I collagen fibers and bone formation. Therefore, MT might be used therapeutically and locally, for instance in the oral cavity damage of mechanical, bacterial, fungal or viral origin, in post-surgical wounds caused by tooth extractions and other oral surgeries and in helping bone formation in various auto-immunological disorders such as Sjögren's syndrome and periodontal diseases.⁽⁶⁾ The major and important property of MT is its ability to serve as a very potent free radical scavenger.^(7,8) A very large body of evidence indicates that melatonin is a major scavenger of

both oxygen- and nitrogen-based reactive molecules, including ONOO⁻. Melatonin has scavenging actions at both physiologic and pharmacologic concentrations.⁽⁹⁾

Melatonin also supports several intracellular enzymatic antioxidant enzymes, including SOD and glutathione peroxidase (GSH-Px). Moreover, melatonin induces the activity of γ -glutamylcysteine synthetase, thereby stimulating the production of another intracellular antioxidant, glutathione (GSH). A number of studies have shown that melatonin is significantly better than the classic antioxidants in resisting free radical-based molecular destruction. In these in vivo studies, melatonin was more effective than vitamin E, β -carotene, and vitamin C. Beneficial antioxidant effects of melatonin have been recently shown in clinical settings for several chronic diseases, including patients with rheumatoid arthritis, elderly patients with primary essential hypertension and females with infertility.⁽⁹⁾

Free radical bursts from the phagocytic cells, such as neutrophils and macrophages migrating to the inflammation site, damage the gingival tissue significantly.⁽⁶⁾

Melatonin promotes osteoblast differentiation and bone formation. At micromolar concentrations, melatonin stimulates the synthesis of type I collagen fibers in human osteoblasts in vitro; in addition, it increases the genetic expression of bone sialoprotein and other protein markers of bone, including alkaline phosphatase, reducing the osteoblast

differentiation period from 21 days (which is normal) to 12 days. Other possible target cells for melatonin are osteoclasts which reabsorb existing bone through the generation of free radicals.⁽⁵⁾

Another very important potential use of the anti-inflammatory abilities of melatonin is the treatment of periodontal diseases. It has already been established that periodontal tissues are destroyed in the course of periodontitis by a disproportionate immunological response to the triggering agent, such as bacteria of the plaque. Damage to periodontal tissues results from a direct effect of the toxic products released by the bacteria and from the action of the immune system stimulated by the bacterial infection. A notable feature of PD is the generation of free radicals, some of which are derived from the bacteria themselves, whereas others are a consequence of the immune response. The increase in free radical generation coexists with a decrease in the antioxidant defense mechanisms. This imbalance between the pro-oxidant and antioxidant systems may lead to a further oxidative attack and a marked deterioration of periodontal tissues.⁽⁴⁾

Gender does not influence the levels of melatonin, whereas factors such as smoking, exposure to light, alcohol consumption, and aging decrease the levels of salivary MT.⁽¹⁰⁾ Therefore, this study aimed to evaluate the presence of melatonin in saliva and assess the levels of salivary MT in periodontal health and disease.

MATERIALS AND METHODS

A total of 50 subjects (24 females and 26 males; mean age of 40.44 ± 6.38 years) were included in the present study, which was conducted in February and March, 2011, at Ahvaz Dental School. Informed consent was obtained from all the patients before participation in the study, which was approved by the Ethics Committee of Ahwaz Jundishapur University. The subjects divided into 5 groups: 10 healthy subjects (control group), 10 subjects with gingivitis, 10 subjects with localized moderate chronic periodontitis, 10 subjects with generalized moderate chronic periodontitis, 10 subjects with generalized severe chronic periodontitis. All the groups were age- and sex-matched. The criteria presented in this study for grouping the subjects were based on the 1999 International Workshop for the Classification of Periodontal Diseases organized by the American Academy of Periodontology (11). Patients who had clinical attachment loss in more than 30% of their sites were named “generalized”, and patients who had clinical attachment loss in more than 30% of their sites were named “localized”. To determine the severity of disease, patients with 3–4 mm of clinical attachment loss were grouped in “moderate periodontitis” and patients with 5 mm or more clinical attachment loss were grouped in “severe periodontitis” groups. Subjects with no clinical attachment loss and just with inflammation in gingival tissues were grouped in the “gingivitis group”; 10 subjects who had no inflammation in gingival tissues and

clinical attachment loss were selected in the study as the control group. Subject inclusion criteria were as follows:

- 1) Patients with a varying degree of periodontal disease
- 2) Good general health
- 3) No invasive periodontal therapy during the previous 6 months

Subject exclusion criteria were as follows:

- 1) Systemic diseases such as diabetes mellitus
- 2) Neurologic disorders such as epilepsy and schizophrenia
- 3) Pregnancy
- 4) Smoking and alcoholism
- 5) Presence of a disease with possible effects on the immune system, e.g. chronic infection or cancer
- 6) Treatment with any drug that might alter MT levels (e.g. diazepam)
- 7) Use of any antibiotics during the previous 6 months, and patients who had undergone non-invasive periodontal therapy (scaling and root planing)

DETERMINATION OF SALIVARY MELATONIN

Collection of saliva

Participants were instructed to refrain from eating, drinking, and practicing oral hygiene habits within 90 minutes before saliva sampling; 3–4 mL of unstimulated saliva was collected using a collection device (avoiding any possible contamination). Samples were collected under

dim light. Low-intensity light was used because a high-intensity light source diminishes secretion of melatonin. The saliva samples were centrifuged at 3000 rpm for 20 minutes and clear supernatant was stored at -20°C until an assay was performed.

Melatonin level determination

Melatonin levels in saliva samples were measured by Enzyme-linked Immunosorbent Assay (ELIZA) (IBL, Hamburg GmbH, and Germany).

Statistical Analyses

Data were analyzed using SPSS 16 computer software. Two-way and one-way ANOVA and Tukey test were used to analyze relationships between the variables. Statistical significance was established at $P < 0.05$.

RESULTS

Means and standard deviations of age in the study groups are presented in Table 1. One-way ANOVA showed that age was not significantly different between the groups ($P > 0.05$). Spearman's correlation between age and salivary melatonin levels was $r = -0.156$, which showed an inverse weak correlation. Then, the age factor in the salivary melatonin levels was not evaluated.

Table 1: Comparison of mean age between the study groups

Group	Mean \pm SD
Healthy	40.5 \pm 6.02
Gingivitis	37.2 \pm 6.79
Localized moderate chronic periodontitis	38.6 \pm 6.58
Generalized moderate chronic periodontitis	41.2 \pm 6.56
Generalized severe chronic periodontitis	44.7 \pm 4.08

Two-way analysis of variance also indicated that gender had no significant effect on salivary melatonin levels (Table 2).

Table 2: Salivary melatonin levels (pg/mL) based on gender in the subjects

Group	Male		Female	
	S.D	mean	SD	Mean
1	0.38	5.22	0.64	5.35
2	0.38	3.34	0.23	4.36
3	0.32	3.77	0.26	4.01
4	0.31	3.75	0.42	3.96
5	0.10	3.35	0.10	3.91

All the samples in each group showed the presence of melatonin. Tukey test revealed that the highest concentration was in the healthy group (5.29 \pm 0.50), and the lowest mean level of melatonin was in the generalized severe chronic periodontitis group (3.39 \pm 0.10). Healthy subjects had significantly higher salivary melatonin level (5.29 \pm 0.50 pg/mL) compared to

patients with gingivitis (4.35 \pm 0.30 pg/mL) (P<0.001). The difference between salivary melatonin level in patients with gingivitis and periodontitis was significant (P<0.001). The means and standard deviations of salivary melatonin levels for all the groups are presented in Table 3.

Table 3. Melatonin levels (pg/mL) in saliva in all the subjects

Group	Mean \pm SD
Healthy	5.29 \pm 0.50
Gingivitis	4.35 \pm 0.31
Localized moderate chronic periodontitis	3.86 \pm 0.30
Generalized moderate chronic periodontitis	3.85 \pm 0.36
Generalized severe chronic periodontitis	3.39 \pm 0.10

DISCUSSION

Data from this study indicated that the amount of salivary melatonin varied according to the severity and extension of periodontal disease: generalized severe chronic periodontitis had the least salivary melatonin level. This finding suggests that melatonin might possess the ability to fight against infection and inflammation, probably due to its antioxidant, anti-aging, and immunoenhancing action.

Periodontal disease is well known to be associated with inflammation of periodontium that destroys periodontal ligament and alveolar bone by resorptive processes. These processes mainly involve osteoclasts, which are mediated by cytokines and local factors released by neighboring defensive cells in response to established bacterial aggression. Melatonin has a critical function in the regulation of proteins

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implicated as mediators of these processes. The receptor activator of nuclear factor-kappa B ligand (RANKL) is a highly important protein in osteoclastic differentiation and proliferation.⁽¹²⁾ Another protein, osteoprotegerin (OPG), interferes with its biologic potential. Liu et al. demonstrated that these proteins play a critical role in the development of periodontal disease, with periodontal bone destruction produced by the upregulation of RANKL and downregulation of OPG.⁽¹³⁾ Melatonin can modulate these events because it is closely related to orchestration of the molecular triad OPG/RANK/RANKL.⁽¹⁴⁾ Similar findings have been observed in other studies.^(10,15,16) These studies have indicated that the amount of salivary melatonin decreases from clinically healthy subjects to subjects with periodontitis.

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