Hindawi Publishing Corporation
Journal of Oral Diseases
Volume 2014, Article ID 307402, 4 pages
http://dx.doi.org/10.1155/2014/307402

Research Article

Salivary Melatonin and the Severity of Attachment Loss: A Case-Control Study

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Received 8 March 2014; Revised 16 April 2014; Accepted 22 April 2014; Published 9 June 2014

Academic Editor: Gul Atilla

Background. Melatonin (MT: N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone secreted mainly by the pineal gland in the brain. MT is produced with a circadian rhythm characterized by elevated blood levels during the night. In healthy individuals, maximal secretion of MT occurs between midnight and 2:00 am, whereas the minimal production occurs during the day. MT can be determined by repeated measurement of plasma or salivary MT or urine sulfatoxy-melatonin. 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. The purpose of this study was to examine the correlation between salivary melatonin level and periodontal diseases.*Methods*. Fifty subjects by 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 among 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 ($P < 0.01$).*Conclusions*. This study determined that salivary melatonin level in patients with periodontal diseases is lower than that in healthy subjects. Consequently we conclude that there is a negative correlation between melatonin level and the severity of disease, suggesting that melatonin might have a protective role against periodontal diseases, although further research is required to validate this hypothesis.

1. Introduction

Melatonin (MT: N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone secreted mainly by the pineal gland in the brain [1]. MT is produced with a circadian rhythm characterized by elevated blood levels during the night [2]. In healthy individuals, maximal secretion of MT occurs between midnight and 2:00 am, whereas the minimal production occurs during the day [3]. MT can be determined by repeated measurement of plasma or salivary MT or urine sulfatoxy-melatonin [4]. MT also is secreted in the saliva, although its role into the mouth is not known well.

The major and important property of MT is its ability to serve as a very potent free radical scavenger [5, 6]. A very large body of evidence indicates that MT is a major scavenger of both oxygen- and nitrogen-based reactive molecules, including $ONOO^-$, at both physiologic and pharmacologic concentrations [7]. It is a potent antioxidant with immune-modulatory, protective, and anticancer properties. It also stimulates synthesis of type I collagen fibers and bone formation. Thus it can be beneficial as a treatment measure in postsurgical wounds caused by tooth extractions and other oral surgeries and in helping bone formation in various autoimmunological disorders such as Sjögren's syndrome and periodontal diseases [8]. Although the beneficial antioxidant effect of MT in the treatment of several chronic diseases, including rheumatoid arthritis, elderly patients with primary essential hypertension, and females with infertility has been recently suggested in clinical studies [7], the body of literature concerning the oral pathologies is very scarce. MT could be a potential local therapeutic measure in the mechanical, bacterial, fungal, or viral pathologies of the oral cavity as well.

Damage to periodontal tissues results from free radicals, some of which are derived from the plaque bacteria, whereas others are a consequence of the immune response. The increased generation of free radicals is associated with decreased antioxidant defense mechanisms. This imbalance between the prooxidant and antioxidant systems may lead to a further oxidative destruction and a marked deterioration of the periodontal tissues [3]. Gender does not influence the levels of MT, whereas factors such as smoking, exposure to light, alcohol consumption, and increasing age decrease the levels of salivary MT [9]. The potential association of different MT levels and periodontal diseases has been the subject of a few studies [3, 10–12]. More specifically, no studies have been conducted concerning the association of salivary MT levels and the severity and extent of attachment loss. The present study then aimed to evaluate the presence of MT in saliva and assess the levels of salivary MT in periodontal health and disease.

2. Methods

The study population of the present case-control study, conducted during February and March of 2011, included 50 subjects (24 females and 26 males) having an age from 18 to 65 years with the mean age of 40.44 ± 6.38 years. All subjects signed an informed consent before participation in the study. The study was approved by the ethics committee of Ahvaz Jundishapur School of Dentistry.

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, and 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.

2.1. Subject Inclusion Criteria. The inclusion criteria were as follows:

(1) a varying degree of periodontal disease,

(2) good general health,

(3) no invasive periodontal therapy during the previous 6 months.

2.2. Subject Exclusion Criteria. The 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, for example, 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 having undergone noninvasive periodontal therapy (scaling and root planing).

2.3. 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.

3. Results

All samples in each group showed the presence of MT. One-way analysis of variance showed no statistically significant difference between all study groups in terms of age ($P > 0.05$). Means and standard deviations of age and the salivary levels of MT for each group are presented in Table 1.

In general, the salivary MT level of the patients in the control, gingivitis, and chronic periodontitis groups (sum of all the patients of the present study with chronic periodontitis) was 5.29 ± 0.50, 4.35 ± 0.31, and 3.70 ± 0.35, respectively. Healthy subjects had significantly ($P < 0.001$) higher salivary MT level compared to patients with gingivitis. The difference

Table 1: Comparison of age and salivary melatonin levels based on gender and study subjects in all groups.

	Healthy	Gingivitis	Localized moderate CP	Generalized moderate CP	Generalized severe CP
Age	40.5 ± 6.02	37.2 ± 6.79	38.6 ± 6.58	41.2 ± 6.56	44.7 ± 4.08
Male MT level (pg/mL)	5.22 ± 0.38	4.34 ± 0.38	3.77 ± 0.32	3.75 ± 0.31	3.35 ± 0.10
Female MT level (pg/mL)	5.35 ± 0.64	4.36 ± 0.23	4.01 ± 0.26	3.96 ± 0.42	3.41 ± 0.10
Total MT level (pg/mL)	5.29 ± 0.50	4.35 ± 0.31	3.86 ± 0.30	3.85 ± 0.36	3.39 ± 0.10

C.P: chronic periodontitis.

between salivary MT level in patients with gingivitis and periodontitis was also significant ($P < 0.001$).

Two-way variance analysis showed a statistically significant difference in the salivary MT levels between the different study groups ($P < 0.001$). Tukey's test revealed that the mean concentration of salivary MT in the healthy group was statistically the highest of all among the study groups ($P < 0.001$). Also patients with generalized severe chronic periodontitis showed the statistically lowest mean MT values ($P < 0.001$). The mean salivary MT levels were not significantly different between the patients with moderate localized and generalized chronic periodontitis ($P > 0.05$). However, these values were statistically higher than that of the patients with severe generalized periodontitis ($P < 0.01$).

4. Discussion

Findings of the present study indicate that the amount of salivary MT may vary according to the severity and extension of periodontal disease. Similar findings were observed in other studies [9, 10, 12]. These studies indicated that the amount of salivary MT decreased from clinically healthy subjects to subjects with periodontitis. In our series, generalized severe chronic periodontitis had the least salivary MT level. This finding suggests that MT may possess the ability to fight against infection and inflammation, probably due to its antioxidant, antiaging, and immune-enhancing action. Further studies with different extent and severity of chronic periodontitis are needed with even more patient population in order to help assess the reliability of such hypothesis with higher precision. In accordance with the literature [9, 11], gender was not influential in the salivary level of MT.

A few studies had concerned the association between salivary MT levels and the presence of periodontal diseases [9–12]. Decreased salivary level of MT in the diseased groups, compared to the healthy group, was consistent with those of Cutando et al. [3, 10, 11] and that of Srinath et al. [9]. Also, consistent with the findings of Srinath et al. [9], the salivary level of MT was lower in the patients with gingivitis compared to healthy adults. However, these studies included patients based on community periodontal index (CPI) in which scores reflected the periodontal status. Cutando et al. [10] suggested that salivary levels of MT were lower in patients with a CPI index of 2 or less. This value increases with an increase in CPI score, reaching the highest levels in patients with a CPI index of 4. The problem with the application of CPI is that it does not measure the clinical attachment loss. This is of utmost importance in cases where gingival recession accompanies attachment loss and conceals the progressive disease [13]. The mean salivary MT level in our group of patients was higher in subjects with moderate periodontitis than in those with severe periodontitis. Also this value was higher in healthy individuals compared to those with moderate periodontitis. However, the mean salivary MT level was not statistically different in patients with the same severity but different extension of the periodontal involvement (localized versus generalized moderate chronic periodontitis). On the other hand, the mean salivary MT level was statistically higher in patients with higher severity and the same extension of the periodontal disease (moderate versus severe generalized chronic periodontitis). These findings might suggest that the extent of periodontitis is less a factor of salivary MT level than its severity. Authors of the present paper are currently conducting a study, including a comparison of the patients with localized moderate chronic periodontitis, localized severe chronic periodontitis, and generalized severe chronic periodontitis, to further examine the reproducibility of the results.

The resorptive process of attachment loss mainly involves osteoclastic activity and is mediated by cytokines and local factors released by defensive cells in response to bacterial aggression. MT has a critical function in the regulation of proteins 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 [14]. Another protein, osteoprotegerin (OPG), interferes with its biologic potential. Liu et al. [15] demonstrated that these proteins play a critical role in the development of periodontal disease, with periodontal bone destruction produced by the upregulation of RANKL with the downregulation of OPG. MT can modulate these events because it is closely related to orchestration of the molecular triad OPG/RANK/RANKL [16].

Recent studies have shown that MT is synthesized not only in the brain but also by the numerous cells, including retina, ovary, Harderian gland, placenta, kidneys, respiratory tract, and, finally, the gastrointestinal system, where MT has been found to be generated in EE cells in about 500 times larger amounts than in pineal bodies. This gland produces

MT in a circadian manner, synchronizing a number of biologic processes in a day-night rhythm [11]. It will then be beneficial to detect the possible association of the MT levels of individuals with night shift jobs with their periodontal status.

Free radicals that burst coming from the phagocytic cells, such as neutrophils and macrophages, migrating to the inflammation place, damage significantly the gingival tissue [8]. MT promotes osteoblast differentiation and bone formation. At micromolar concentrations, MT stimulates the synthesis of type 1 collagen fibers in human osteoblasts in vitro; in addition, it increases the genic 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 MT are osteoclasts, which reabsorb existing bone through the generation of free radicals [4]. MT also supports several intracellular enzymatic antioxidant enzymes, including SOD and glutathione peroxidase (GSH-Px). Moreover, MT induces the activity of γ-glutamylcysteine synthetase, thereby stimulating the production of another intracellular antioxidant, glutathione (GSH). A number of studies had shown that MT is significantly better than the classic antioxidants in resisting free-radical-based molecular destruction. In these in vivo studies, MT was more effective than vitamin E, β-carotene, and vitamin C [8].

To the best of our knowledge, no studies have been conducted concerning the in vivo administration of MT to detect its possible preventive or protective effect. The present literature on the salivary or systemic melatonin and its association with the state of health of periodontal apparatus is scarce and at best suggests that an association between MT level and gingival and periodontal health exists. However, there are no studies to date concerning a possible cause and effect relation between the salivary level of MT and the periodontitis. On the other hand, since attachment loss is difficult in treatment such studies may face substantial methodological challenges. We should, however, remember that gingivitis is the primary manifestation of periodontal involvement. It will then be possible to conduct such interventions on the treatable gingivitis patients.

Within the limitations of the present study, different salivary levels of MT were associated with different states of health in periodontal apparatus. Salivary levels of melatonin were seemingly associated reversely with the extent of periodontal attachment loss while such an association is not supported for the extent of the periodontal attachment loss.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank and appreciate the Research Center of Ahvaz University of Medical Sciences and deny any competing conflict of interest.

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