

STUDY ON THE EFFECTS OF MELATONIN ADMINISTRATION ON INFLAMMATORY AND OXIDATIVE STRESS MARKERS IN PATIENTS WITH TYPE II DIABETES AND PERIODONTAL DISEASE

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Abstract:

The aim of this study was to evaluate the effects of melatonin supplementation accompanied by non-surgical periodontal therapy on serum melatonin, as well as inflammatory and oxidative stress markers in patients with type 2 diabetes with chronic periodontitis. The study was conducted on 50 subjects with periodontitis and diabetes mellitus type 2 (study group n = 25: patients with scaling and root planing + melatonin; control group = 25: patients with scaling and root planing + placebo). The melatonin, interleukin 6 (IL-6), tumour necrosis factor alpha (TNF- α) and high-sensitivity C-reactive protein (hs-CRP), total antioxidant capacity (TAC) and total serum oxidant (TOS) levels were measured at baseline and after 8 weeks. Melatonin systemic intake reduced inflammation and oxidative stress markers in diabetic patients with chronic periodontitis.

Keywords: *periodontitis, diabetes mellitus, melatonin, inflammation, oxidative stress*

Introduction

Periodontal disease is one of the most common oral infectious diseases in humans; it involves the destruction of the tissues that support the teeth (gingival tissue, periodontal ligament, root cementum and alveolar bone) due to the accumulation and maturation of oral bacteria, as well as the subsequent immune response displayed by the host [1]. Periodontitis is defined as a "complex heterogeneous biological phenomenon, derived from the interaction between genetic and epigenetic factors together with environmental determinants that lead to an imbalance of oral microbiome homeostasis and an inadequate immune response" [2]. This condition is aggravated

by an over production of reactive oxygen species (ROS) that leads to membrane peroxidation, which damages cellular structures [3,4]. Once established, this condition evolves with the reduction of collagen fibres, loss of attachment to the root surface and resorption of alveolar bone [5], eventually leading to tooth loss. [6,7]

Type 1 and type 2 diabetes mellitus (DM) are associated with elevated levels of systemic markers of inflammation [8]. High inflammatory status in diabetes contributes to microvascular and macrovascular complications, and it is clear that hyperglycaemia can lead to activation of pathways that increase inflammation,

oxidative stress, and apoptosis [9]. Elevated serum levels of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α) have been found in diabetes and obesity subjects, and serum levels of IL-6 and C-reactive protein (CRP) have been shown to predict the onset of type 2 diabetes [10]. Elevated CRP levels are associated with insulin resistance, type 2 diabetes and cardiovascular disease [11]. TNF- α and IL-6 are major inducers of acute phase proteins, including CRP, and both have been shown to affect intracellular insulin signalling that contributes to insulin resistance [12].

In addition, studies have consistently demonstrated defects in polymorphonuclear leukocytes (PMN) in patients with diabetes, including chemotaxis, phagocytosis, and impaired antimicrobial function [13]. PMNs need energy to function, and these defects may be related to metabolic changes that occur in diabetes.

The relationship between diabetes and periodontitis has been widely studied. Diabetic patients with severe periodontitis have been shown to have impaired PMN chemotaxis compared to patients with diabetes and mild periodontitis [13], as well as defects in PMN apoptosis [14] that can lead to increased retention of PMN in periodontal tissue, generating severe tissue destruction through the continuous release of matrix metalloproteinases (MMPs) and reactive oxygen species (ROS). Diabetes prolongs the inflammatory response to *Porphyromonas gingivalis*, with an increased production of TNF- α . Periodontal treatment has been shown to reduce serum concentrations of inflammatory mediators, including IL-6,

TNF- α , CRP and MMPs, in patients with and without diabetes [15].

On the other hand, it is indicated that periodontal disease is an inflammatory reaction that affects glycaemic status and DM control. According to studies, periodontitis can increase DM complications [16]. Several data indicate the possible effects of melatonin on pro-inflammatory cytokines and oxidative stress in experimentally induced inflammation [17].

It is suggested that melatonin may be useful in the treatment of periodontal disease by reducing oxidative stress, limiting tissue damage, stimulating the immune response, and reducing progressive alveolar bone loss [18,19]. It also decreases the production of pro-inflammatory cytokines by suppressing mRNA expression of TNF- α , interleukin-1 β (IL-1 β), IL-6 and iNOS [20]. In a clinical study, it was found that non-surgical periodontal therapy was associated with a significant decrease in serum TNF- α and interleukin-17 (IL-17) levels in patients with periodontal disease. In general, oxidative stress is thought to play a central role in the pathogenesis of diabetes and periodontal disease [21].

The aim of this study was to evaluate the effects of melatonin supplementation accompanied by non-surgical periodontal therapy on serum melatonin, as well as inflammatory and oxidative stress markers in patients with type 2 diabetes with chronic periodontitis.

Materials and method

Inclusion and design criteria

In this double-blind, placebo-controlled, single-center study, 74 patients with type 2 diabetes with symptoms of periodontal disease were recruited.

Subjects were called to the dental clinic for further diagnosis to confirm periodontal disease. Out of 74 participants, 20 patients were initially excluded. Fifty-four subjects were randomly assigned to the study group (n = 27) or placebo (n = 27) by a randomized block procedure (block design) based on the combined analysis.

The inclusion criteria were as follows: men and women, age 30 to 60 years, body mass index 18.5-30 kg / m², confirmed DM (not more than 5 years after diagnosis), subjects with mild and moderate periodontitis (PD ≥ 4 mm and CAL = 1-4 mm). Exclusion criteria included renal failure, pregnancy, lactation, thyroid disease, travel for more than 2 weeks, smoking, use of immunosuppressive drugs, insulin use, use of antibiotics, significant change in drug use and treatment of diabetes, history of periodontal treatment in the last 6 months, people with severe periodontitis, who use any antioxidants, anti-inflammatory agents and considerable changes in diet in the last 6 months. By definition, people who had FBS ≥ 126 mg / dl and HbA1c ≥ 6.5% were defined as diabetic [22].

Subjects in the study group received two melatonin tablets (250 mg) containing starch glycolate, sodium stearate and 3 mg net melatonin, and the control group received two placebo tablets (250 mg) containing cellulose, silicon dioxide, magnesium and starch for 8 weeks. Placebo tablets were similar to melatonin tablets in shape, colour, size and taste.

Melatonin and placebo tablets were prescribed two tablets once a day, 1 hour before bedtime. All subjects were asked to report any side effects of supplementation during the study. Subject

compliance was assessed by counting the remaining tablets. Subjects who consumed less than 90% of the prescribed tablets were excluded.

Biochemical measurements

Saliva samples were obtained in the morning, after an overnight fast, and all subjects were asked not to drink anything except water. The subject was asked to accumulate saliva and then spit in a polypropylene tube. The saliva samples were processed by centrifugation (1500 g) for 15 min and aliquoted into 0.3 ml portions. Samples were stored at -70°C until the end of the sampling period. Blood samples (5 ml) were collected after 12 hours of fasting during the night before and after the intervention and were poured into anticoagulant tubes to extract serum samples and sent to the laboratory in transport boxes. All samples were stored at -70°C until biochemical analysis. All reagents and samples were brought to room temperature and used according to the manufacturer's instructions.

Salivary and serum levels of melatonin, IL-6 and TNF- α and high-sensitivity C-reactive protein (hs-CRP) were measured by ELISA, using laboratory kits (Sigma-Aldrich, USA), according to the manufacturer's instructions.

Total antioxidant capacity (TAC) in serum and saliva samples were measured colorimetrically using a TAC kit (Rel Test Testing), according to the TAC method of Erel (2004). Total serum oxidant levels (TOS) and saliva were measured using Erel's (2005) TOS method.[23]

Statistical analysis

All data are presented as mean \pm standard deviation. The normality of the data distribution was examined using the Kolmogorov - Smirnov test. Statistical significance between groups at different study time points was analysed using the independent t test or the Mann-Whitney U test. Intragroup comparisons were completed using the paired t test or the Wilcoxon signed rank test. Repeated measures ANOVA was used to test variations within the group at baseline and at 3 and 6 months after treatment. The Bonferroni correction was applied for multiple comparisons. The Pearson Bivariate correlation was performed to determine the relationships between the variables.

Results

All data from this study had a normal distribution. Fifty-four patients were recruited for the study. Four subjects in the intervention and control groups consumed less than 90% of the prescribed tablets and were excluded from the study.

In total, 50 subjects (study group n = 25; control group = 25) completed the study.

We demonstrated that mean serum and salivary melatonin levels were significantly increased after surgery (4.21 ± 0.9 and 5.42 ± 1.1 pg / ml, respectively). In addition, mean pre- and post-intervention melatonin changes were significantly higher in the intervention group compared to the control group ($p < 0.001$) (Table 1, 2).

IL-6 and hs-CRP showed similar salivary and serum values between groups at baseline but these values decreased after therapy; the decreases, however, reached a threshold of statistical significance only for the group that followed additional melatonin therapy. We could not demonstrate the same significance for TNF- α , although both types of treatment generated slight detectable decreases in saliva and serum (Table 1, 2).

Table 1. Saliva markers values

	Control group (n=25)		Study group (n=25)		p Value between groups at baseline	p Value between groups at 8 weeks
	Baseline	At 8 weeks	Baseline	At 8 weeks		
Melatonin (pg/ml)	4.34 \pm 0.41	4.21 \pm 0.9	4.41 \pm 0.20	5.42 \pm 1.1	0.1	<0.001
p Value	0.09		<0.001			
hs-CRP (ng/ml)	2.21 \pm 0.31	2.17 \pm 0.21	2.73 \pm 0.71	1.24 \pm 0.46	0.08	<0.001
p Value	0.4		<0.001			
TNF- α (pg/ml)	8.55 \pm 1.8	8.42 \pm 1.9	8.72 \pm 3.1	8.01 \pm 3.3	0.6	0.09
p Value	0.8		0.07			
IL-6 (pg/ml)	2.21 \pm 0.43	2.11 \pm 0.21	2.09 \pm 0.60	1.31 \pm 0.32	0.09	<0.001
p Value	0.4		<0.001			
TAC (μ mol)	0.52 \pm 0.10	0.58 \pm 0.25	0.57 \pm 0.11	1.32 \pm 0.26	0.8	<0.001
p Value	0.09		<0.001			
TOS (μ mol)	0.075 \pm 0.002	0.072 \pm 0.002	0.073 \pm 0.009	0.031 \pm 0.001	0.9	<0.001
p Value	0.7		<0.001			

hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin 6; TAC: total antioxidant capacity; TOS: total serum oxidant levels; values are expressed as Mean \pm Standard Deviation; $p < 0.05$ was considered statistically

significant

Table 2. Serum markers values

	Control group (n=25)		Study group (n=25)		p Value between groups at baseline	p Value between groups at 8 weeks
	Baseline	At 8 weeks	Baseline	At 8 weeks		
Melatonin (pg/ml)	11.21±2.1	11.06±1.9	11.17±2.0	18.21±2.5	0.7	<0.001
p Value	0.4		<0.001			
hs-CRP (ng/ml)	2.11±0.42	1.97±0.21	2.24±0.51	1.14±0.62	0.08	<0.001
p Value	0.09		<0.001			
TNF- α (pg/ml)	22.5±3.2	21.4±3.6	22.6±3.1	21.1±3.3	0.6	0.1
p Value	0.6		0.1			
IL-6 (pg/ml)	19.81±2.5	18.99±2.1	20.54±2.0	10.11±1.6	0.06	<0.001
p Value	0.7		<0.001			
TAC (μ mol)	1.23±0.12	1.22±0.15	1.27±0.21	1.41±0.24	0.12	<0.05
p Value	0.6		<0.001			
TOS (μ mol)	0.015±0.001	0.014±0.002	0.016±0.004	0.01±0.004	0.6	<0.001
p Value	0.8		<0.05			

hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin 6; TAC: total antioxidant capacity; TOS: total serum oxidant levels; values are expressed as Mean \pm Standard Deviation; p<0.05 was considered statistically significant

The initial concentration of salivary and serum TAC was not statistically different between the study groups ($p = 0.8$, $p = 0.12$, respectively). At 8 weeks after baseline, the study group showed a significantly higher TAC concentration than the control group ($p < 0.001$). Regarding the TOS value in serum and saliva, we did not notice significant differences at baseline ($p = 0.6$, $p = 0.9$, respectively). At 8 weeks we observed serum and salivary decreases in TOS for both groups but these decreases were significant only for the study group, with a significant difference between groups ($p < 0.001$) (Table 1, 2).

Discussions

There is evidence from clinical trials to support the high levels of pro-inflammatory mediators in poorly controlled diabetes (IL-1 β , TNF- α , IL-6, RANKL / OPG ratio and oxidative stress) in human gingival tissues (or animal

models) ([4,24], with diabetes plays a role in the observed increased periodontal destruction; this is supported by studies using cell cultures exposed to high glucose levels [25].

Improving the diabetes control reduces oxidative stress, improves lipid profiles, and reduces circulating cytokine levels [25]. There is evidence from several human studies showing that a successful periodontal treatment reduces the circulating level of CRP and TNF- α in people with diabetes [26]. There have been no studies addressing the impact of successful long-term periodontal therapy on the mechanisms involved in diabetes complications.

Studies indicate that high levels of oxidants can promote melatonin consumption, even in organisms that produce melatonin in high concentrations [27]. On the other hand, in diabetic patients, norepinephrine is the main stimulus of pineal melatonin synthesis

[28]. Several studies have shown that the pineal glands of the diabetic animal model contain less norepinephrine and produce less melatonin in response to norepinephrine. Melatonin synthesis begins with tryptophan; however, the net concentration of tryptophan is low in the pineal glands of diabetic animals. Tryptophan deficiency may decrease pineal and plasma melatonin concentrations [29]. It is generally suggested that the comorbidity of diabetes and periodontal disease may lead to a further reduction in melatonin levels.

In this study, we found that melatonin administration for 8 weeks significantly decreased the mean serum IL-6 and hs-CRP [30], found that melatonin administration for 6 weeks significantly decreased the mean serum hs-CRP level in patients with non-alcoholic hepatic steatosis [30]. Similarly, Cutando et al. (2015) [31] showed that local application of melatonin (1% Orabase cream) in patients with diabetes and periodontal disease led to a significant reduction in serum hs-CRP and IL-6 levels [26].

In another study by [32] showed that daily intake of 6 mg melatonin along with a low-calorie diet for 40 days significantly reduced serum hs-CRP and IL-6 levels in obese women.

Moreover, the results of a study by [32] showed a significant reduction in serum TNF- α levels, which did not agree with the results of the study. In the present study, melatonin did not significantly alter serum TNF- α levels. It is suggested that the difference in disease type, research method, sample size and duration of intervention are possible factors leading to the diversity of results. It is suggested that the effects of melatonin on diabetic

patients can take two forms: first, the inherent anti-inflammatory and antioxidant properties of melatonin reduce inflammation in periodontal tissues [19,33]; and second, melatonin combats ROS produced in DM and therefore reduces the inflammatory effects of diabetes on the periodontium [34].

It is commonly accepted that oxidative stress contributes to the onset and progression of most oral conditions and, in particular, periodontal disease. This process plays a key role in the progression of chronic inflammation, degradation of the extracellular matrix of the periodontium and bone remodelling. Oxidative stress is a major source of oral inflammation [35].

It has been shown that the rate of ROS production in the oral cavity is determined by the number and functional status of neutrophils participating in phagocytosis. It has been shown that overproduction of ROS by neutrophils can lead to collagen degradation, disturbances in proteoglycan synthesis, depolymerization of hyaluronic acid, leading to loss of periodontal tissue integrity and decreased biochemical properties [36].

Moreover, oxidative stress is one of the main factors responsible for damage to the salivary glands during metabolic diseases (obesity, type 1 and 2 diabetes), autoimmune diseases (Sjögren's disease, rheumatoid arthritis, systemic scleroderma), neurodegenerative diseases (dementia, Alzheimer's disease), oral cancer and oral precancerous conditions (keratosis) [37,38].

It has been shown that oxidative stress can lead to morphological changes in the parenchyma of the salivary glands, which leads to decreased salivary

secretion and biochemical changes in saliva [39]. This condition can lead to other oral conditions, such as xerostomia, mouth burn syndrome and precancerous lesions.

Elevated concentrations of markers of protein, lipids and oxidizing DNA were observed [40], as well as changes in the concentrations / activity of enzymatic and non-enzymatic antioxidants in the saliva and / or gingival fluid of patients with periodontitis, diabetes, insulin resistance and dementia [41].

The initial concentration of salivary and serum TAC was not statistically different between the study groups but at 8 weeks after the initial time, the study group showed a significantly higher TAC concentration than the control group. Regarding the value of TOS in serum and saliva, we did not notice significant differences at the initial time. At 8 weeks we observed serum and salivary decreases in TOS for both groups but these decreases were significant only for the study group, with a significant difference between the groups.

Chronic systemic inflammation and oxidative stress are common features for both diabetes and periodontitis [42]. It has been shown that following scaling and root planing, TOS is low and antioxidants increase [43], results that are consistent with the data in the present study.

Decreases in salivary TOS levels are more important than decreases in serum levels because saliva is a local biological fluid that includes markers of periodontal disease. Moreover, it has been suggested that the antioxidant enzyme system may initiate a compensatory mechanism in the presence

of metabolic disorders [44]). In addition, increased oxidative stress from hyperglycaemia in diabetic patients with periodontitis may induce the expression of proinflammatory cytokines [45,46].

In addition, it is suggested that melatonin (a hormone with antioxidant qualities) may be considered a useful diagnostic marker in periodontitis. Ghallab et al. (2016) [19,47] demonstrated that the determination of oxidative stress indices in gingival fluid can be used for the differential diagnosis of chronic and aggressive periodontitis. Given that oxidative stress plays an important role in the pathogenesis of periodontitis, it has been suggested that antioxidant supplementation may reduce / slow down periodontal injury. [46,48].

Despite the need for large randomized clinical trials to examine the use of free radical scavengers in periodontal disease, research provides positive evidence of antioxidant therapies in periodontitis [35,49]. Systematic analysis of clinical trials, performed by Muniz et al. (2015) [50]. demonstrated the efficacy of only lipophilic antioxidants (such as vitamin E), as opposed to hydrophilic antioxidants.

Conclusions

Melatonin administration can reduce inflammation and oxidative stress markers in diabetic patients with chronic periodontitis. The antioxidant, anti-inflammatory and immune modulating properties, together with its osteogenic actions on maxillary bone metabolism determine that melatonin could be a good therapeutic strategy for treating periodontitis.

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