Evaluation of melatonin levels in saliva in gingivitis and periodontitis cases: A pilot study

Karuna Lodhi, Charanjit Singh Saimbi, Mohammad Akhlaq Khan, Chandishwar Nath[1], Rakesh Shukla[1]

Abstract

Objective: To evaluate the melatonin levels in saliva in gingivitis and periodontitis cases. **Background:** Melatonin has strong antioxidant, free radical scavenging, and immunomodulating properties, acts on osteoblasts directly to stimulate cell proliferation and synthesis of Type I collagen, and promotes bone formation. **Materials and Methods:** A total of thirty participants were selected and divided into three groups (control group, gingivitis group, and periodontitis group). In each group, ten participants were taken. Salivary melatonin was estimated in each of the three groups. **Results:** Results from this study showed that the mean levels of salivary melatonin increased as severity increased from control to periodontitis, i.e., the mean levels were highest in periodontitis followed by gingivitis and least in control group. The melatonin level of all participants was positively and significantly ($P < 0.01$) correlated with their gingival index ($r = 0.85$, $P < 0.01$) and probing depth ($r = 0.72$, $P < 0.01$). **Conclusion:** Salivary melatonin level varied with the severity of gingivitis and periodontitis. With increased severity of periodontal disease, the level of salivary melatonin also increased suggesting that salivary melatonin may act as a diagnostic biomarker for periodontal diseases.

Keywords: Gingivitis, melatonin, periodontitis, saliva

Introduction

Periodontal disease is an inflammatory process characterized by periodontal pockets, attachment loss, and bone loss.[1] The status of disease ranges from gingivitis to advanced periodontitis with destruction of connective tissue attachment and alveolar bone which can ultimately lead to tooth loss.[2] Periodontal tissue damage is caused by direct effect of the toxic products released by the bacterial plaque and from the action of the host immune system, stimulated by the bacterial infection.[3] The important characteristic of periodontal disease pathogenesis is the generation of free radicals, some of which are derived from the bacteria themselves and others are derived from the host immune response.[4] It has been suggested that increase in both reactive oxygen species (ROS) which are hydroxyl (OH) radical, hydrogen peroxide (H_2O_2), singlet oxygen (O_2^-), and hypochlorous acid (HOCl) and reactive nitrogen species which are nitric oxide, peroxynitrite anion ($ONOO^-$), and peroxynitrous acid (ONOOH) during periodontitis is responsible for the oxidative damage to periodontal tissues.[5] Thus, raised free radical generation coexists with decreased antioxidant defense. This disturbed balance between the pro-oxidant and antioxidant systems may result in added oxidative attack and extensive worsening of the periodontal tissues.

Melatonin is mainly secreted by the pineal gland in circadian manner. It affects many physiologic processes, such as the control of circadian rhythm, the regulation of the body temperature, and the activation of the immune system.[6] After the release of melatonin in blood stream, it diffuses into saliva passively. The fraction of plasma melatonin passing through salivary glands into the mouth appears to be constant ranging from 24% to 33%.[7] About 70% of plasma melatonin is bound to albumin. Thus, free fraction of melatonin is present in saliva.[8] This study was conducted with the aims and objectives to observe the melatonin levels in saliva in gingivitis and periodontitis cases.

Department of Periodontics, Faculty of Dental Sciences, C. S. M. Medical University, [1]Division of Pharmacology, CDRI, Lucknow, Uttar Pradesh, India

Correspondence: Dr. Karuna Lodhi,
SRA 26A, Shipra Riviera, Gyankhand 3, Indirapuram,
Ghaziabad - 201 014, Uttar Pradesh, India.
E-mail: karunaweb11@gmail.com

Access this article online	
Quick Response Code:	Website: www.contempclindent.org
	DOI: 10.4103/0976-237X.194115

How to cite this article: Lodhi K, Saimbi CS, Khan MA, Nath C, Shukla R. Evaluation of melatonin levels in saliva in gingivitis and periodontitis cases: A pilot study. Contemp Clin Dent 2016;7:519-23.

Materials and Methods

A total of thirty participants with age group between 18 and 65 years were selected for this study from the Department of Periodontics, Faculty of Dental Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India. Ten participants each were taken from control group (without disease), gingivitis group, and periodontitis group on the basis of disease severity. Participants without the presence of diseases which may affect the immune system (such as chronic infections and oncological disorders) and treatment which can change the melatonin levels were enrolled. Before starting the study, informed written consent from all the participants was obtained. The Institutional Ethical Committee approval was taken. All the participants selected for this study underwent oral examination for periodontal assessments, and medical history was taken. Gingival index by Loe and Silnes method and probing depth measured by UNC-15 probe were recorded for each participant.

Assessment of salivary melatonin levels

Participants were refrained from eating during sampling period. Beverages containing artificial colorants as well as alcohol, coffee, and bananas were avoided during sampling period. Salivary flow stimulation by chewing gums and eating lemons was also avoided to prevent any possible interference.

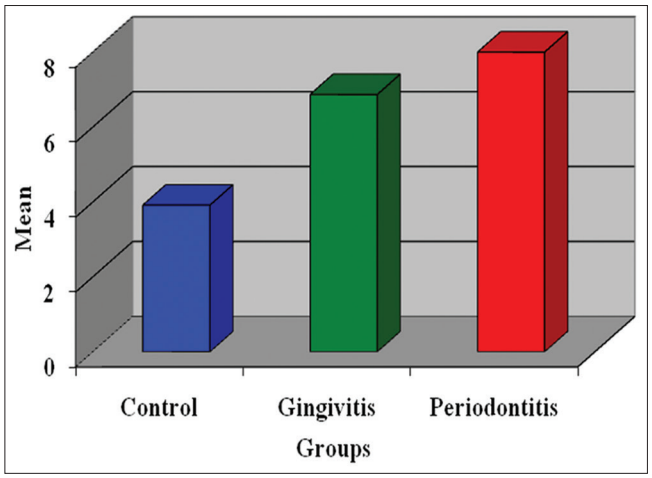
Samples were collected at 9:15 A.M. using the BUHLMANN saliva collection device. Cotton swab provided in the device was kept in the patient's mouth below the tongue. One cotton swab could absorb up to 3 ml of saliva. The samples were stored frozen at ≤ -20°C. Frozen samples were thawed and centrifuged at 1000 $\times g$ and 2°C–8°C for 5 min before assay. Clear supernatant was transferred to a new vial. The collected sample was then transferred for analysis. The amount of melatonin in saliva was determined by the BUHLMANN direct saliva melatonin ELISA, a competitive immunoassay using a capture antibody (Ab) technique, according to manufacturer's instructions. The antimelatonin Ab was coated onto the microtiter plate. After the first 16–20 h overnight incubation, melatonin present in cases and controls as well as in the calibrators competed with biotinylated melatonin during the second 3-h incubation for the binding sites of this highly specific Ab. After washing, the enzyme label, streptavidin, conjugated to horseradish peroxidase is added, this binds during the third incubation step (1 h) to the melatonin-biotin Ab complexes captured on the coated wells. The unbound enzyme label was then removed by the second washing step, and tetramethylbenzidine substrate was added to the wells. In the fourth 30-min incubation step, a chromophore was formed in inverse proportion to the amount of melatonin present in the sample. The intensity of the color was measured at 450 nm. The concentration of melatonin was determined in picograms/milliliter (pg/ml).

Statistical analysis

Due to small sample size, heterogeneous variance, and skewed nature of the data, the studied variables were analyzed using nonparametric tests. Groups were compared by Kruskal–Wallis (H) one-way analysis of variance (ANOVA), and the multiple comparisons of mean ranks between groups were done by *Z*-test. Spearman rank-order correlation (r_s) method was used to assess relative association among the variables. A two-tailed ($\alpha = 2$) $P < 0.05$ was considered to be statistically significant, $P < 0.01$ as highly significant, and $P > 0.05$ the not significant. GraphPad Prism (version 3, GraphPad Software. Inc., California) and STATISTICA (version 7.0, StatSoft.Inc. (now a part of Dell)) were used for the analysis.

Results

The salivary melatonin level, gingival index, and probing depth of three groups of participants were shown in Graphs 1 and 2 and summarized in Tables 1 and 2, and clinically correlating photographs are given in Figures 1-3. The salivary melatonin levels of control group ranged from 0.80 pg/ml to 6.80 pg/ml with an average (±standard error [SE]) 3.92 ± 0.58 pg/ml while it ranged from 3.30 pg/ml to 10.90 pg/ml with an average (±SE) 6.87 ± 0.82 pg/ml in



Graph 1: Mean salivary melatonin levels (pg/ml) in participants of control group, gingivitis group, and periodontitis group

Table 1: Summary of melatonin level (pg/ml) of control group, gingivitis group, and periodontitis group participants

Statistic	Control group	Gingivitis group	Periodontitis group
Minimum	0.80	3.30	5.20
Maximum	6.80	10.90	14.80
Range	6.00	7.60	9.60
Mean	3.92	6.87	10.20
SD	1.85	2.61	3.11
SE	0.58	0.82	0.98

SD: Standard deviation; SE: Standard error

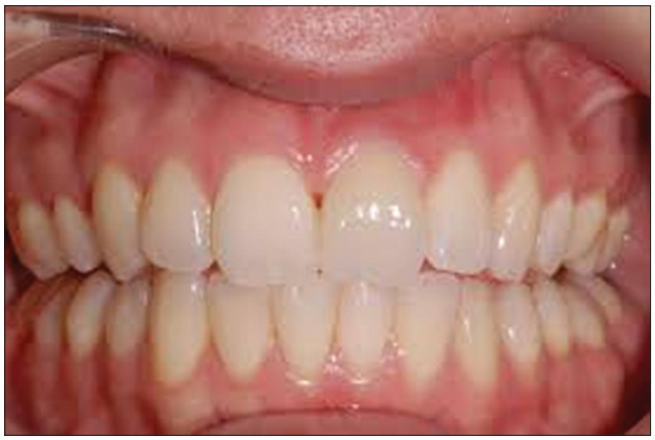


Figure 1: Clinical photograph of control group participant in which salivary melatonin levels (pg/ml) were lesser than other two groups (mean = 3.92)

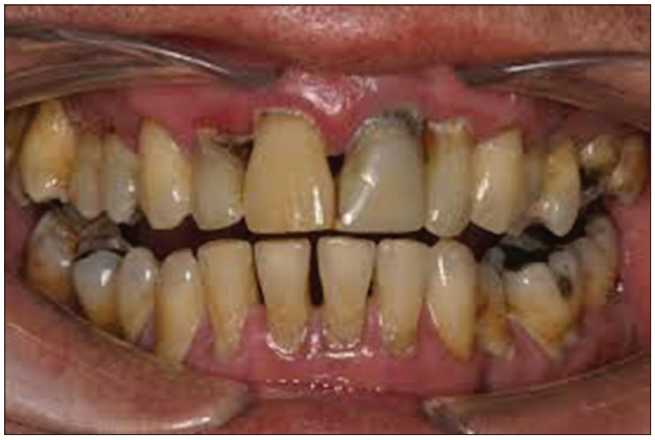


Figure 3: Clinical photograph of periodontitis group in which highest levels of salivary melatonin (pg/ml) were found (mean = 10.20)

gingivitis group and from 5.20 pg/ml to 14.80 pg/ml with an average (±SE) 10.20 ± 0.98 pg/ml in periodontitis group.

Comparing the levels of salivary melatonin of all three groups together, the ANOVA [Table 2] revealed that the mean levels of salivary melatonin among groups differed significantly ($F = 14.93$, $P < 0.01$). The correlation between severity (severity of disease of control group was ranked 1, gingivitis as 2, and periodontitis as 3), melatonin level, gingival index, and probing depth in all three groups of participants was summarized in Table 3. All variables showed positively significant ($P < 0.01$) correlation with each other.

Estimation of melatonin level from each gingival index and probing depth was also done separately by simple linear regression analysis, considering the melatonin level as a dependent variable and gingival index and probing depth the independent variables [Graph 3]. The regression

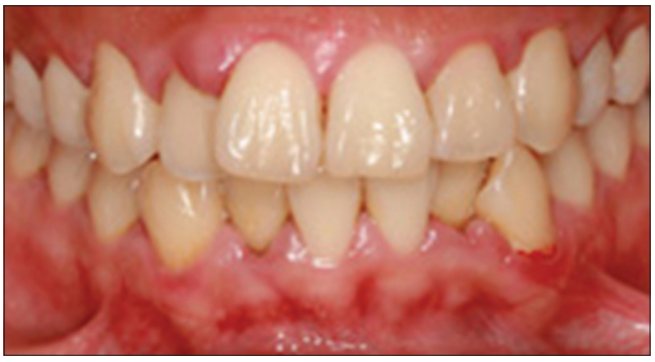
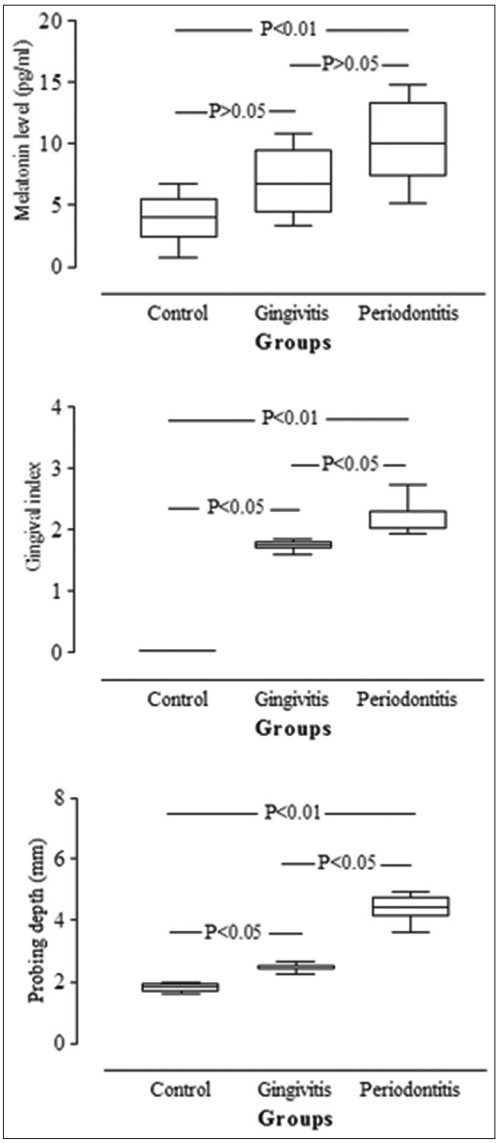


Figure 2: Clinical photograph of gingivitis group participant in which salivary melatonin levels (pg/ml) were greater than control group and lesser than periodontitis group (mean = 6.87)



Graph 2: Box and whiskers plot showing the distribution of melatonin level (pg/ml), gingival index, and probing depth (mm) in three groups of participants

Table 2: One-way analysis of variance of salivary melatonin levels of control group, gingivitis group, and periodontitis group participants

SV	SS	df	MS	FR
Between groups	197.70	2	98.84	14.93**
Residual	178.70	27	6.62	
Total	376.40	29		

**P<0.01. SV: Source of variation; SS: Sum of square; df: Degrees of freedom; MS: Mean square; FR: F ratio

Table 3: Correlation (n=30) between variables of three groups of participants

Variables	Severity	Melatonin	Gingival index	Probing depth
Severity	1.00			
Melatonin	0.75**	1.00		
Gingival index	0.95**	0.85**	1.00	
Probing depth	0.94**	0.72**	0.90**	1.00

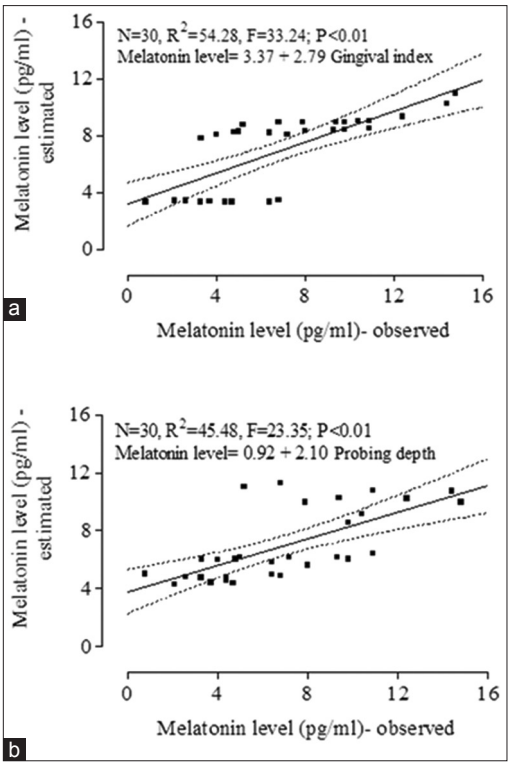
**P<0.01

analysis showed significant effect of both gingival index ($F = 33.24$; $P < 0.01$) and probing depth ($F = 23.35$; $P < 0.01$) on melatonin level which can account (estimate) 54.3% and 45.5% of the total variations (i.e., coefficient of determination; R^2) of melatonin level, respectively. The best fit regression equation to estimate melatonin level from gingival index and probing depth follows as:

- Melatonin level (pg/ml) =3.37 + 2.79 gingival index (score) (i)
- Melatonin level (pg/ml) =0.92 + 2.10 probing depth (mm). (ii)

Discussion

Periodontal diseases are known to be exacerbated by free radicals and by altered host immune response to microorganisms present in plaque. In periodontitis, polymorphonuclear neutrophils infiltration is a key finding, which produces high amounts of ROS. In addition, a considerable migration of neutrophil to the gingiva and gingival fluid during periodontitis leads to excessive production of ROS.[9] Hence, this increase in the free radical production in periodontal diseases can lead to rise in melatonin level due to its free radical scavenging and antioxidant properties. Melatonin can alter the development of the periodontal disease because it can act on PGE_2, thereby inhibiting the differentiation of the osteoclasts induced by cell-to-cell contact between osteoclasts and osteoblasts.[10] Melatonin can also modulate all proteins that regulate the resorption process in the periodontal disease and interact with other biologic agents such as calcitonin, parathormone and 1α 25$(OH)_2D_3$, or interleukin (IL)-2, IL-1β, and IL-6, and osteoprotegerin/receptor activator of nuclear factor-κB ligand system.[11-13] Thus, melatonin diminishes bone resorption or promotes the bone marrow cell differentiation. Melatonin



Graph 3: Best fit regression equation to estimate melatonin level (pg/ml) from gingival index (scores) (a) and probing depth (mm) (b) with 95% confidence interval for β (broken lines)

also has antimicrobial activity against some bacterial species present in plaque.[14]

In contrast to previous studies which reported decreased salivary and serum melatonin levels in periodontitis, in the present study, authors reported increased salivary melatonin levels with increased severity of periodontal disease and suggested that this increase may be result of a signal(s) derived from periodontal inflammation in oral cavity.[15,16]

Conclusion

Salivary melatonin may act as a diagnostic biomarker for periodontal diseases. Further research is required to make clear the role of melatonin in the pathogenesis of periodontal disease and to understand its clinical relevance.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Ridgeway EE. Periodontal disease: Diagnosis and management. J Am Acad Nurse Pract 2000;12:79-84.
2. Loesche WJ. Bacterial mediators in periodontal disease. Clin Infect Dis 1993;16 Suppl 4:S203-10.

3. Zambon JJ, Reynolds H, Fisher JG, Shlossman M, Dunford R, Genco RJ. Microbiological and immunological studies of adult periodontitis in patients with noninsulin-dependent diabetes mellitus. J Periodontol 1988;59:23-31.
4. Gustafsson A, Asman B. Increased release of free oxygen radicals from peripheral neutrophils in adult periodontitis after Fc delta-receptor stimulation. J Clin Periodontol 1996;23:38-44.
5. Kimura S, Yonemura T, Kaya H. Increased oxidative product formation by peripheral blood polymorphonuclear leukocytes in human periodontal diseases. J Periodontal Res 1993;28:197-203.
6. Laakso ML, Porkka-Heiskanen T, Alila A, Stenberg D, Johansson G. Correlation between salivary and serum melatonin: Dependence on serum melatonin levels. J Pineal Res 1990;9:39-50.
7. McIntyre IM, Norman TR, Burrows GD, Armstrong SM. Melatonin rhythm in human plasma and saliva. J Pineal Res 1987;4:177-83.
8. Reiter RJ. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr Rev 1991;12:151-80.
9. Battino M, Bullon P, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: The challenge of anti-oxidants to free radicals and reactive oxygen species. Crit Rev Oral Biol Med 1999;10:458-76.
10. Takahashi N, Nobuyuki U, Takami M, Suda T. Cells of bone. Osteoclast generation. In: Bilezikian J, Raisz L, Rodan G, editors. Principles of Bone Biology. 2nd ed. San Diego: Academic Press; 2002. p. 109-26.
11. Sandyk R, Anastasiadis PG, Anninos PA, Tsagas N. Is postmenopausal osteoporosis related to pineal gland functions? Int J Neurosci 1992;62:215-25.
12. Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: Involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev 2004;15:457-75.
13. Reiter RJ, Rosales-Corral SA, Liu XY, Acuna-Castroviejo D, Escames G, Tan DX. Melatonin in the oral cavity: Physiological and pathological implications. J Periodontal Res 2015;50:9-17.
14. Srinath R, Acharya AB, Thakur SL. Salivary and gingival crevicular fluid melatonin in periodontal health and disease. J Periodontol 2010;81:277-83.
15. Giordano M, Palermo MS. Melatonin-induced enhancement of antibody-dependent cellular cytotoxicity. J Pineal Res 1991;10:117-21.
16. Morrey KM, McLachlan JA, Serkin CD, Bakouche O. Activation of human monocytes by the pineal hormone melatonin. J Immunol 1994;153:2671-80.