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Efficacy of Melatonin Supplementation as an Adjunct to Periodontal Therapy

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Original Article

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ABSTRACT

Background: Melatonin because of its numerous physiological actions is considered a powerful cell protector against molecular damage. It appears to play a key role in the control of periodontitis due to its protective, antioxidant, anticancer, and immunomodulatory properties.

Aim: To evaluate the efficacy of melatonin by oral supplementation (3mg per day for 20 days) in periodontal disease and its effect on the level of IL-8 in the serum sample.

Settings and Design: Non-randomized clinical trial.

Methods and Material: Thirty subjects were categorized into control (periodontally healthy) and experimental groups (generalised periodontitis). Non-surgical periodontal therapy (NSPT) was performed in the given groups. The experimental group received oral administration of 3mg melatonin tablet once daily at bedtime for 20 days. The parameters of the gingival index, plaque index, probing depth, clinical attachment level, and serum level of IL-8 were evaluated in all participants at baseline and after 20 days in the experimental group.

Statistical analysis used: Wilcoxon signed-rank test, Chi-square ($\chi 2$) test, and Kolmogorov-Smirnov test were performed on the data acquired.

Results: After the intervention of melatonin, a statistically significant reduction in probing depth, plaque index, clinical attachment level, and the gingival index was observed. The increased levels of serum IL-8 were found after treatment with the melatonin supplementation group though the p-value was not found statistically significant.

Conclusions: Oral melatonin supplementation (3mg per day for 20 days) may improve the periodontal status of patients. Therefore, melatonin supplements may be considered a part of the therapeutic approach for non-surgical periodontal therapy.

Key Messages: Oral administration of melatonin (3mg per day for 20 days) may improve periodontal status of patients.

Keywords: IL-8, Melatonin, Non-surgical Periodontal Therapy, Periodontal Disease.

Introduction

Periodontal disease is one of the most common diseases of the oral cavity which is characterized by the destruction of the supporting periodontal tissue that may lead to tooth loss.[1] One of the causes of periodontitis is bacterial biofilm growth on the tooth surface. In addition, local factors like calculus, plaque, environmental factors, genetics, and lifestyle habits further determine the progression of the disease.[2] Both surgical and non-surgical measures have been used to treat periodontitis. Non-surgical periodontal therapy (NSPT) is considered to be the gold standard for initial treatment as studies have shown it to improve clinical attachment levels (CAL) and pocket depths (PPD) for a moderate level of periodontitis i.e., up to 6mm PPD.[3] Oral supplementation of immunomodulant agents like melatonin as an adjunct to NSPT has been shown to be more effective in improving periodontal status. Melatonin is mostly known for its physiological function as a regulator of circadian and circannual rhythm.[4] These rhythms are coordinated by the brain's suprachiasmatic nucleus and both are important in the regulation of biological events. It also possesses antioxidant, anti-inflammatory as well as bone formative, and resorptive properties[5] which can aid in periodontitis treatment. Though melatonin is available in the diet, dietary intake estimation methods are usually susceptible to memory errors and bias.[6] Therefore, oral supplementation of melatonin was considered, and adding on a dosage of 1mg/day for the period of one month has shown considerable improvement in the periodontal status.[7] However, oral melatonin supplementation has shown poor melatonin bioavailability.[8] Therefore, an increased dosage of melatonin can be beneficial in improving the periodontal status even further and perhaps for a shorter duration. Therefore, this study is aimed to assess the efficacy of melatonin by oral supplementation of 3mg per day for 20 days in subjects suffering from periodontal disease. Clinical attachment level (CAL), Pocket depth (PPD), Gingival Index(GI), and Plaque Index(PI)are selected as parameters to assess the periodontal status. In addition, measuring interleukin -8 (IL-8) serum levels are considered as it is known to indicate the level of inflammation at the molecular level.

Materials and Methods

The study was a non-randomized clinical trial. The study protocol was approved by the institutional Ethical Committee and has been registered in the Clinical Trial Registry of India. This study was carried out from May 2020 to 2021.

A detailed medical history notably gastrointestinal disorders were taken and systemically healthy adult patients between 25-70 years having generalized periodontitis according to the definition set by World Workshop in 2017 for the classification of peri-implant and periodontal diseases and conditions were included in the study.[9] Smokers, patients with any systemic disorders like immunosuppression (pathological or drug-induced), gastrointestinal disorders, patients on anti-depressants, anti-hypertensive, anti-psychotic, and anti-coagulant medications that are known to cause drug interactions thus minimizing melatonin bio-availability, patients on antiresorptive drugs (bisphosphonates) and are currently on antibiotic therapy or other medications, pregnant and breastfeeding women and patients who have undergone periodontal therapy in last 3 months were excluded.

Thirty subjects were selected based on the above-mentioned criteria and their written informed consent was taken for participation in the study. They were further categorized into control and experimental groups after precise case history recording. The control group comprised 15 periodontally healthy subjects and the experimental group comprised 15 generalized periodontitis patients. NSPT was done with ultrasonic instruments by a single periodontist for both groups. Scaling was done as a part of NSPT in the control group of patients who were having local deposits. The control group was introduced in order to understand the mean values of IL-8

levels in periodontally healthy subjects. The experimental group was further put on a 3mg melatonin tablet once daily at bedtime for 20 days.

The clinical parameters such as gingival index (Loe and Silness 1963), probing depth, plaque index (Silness and Loe 1967), clinical attachment level, and IL-8 level were recorded at the baseline. The same parameters were recorded in the experimental group after 20 days.

All patients were advised to maintain oral hygiene and were instructed to rinse their mouth twice a day with a 0.2% solution of chlorhexidine for 14 days which helped in adequate plaque control. The patients were advised to report any side effects like sleepiness, circadian rhythm disruption, daytime fatigue, headache, and irritability observed during the treatment period.

A blood sample of 5 ml was collected from the antecubital vein at baseline in both the groups as well as after 20 days in the experimental groups. The serum was collected using centrifugation (10 min at 1300 rpm) and was stored at -80°C until analysis. Further, the serum samples were processed using commercially available Human IL-8 ELISA in accordance with the manufacturer's instructions (Human IL-8 GENLISA™ ELISA KB1070#).

All the characteristics were summarized descriptively. Mean ± standard deviation (SD) was the statistical tool used for continuous variables. Frequency and percentage were used for data summarization and diagrammatic representation for categorical data. Chi-square ($\chi 2$) test was used to observe if there was any significant association between the two categorical variables. The difference in the means of analysis variables between the two independent groups was tested by the Kolmogorov-Smirnov test. Wilcoxon signed-rank test was used to compare paired observations. The p-value was set to 0.05 thus, the results were considered to be statistically significant if Chi-square ($\chi 2$) test results came less than the defined p-value or else it was statistically insignificant. Data were analyzed using SPSS software v.23 (IBM Statistics, Chicago, USA) and Microsoft office 2007.

Results

During the study period, none of the patients reported any side effects. The subjects selected in the control and experimental groups were aged an average of 29.4 years and 37.7 years respectively.

At the baseline, the difference in the age in both control and experimental groups was found to be statistically significant. The demography characteristics were statistically significant in both groups. Patients in the experimental group had significantly higher levels of PI,GI,PD, and CAL than control subjects. There was a significantly lower IL-8 level among patients with periodontitis at baseline than in the control group (Table 1).

Parameters	Experimental group (n=15)			Control group (n=15)			p value
	Mean	SD	Median	Mean	SD	Median	
Age(years)	37.7	9.3	35.0	29.4	3.1	29.0	0.009*
Gender Male (N)(%)	9 (60%)			5 (33.3%)			0.143
Female (N)(%)	6 (40%)			10 (66.7%)			
Plaque index	1.66	0.45	1.70	0.25	0.17	0.20	<0.001*
Gingival index	1.85	0.67	1.80	0.00	0.00	0.00	<0.001*
Pocket depth	4.96	1.11	4.95	1.41	0.45	1.45	<0.001*

Clinical attachment level	5.29	1.23	5.09	1.48	0.24	1.45	<0.001*
IL-8 Level pg/ml	2.89	3.09	1.52	19.52	20.09	9.09	0.009*

Note: SD Standard Deviation, p value* significant at 5% level of significance (p<0.05), pg/ml picogram per milliliter, n- number of subjects, IL- Interleukin.

Table 1: Baseline characteristics of the subjects

After 20 days of melatonin intervention, the experimental group showed a significant reduction in PI, GI, and PPD.CAL gain was noted as compared to the baseline values. The observations were found to be statistically significant (P=) as well. Increased IL-8 serum levels were also observed after the treatment with melatonin, but the p-value was not found statistically significant (Table 2 and Figure 1).

Parameters	Baseline			After 20 days			p value
	Mean	SD	Median	Mean	SD	Median	
Plaque index	1.66	0.45	1.70	0.40	0.29	0.30	0.001*
Gingival index	1.85	0.67	1.80	0.66	0.38	0.70	0.001*
Pocket depth	4.96	1.11	4.95	3.44	0.75	3.43	0.001*
Clinical attachment level	5.29	1.23	5.09	3.83	0.90	3.70	0.001*
IL-8 Level (pg/ml)	2.89	3.09	1.52	5.05	8.87	1.29	0.691

Note: SD Standard Deviation, p value* significant at 5% level of significance (p<0.05), pg/ml picogram per milliliter, IL- Interleukin.

Table 2: Distribution of parameters between baseline to 20 days among experimental group

Discussion

Periodontal disease is a complex disease arising from the interaction of bacterial infection and host response along with environmental, acquired, and genetic risk factors. Numerous host-modulating agents have been tried to restore the balance between pro-inflammatory and anti-inflammatory mediators in periodontal disease. Melatonin is an indoleamine produced by the pineal gland possessing significant free radical scavenging, anti-microbial, anti-inflammatory, osteo-promoting, and bone loss inhibitory properties.[5]

Melatonin is highly lipophilic. It reaches every cell in the organism. It has been used in various dosages as an adjunct to treat periodontitis.1% melatonin topical application has shown improvement in diabetic patients with periodontitis.[10] It has also been reported that supplementation of melatonin capsules 1mg per day for a period of 30 days after NSPT resulted in improvement of clinical parameters in stage III periodontitis patients.[7] The efficacy of oral supplementation of melatonin dosage 6mg for 8 weeks in addition to NSPT has been investigated on periodontitis patients with type 2 diabetes mellitus. The study revealed improvement in inflammatory and periodontal status upon the higher dosage of melatonin oral supplementation.[11] Hence this study focused on evaluating the efficacy of tablet melatonin (3mg per day) as an adjunct to NSPT for a shorter period (20 days) in systemically healthy patients with periodontitis. According to the author's knowledge, this is the first trial with 3mg of melatonin supplementation.

The results of this clinical trial confirm the beneficial application of clinical parameters for periodontal disease. The influence of scaling and root planing has shown a reduction in the amounts of interleukin IL-1b, IL-8, and MMP-8 in gingival crevicular fluid and improvement in clinical parameters being plaque index, gingival index, pocket depth, and clinical attachment loss from patients with chronic periodontitis when assessed at baseline and at 1 and 4 weeks after treatment.[12] In another study, Anderson R et al., compared the effectiveness of a photo disinfection process to that of scaling and root planing for NSPT and evaluated clinical parameters at baseline, three weeks, six weeks, and 12 weeks following therapy.[13] Hence in this study, an evaluation of the parameters was done 20 days after the intervention in order to comprehend the effectiveness at a shorter duration.

The present study predominantly revealed a significant decrease in PI,GI,PD, and CAL gain after the intervention. This might be due to the known antimicrobial activity of melatonin against periodontal pathogens like *Porphyromonas gingivalis, Streptococcus mutans,* and *Prevotella intermedia* which are primarily involved in periodontal disease.[14] Melatonin's lipophilic nature adds to its antimicrobial property as it can cross the bacterial cell wall and inhibit growth. It has also been reported that it is very effective in inhibiting Gram-negative organisms.[15] This corroborates with another study which reported that melatonin had decreased the formation of microbial plaque.[11] The significant improvement of the periodontal status in our investigation allows us to speculate that melatonin is acting as an anti-inflammatory agent that accelerates the healing process. This assumption can be promoted by the ability to bind to cyclooxygenase isoenzymes, COX-1 and COX-2.[16] These enzymes catalyze the formation of prostaglandins, thromboxane, and levuloglandins.[17] Melatonin is known to bind to their active sites indicating its probable role as a natural inhibitor of these enzymes and thereby exhibiting anti-inflammatory action.[16] Melatonin also regulates receptor activators of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin synthesis, thus inducing osteoblastogenesis and inhibiting osteoclastogenesis.[7] Hence, it helps in bone formation as well as protects bone cells from oxidative stress.

Studies have shown a direct correlation between the levels of cytokines such as IL-6 and IL-1β with melatonin.[18] We evaluated the level of IL-8 which is the foremost chemokine in periodontal diseases. IL-8 is a known pro-inflammatory cytokine which regulates the body's inflammatory response. It is associated with the activation of neutrophils at the inflammatory site. Thus, it manifests its defensive action against periodontal pathogens, which migrates from the peripheral blood to the gingiva.[19] The present study reported a slight increase in IL-8 levels from baseline to 20 days after intervention in the periodontitis group. This increased serum IL-8 level could be due to hidden infections. Studies have reported serum IL-8 level as the earliest cytokine to increase inflammatory conditions and could persist for a longer period due to hidden infection. The current study contradicted other short-interval studies, which measured serum IL-6 levels after 20 days of topical application of melatonin.[18] Even though short-term studies can demonstrate improvements in clinical parameters; it may not be the same with the serum level of every cytokine. This concept is advocated in a study which stated that 12 weeks of NSPT may not be adequate to establish any biochemical changes.[20] Furthermore, a study conducted observed a decline in the levels of systemic biomarkers such as CRP, interleukin-6, von Willebrand factor, and soluble E-select in post-NSPT in 6 months.[18] A systematic review proposed that post-NSPT's short-term inflammatory response occurred through a steady decline in systemic inflammation as well as amelioration of endothelial function.[21] Therefore, considering the shorter period used in this trial to analyse the change in serum IL-8 levels post-melatonin treatment may be inadequate to conclude the effects of melatonin along with NSPT on pro-

inflammatory IL-8 serum levels in periodontitis subjects.[19] Though periodontitis is an inflammatory response to the bacteria, the inherent susceptibility of an individual determines the final result of the disease process through the release of mediators.[22]

Conclusion

The study proposes that oral supplementation of melatonin (3mg daily for 20 days) may improve the periodontal status of patients. Therefore, melatonin oral supplementation as an adjunct to NSPT can be considered as a therapeutic approach for periodontal diseases.

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