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The effect of adjunctive use of melatonin as a supplement on serum ferritin level in periodontal patients: A randomized, controlled trial

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ABSTRACT

Background: Ferritin is an acute-phase protein that is increased in inflammatory diseases. Melatonin has been studied due to its antioxidant effects and the elimination of free radicals. The aim of this study was to evaluate the effect of melatonin supplement as an adjunct to routine periodontal treatment on serum ferritin levels in patients with periodontitis.

Materials and Methods: Forty patients with chronic periodontitis were included in this randomized controlled clinical trial study. Twenty patients received nonsurgical periodontal treatment and twenty patients received nonsurgical periodontal treatment with adjunctive use of melatonin. Serum ferritin concentrations and periodontal parameters were recorded at baseline and 3 months after periodontal therapy. Serum ferritin level and periodontal parameters comparison at baseline and 3 months after periodontal treatment was done by Wilcoxon signed-rank test and paired sample *t*-test, respectively. Differences between test and control groups were evaluated by Mann–Whitney U-test for ferritin level and independent *t*-test for periodontal parameters, and a $P \leq 0.05$ was considered statistically significant.

Results: Serum ferritin level decreased in both the control and test group in 3 months follow-up compared to baseline ($P < 0.001$). The reduction in ferritin levels in the test group was more than the control group, but this difference was not statistically significant ($P = 0.414$). Improvement in periodontal parameters were not significant between two groups ($P = 0.489$), but improvement rates in the test group were more than control.

Conclusion: Melatonin, as an adjunct to periodontal therapy, showed additional benefits in the reduction of serum ferritin levels and improvement of periodontal parameters.

Key Words: Chronic periodontitis, dental scaling, ferritin, inflammation melatonin

INTRODUCTION

Periodontal disease is a chronic infectious disease characterized by bone and connective tissue destruction.[1] The main etiological factor in this condition is the anaerobic bacteria found in the subgingival plaque. However, the host response to bacterial products is critical in this process.[2] An important characteristic of periodontal infection is the production of free oxygen radicals by bacteria and host immune response. An imbalance between these antioxidants and pro-oxidants can lead to significant tissue degradation in periodontitis.[3] Melatonin is an endogenous hormone rhythmically generated in the

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pineal gland under the control of the Supra Chiasmatic Nucleus and the light/dark cycle. It plays a crucial role in many physiological processes, including blood pressure regulation, ovarian physiology, immune function, etc., Melatonin prescribed in several ways and is available as a supplement.[4] Melatonin has been studied in relation to bone remolding, osteoporosis, osseointegration of dental implants, and dentine formation. Melatonin has strong antioxidant effects that can play an important role in protecting cells against inflammatory processes and oxidative damage. After the release of melatonin into the bloodstream, it is secreted into the saliva. The proportion of plasma melatonin passing into the mouth via salivary glands seems to be almost stable, ranging from 24% to 33%.[5] It has been shown that salivary melatonin level depends on the severity of the periodontal disease. With the increasing severity of the periodontal disease, salivary melatonin level also increases. It is even suggested that salivary melatonin levels may be a diagnostic biomarker in periodontal disease. This association reflects the fact that melatonin may protect the body from bacterial invasions.[6] The protective role of melatonin in periodontal tissues can be attributed to antimicrobial properties,[7] immune regulation,[8] anti-inflammatory effects,[9] and the removal of free radicals created during the periodontal disease process.[10,11] Studies have shown that systemic and topical administration of melatonin in rats with periodontitis induced by ligature significantly reduced the level of inflammatory enzymes compared with the control group.[12,13] Furthermore, the administration of melatonin in diabetic rats with periodontitis reduced osteoclastic activity and bone loss.[14] Based on the results of the research regarding adjunctive use of melatonin in improving the clinical outcomes of nonsurgical periodontal treatment, treatment with melatonin as an adjunct to scaling and root planning (SRP) can improve periodontal indices compared to SRP alone.[15]

Ferritin is an acute-phase protein that increases in inflammation, autoimmune disorders, and chronic infections.[16-18] In addition to its role as an acute-phase protein, it is also involved in the storage and recycling of iron. Ferritin stores iron into non-toxic and soluble form and releases it in a controlled order way.[19] Ferritin also plays an important role in the immune response of the host, and an increased immune response leads to an increase in ferritin migration into cells to counter with infectious agents.[20] Two key factors that regulate ferritin levels are iron[21] and proinflammatory cytokines.[22] Oral infections result in a significant increase in systemic inflammatory responses that release acute phase cytokines and acute-phase proteins.[23] According to the research of Chakraborty *et al*.[24] Serum ferritin levels increased in patients with chronic periodontitis, and reduced to control level after periodontal treatment. In a study on hemodialysis patients who showed elevated levels of ferritin after anemia treatment, oral melatonin was prescribed for 30 days at night. After taking melatonin, ferritin level decreased significantly.[25] Melatonin acts as an effective agent in preventing the effects of iron overload that can lead to oxidative stress.[26]

Considering the effect of melatonin on periodontal disease and the relation of ferritin with this disease, the aim of this study was to evaluate the effect of systemic melatonin and non-surgical periodontal therapy on serum ferritin level in patients with periodontitis.

MATERIALS AND METHODS

Ethics and study design

This randomized clinical controlled trial was approved by the Research Ethics Committee of Tabriz-Iran University of Medical Sciences (protocol number: 1397.664) and registered with the local World Health Organization Registry Network (IRCT). Participants were selected from the patients referred for periodontal treatment to the Department of Periodontics from April 2017 to February 2018. Written informed consent was obtained from all patients before their participation in this study.

Subjects

A total of 40 participants (19 males and 21women, aged 25 to 45 years) participated in this study. The included patients presented with moderate-to-severe chronic periodontitis, as defined by Armitage.[27] Patients included in the study had the following criteria: (1) Good general health; (2) ≥12 natural teeth with a minimum of three in each quadrant; (3) ≥4 mm attachment loss in about a minimum of 30%of the existing teeth; and (4) 30% ≥ teeth with probing depth (PD) of ≥5 mm and bleeding on probing. Patients with the following criteria were excluded: (1) Current or previous smokers; (2) pregnant, menopause, and lactating females; (3) Iron deficiency anemia; (4) those who had received systemic antibiotics or surgical or nonsurgical periodontal treatment within the past 12 months; and (5) those who had a history of poorly

controlled diabetes, liver disease, malignancy, and radiotherapy. Patients were randomly assigned to the following two groups (control and test), including 20 patients in each group using randomization software.

Study intervention

After enrollment, all patients were trained in tooth brushing with modified bass technique and flossing twice a day. Venous blood samples were collected at baseline before recording clinical parameters. All the patients in the two groups underwent non-surgical periodontal treatment including SRP using the ultrasonic device and hand instruments. SRP was carried out by using an ultrasonic device (Various 350, NSK, Japan) and standard Gracey periodontal curettes (Hu-Fridey, Chicago, IL, USA). Periodontal treatment was performed by a single periodontist (MF). In the test group, in addition to nonsurgical periodontal treatment melatonin tablet (3 mg, once a day for 30 days) was prescribed.

All clinical evaluations were performed using a standard probe (UNC-15, Hu-Fridey Instruments, Chicago, IL, USA). Clinical measurements were conducted by a single examiner who was a trained student in this field and was blind to the study groups. Clinical measurements included PD and clinical attachment level (CAL) (were performed at four surfaces of the tooth: mesiofacial, buccal, distofacial, and lingual. The determination of examiner reproducibility was done by carrying out double clinical periodontal data recording on ten patients. Each participant was assessed twice in one appointment, and the repeat measurements were carried out masked to the first measurement. To determine the reproducibility of the examinations, each clinical parameter was recorded twice in 10 patients at a 1-h interval and in one session. Assessment of the mean difference in the scores (with 90% accuracy, *k* value ranging from 0.77 to 0.81) indicated that there was no systematic bias in the measurements. Gingival index (GI) was also recorded.[28] Clinical Resampling of venous blood and reevaluation of clinical parameters was done after 3 months. To avoid circadian rhythm changes, all venous blood samples were obtained early in the morning for the hematologic test.

Blood collection and analysis

Venous blood samples were taken from all participants at baseline and after 3 months by professional operators. The blood samples were transferred into sterile vacuum tubes with no anticoagulant and sent to the laboratory in <2 h. An automated analyzer (Tosoh co., Japan) was used to measure ferritin serum level using an enzymatic immunoassay technique.

Statistical analysis

All statistical analyses were performed using statistical software (SPSS, v. 17.0 Chicago: SPSS Inc) and a *P* value of 0.05 used as a threshold for significance. Kolmogorov–Smirnov test was used to assess the data normality distribution, which showed all periodontal parameters were distributed normally, but ferritin was found to be nonnormally distributed. Comparison of serum ferritin level and periodontal parameters at baseline and 3 months after periodontal treatment were analyzed using Wilcoxon signed-rank test and paired sample *t*-test, respectively. Differences between test and control groups were evaluated using Mann–Whitney U-test for ferritin level and independent *t*-test for periodontal parameters.

RESULTS

All participants showed a significant reduction in serum ferritin levels 3 months after periodontal treatment ($P < 0.001$) [Table 1]. The reduction in ferritin level in the test group was more than the control group, but this difference wasn't statistically significant ($P = 0.414$).

Both control and test group showed significant improvements in PD, CAL, and GI, 3 months after non-surgical periodontal treatment when compared with baseline [Table 1]. The mean PD reduction (in mm) from baseline to 3 months was 0.91 ± 0.14 for test group; 0.87 ± 0.08 for control group ($P < 0.001$). Improvement in this parameter was not significant between two groups ($P = 0.489$), but reduction rate in the test group was more than control. The mean clinical attachment gain (in mm) was 0.87 ± 0.06 at 3 months for the test group, and it was 0.83 ± 0.07 at 3 months for the control group ($P < 0.001$) with no significant difference between two groups ($P = 0.617$) but the attachment gain in the melatonin group was more than control group.

DISCUSSION

The present study was designed to evaluate the efficacy of systemic use of melatonin supplement as an adjunct to non-surgical periodontal treatment

Table 1: Comparison of serum ferritin, probing depth, clinical attachment level, and gingival index at different follow-up periods in test and control groups

Parameters	Evaluation	Control (SRP)	Test (SRP + melatonin)	Inter-group *P*
Serum ferritin (ng/mL)	Baseline	108.55±25.66	107.70±22.03	0.414
	3 months	101.15±22.84	95.20±2271	
	Intra-group *P* value	<0.001	<0.001	
PD (mm)	Baseline	3.63±0.21	3.61±0.24	0.489
	3 months	2.76±0.2	2.70±0.23	
	Intra-group *P* value	<0.001	<0.001	
CAL (mm)	Baseline	3.51±0.12	3.44±0.13	0.617
	3 months	2.68±0.1	2.68±0.1	
	Intra-group *P* value	<0.001	<0.001	
GI	Baseline	1.55±0.09	1.51±0.09	0.910
	3 months	0.63±0.08	0.62±0.07	
	Intra-group *P* value	<0.001	<0.001	

All values are means±SD.Comparisons of variables between two groups were tested with Mann-Whitney U-test for ferritin level and independent *t*-test for periodontal parameters. PD: Probing depth; CAL: Clinical attachment level; GI: Gingival index; SD: Standard division; SRP: Scaling and root planing

on serum ferritin levels and periodontal clinical parameters. It was shown that the serum ferritin in subjects with chronic periodontitis, after the systemic use of melatonin was lower than those that only receive nonsurgical periodontal therapy. But this reduction was not significant. According to previous studies, the serum ferritin level was higher in subjects with CP and decreased to control levels after periodontal treatment.[24] The level of ferritin, which is an acute-phase protein, rises in inflammation and chronic infections.[16] In addition to its role as an acute-phase protein, ferritin plays an important role in the storage of iron. Two effective factors regulate the level of ferritin, iron, and pro-inflammatory factors.[21,22,29] Periodontal disease is involved in the destruction of periodontal supporting tissues through the production of inflammatory cytokines in response to bacterial attack. Pro-inflammatory cytokines such as tumor necrosis factor (TNF), IL-1, and IL-6 have a central role in the destruction of periodontal tissues.[30,31] Human and animal researches showed that TNF could enhance ferritin expression in muscle, fat, and other cell types and suggested a role for TNF in iron metabolism.[22] In the present study, after nonsurgical periodontal treatment and inflammation reduction, serum ferritin levels significantly decreased in both groups. This finding in our study is in agreement with Chakraborty *et al*.[24] showed serum ferritin levels raised in patients with chronic periodontitis and decreased to control levels after treatment. In the present study, we added the systemic administration of melatonin to periodontal routine treatment. In our research, reduction of the level of ferritin in the test group was more than the control group that this can be attributed to the use of melatonin with non-surgical periodontal treatment. Many studies have been conducted on the use of anti-inflammatory properties of melatonin in the treatment of periodontal diseases.[32-35] Melatonin can enter the saliva from the bloodstream and then reach the oral cavity. But its salivary concentration is one-third of its concentration in the blood 5. The anti-inflammatory properties of melatonin are related to its ability to remove oxygen radicals released from the inflammatory process.[32] Cutando *et al*.[5] showed the salivary levels of melatonin are associated with the severity of the periodontal disease. As the severity of periodontitis increased, the salivary level of melatonin increases, and this implies that melatonin protects the body against bacterial invasion. Hence, melatonin may be effective in the treatment of periodontal diseases. In this study, improvement of the results of non-surgical periodontal treatment may be related to the adjective use of melatonin.

The effect of melatonin on the ferritin levels in our study can be justified by the study of Amer *et al*.[26] They evaluated the protective effect of melatonin on iron overload and its toxic prosperities on the brain and testis of male rats. Despite the importance of iron in the body, imbalance in its amount can be associated with hemochromatosis and neuronal damage as a result of producing free radicals.[33] In the study of Amer *et al*.[26] the administration of ferrous sulfate to rats increased the level of serum iron and ferritin, and iron concentration elevated in the brain and testis. An increase in serum ferritin levels may be one of the early clinical signs of iron overload, in which iron is stored in ferritin inside the cells. The results of this study suggested that melatonin might reduce

the production of destructive free radicals produced by iron toxicity. This research demonstrated serum iron, ferritin in the melatonin-treated group were normalized significantly as compared with the iron group.[26] These findings were agreement with Othman *et al*.[34] who suggested that melatonin can prevent oxidation of iron-binding proteins. In one conclusion, the chelation of iron ions by melatonin is important in the anti-free radical effects of melatonin, which prevents or at least decreases iron-mediated tissue oxidative stress.

In this present study, we found that this co-treatment in patients with moderate to severe periodontitis caused more decrease in CAL, PD, and GI than those patients who received only non-surgical therapy. This further improvement can be attributed to the systemic use of melatonin. In a study by Chitsazi *et al*.,[15] they evaluated the combined use of vitamin C and melatonin supplements with non-surgical periodontal therapy. The results of this study on the improvement of periodontal clinical parameters in the melatonin group relative to the scaling alone were in agreement with the present study. Cutando *et al*.[35] were observed similar results in diabetic patients, and there was a significant improvement in clinical parameters (PD, CAL, GI). The more improvements in the study of Cutando *et al*.[35] compared to the present study, can be attributed to the local use of melatonin. The use of the therapeutic effects of melatonin has documented by animal studies and clinical trials.[35-38] These studies suggested the adjunctive use of melatonin in the treatment of periodontal diseases can have a beneficial effect.

Small sample size and short-term follow-up periods were our research limitations. It is recommended that long-term longitudinal studies with a large sample size be performed to determine whether serum ferritin levels can be used as a marker for diagnosis of periodontal disease or as marker for treatment response evaluation. Despite several studies that recommend the use of melatonin in dentistry, there are still some constraints that are needed to be overcome. Studies on antimicrobial effects of melatonin and the effects of this supplement on periodontal inflammatory cytokines are also recommended.

CONCLUSION

According to the present study results, improvement in all periodontal parameters along with a reduction in serum ferritin levels was observed 3 months after periodontal therapy. However, the improvement in the melatonin group was more than the scaling group alone. By taking the data of this study and previous information can be concluded that inflammation may increase ferritin expression in serum of patients with chronic periodontitis; other factors can also participate in increasing serum ferritin, including bacterial load and virulence factors of the bacteria.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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