**Al-Rafidain J Med Sci. 2023;5(Suppl 1):S177-182.**
**DOI:** https://doi.org/10.54133/ajms.v5i1S.385

AJMS
Online ISSN (2789-3219)

## Research Article

# Systemic Melatonin Supplementation as an Adjunct to Non-Surgical Periodontal Treatment in Obese Patients with Periodontitis

Hussam Sami Ismail[1], Maha Shukri Mahmood[2], Athraa Ali Mahmood[3]*

*[1]Department of Periodontics, College of Dentistry, AL-Ayen University, Nasiriyah, Iraq; [2]Department of Periodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq; [3]Department of Oral Surgery and Periodontics, College of Dentistry, Mustansiriyah University, Baghdad, Iraq*

Received: 24 October 2023; Revised: 7 December 2023; Accepted: 17 December 2023

## Abstract

***Background***: Obesity is considered an important risk factor for periodontal disease. It has been reported that reactive oxygen species linking both diseases, systemic melatonin supplementation as antioxidant therapy, was addressed as an adjuvant to scaling and root surface debridement (SRP) to enhance the treatment of periodontitis. ***Objective***: To investigate the efficacy of systemic melatonin administration on periodontitis-obese patients as an adjuvant to scaling and root surface debridement (SRP). ***Methods***: A randomized clinical trial was conducted at a dental-specialized center. Eighty subjects were included and allocated into group-I: twenty periodontium-healthy, normal-weight people; group-II: 30 obese patients with stage-III treated only with SRP, and group-III: 30 obese patients with stage-III periodontitis treated with SRP and 5mg melatonin. periodontitis and subjected to estimation serum levels of Receptor Activator of Nuclear Factor-Kappa B Ligand (RANKL) were estimated in all groups. Probing pocket depth (PPD), Bleeding on probing (BOP), and relative attachment level (RAL) were estimated in Groups II and III at baseline and after a one-month visit. ***Results***: RANKL baseline visits were significantly different between the control and studied groups, with no significant difference in clinical parameters except for PPD. The 2nd visit showed a significant difference in BOP score-1 compared to RAL and BOP score 0. In the 2nd visit, only weak negative and positive significant correlations were found between RANKL and BOP. ***Conclusion***: Daily use of 5mg melatonin improves periodontal parameters and decreased serum RANKL levels.

***Keywords***: Obesity, Melatonin, Periodontitis, Public health, RANKL.

**استخدام الميلاتونين كعامل مساعد لعلاج اللثة غير الجراحي في المرضى ذوي السمنة المفرطة و يعانون من التهاب اللثة**

**الخلاصة**

**الخلفية**: تعتبر السمنة عامل خطر مهم لأمراض اللثة. ثبت بان لجذور الأوكسجين الفعالة علاقة بالمرض. استخدم الميلاتونين كعلاج مضاد للأكسدة و كمساعد لتحجيم وكشط الجذر (SRP) لتعزيز علاج التهاب دواعم السن. **الهدف**: تقييم فعالية إعطاء الميلاتونين الجهازي على مرضى التهاب اللثة والسمنة كعامل مساعد للتحجيم و SRP. الطريقة: أجريت تجربة سريرية عشوائية في مركز متخصص في طب الأسنان. تم إدراج ثمانين شخصا وتوزيعهم في المجموعة الأولى: عشرون شخصا يتمتعون بصحة اللثة والوزن الطبيعي. المجموعة الثانية: 30 مريضا يعانون من السمنة المفرطة في المرحلة الثالثة يعالجون فقط ب SRP، والمجموعة الثالثة: 30 مريضا يعانون من السمنة المفرطة يعانون من التهاب اللثة في المرحلة الثالثة يعالجون ب SRP و 5 ملغ من الميلاتونين. تم تقدير مستويات التهاب دواعم السن والخضوع لتقدير مستويات مصل منشط مستقبلات العامل النووي (RANKL) في جميع المجموعات. تم تقدير عمق جيب الفحص والنزيف عند الفحص ومستوى التعلق النسبي في المجموعتين الثانية والثالثة عند خط الأساس وبعد شهر واحد. **النتائج**: كانت نتائج خط الأساس ل RANKL مختلفة بشكل كبير بين المجموعة الضابطة والمجموعة المدروسة، مع عدم وجود فرق كبير في المعلمات السريرية باستثناءPPD . أظهرت الزيارة الثانية فرقا كبيرا في درجة BOP -1 مقارنة ب RAL و BOP النتيجة 0. في الزيارة 2، تم العثور على ارتباطات معنوية سلبية وإيجابية ضعيفة فقط بين RANKL وBOP . **الأستنتاج**: الاستخدام اليومي للميلاتونين 5 mgيحسن معايير اللثة ويقلل من مستويات RANKL في الدم.

* ***Corresponding author***: Athraa A. Mahmood, Department of Oral Surgery and Periodontics, College of Dentistry, Mustansiriyah University, Baghdad, Iraq; Email: athraa.a.mahmood@uomustansiriyah.edu.iq

***Article citation***: Ismail HS, Mahmood MS, Mahmood AA. Systemic Melatonin Supplementation as an Adjunct to Non-Surgical Periodontal Treatment in Obese Patients with Periodontitis. *Al-Rafidain J Med Sci.* 2023;5(Suppl 1):S177-182. doi: https://doi.org/10.54133/ajms.v5i1S.365

## INTRODUCTION

Periodontal disease is an inflammatory oral process that affects the gingiva, cementum, periodontal ligament and alveolar bone [1]. Periodontitis is considered one of the most common bacterially-induced inflammatory diseases, affecting about 20 to 50 percent of global adults [2,3]**.** Modulation of the immune system may link periodontitis with obesity [4–6]. It is possible for high-triglycerides to irritate periodontal tissue by messing up the inflammatory response by causing glycosylated products to build up in the tissue and inflammatory cytokines to be released [7]. A decrease in HDL-C in obesity causes the accumulation of lipids and cholesterol in the atrial wall and immune cells. Thus, inflammatory responses represented by cytokines will be observed, possibly contributing to periodontal disease and insulin resistance [8,9]. Other studies have shown that increasing the scale of adipocytes will be postulated by the acceleration of free-fatty acid concentration, which may additionally motivate adipose tissue macrophages ensuing from the production of TNF-α, which is followed by the rise of IL-6, contributing to the induction of C-reactive proteins and causing severe inflammation. Many studies have reported that multiplied concentrations of C-reactive proteins, IL-6 and TNFα, play a valuable role in promoting periodontal tissue destruction [10–12]. RANKL mRNA is made by human periodontal ligament cells, mesenchymal cells, osteoblasts, and microvascular endothelial cells when IL-1 and TNFα are present [13,14]. Melatonin significantly reduced periodontal inflammation through the downregulation of RANKL [15,16]. Application of melatonin to the skin also lowers the gingival index and PPD by a large amount. These results suggest that bone is being formed and osteoblasts are differentiating [17].

## METHODS

### *Study design and setting*

The study was conducted in the Department of Periodontology at AL-Diwaniyah dental specialized center. The sample was collected from November 2019 to April 2020. This research was registered at ClinicalTrials.gov with ID: NCT04788979 (https://clinicaltrials.gov/study/NCT04788979?cond=NCT04788979&rank=1)

### *Ethical consideration*

Ethical approval was certified by the local research ethics ethical committee of the College of Dentistry, University of Baghdad, on November 28, 2019, under reference number 128619, and complied with Tokyo and Helsinki declarations on human research.

### *Inclusion criteria*

Participants voluntarily participated in the study after signing a written consent form and completing a questionnaire about their name, gender, age, medical and dental history, sleep pattern, BMI, diet protocol, and smoking habit. Periodontal parameters that were examined involved bleeding on probing (BOP), probing pocket depth (PPD), and relative attachment level (RAL). Women who were pregnant or lactating, patients with prostheses and orthodontic appliances, smokers or on anti-inflammatory, antibiotics and immunosuppressive medications, and people who had night shift work were excluded.

### *Patients group and interventions*

Eighty subjects were chosen to enroll in this study, with 20 serving as control healthy people (PPD≤3 mm, BOP≤10%, no attachment loss) [18]. While 60 patients were reported with obesity and stage III generalized unstable periodontitis (PPD ≥5mm with or without BOP and RAL≥7mm in the middle 1/3 of the root and beyond) [19,20], 30 patients received only SRP by hand periodontal curette and an ultrasonic scaler. In contrast, 30 patients were exposed to scaling and root surface debridement (SRP) and received single 5 mg of melatonin/day before bedtime for one month as a dietary supplement. Intensive oral hygiene instructions were provided for all study groups. After one month of therapy, both study groups returned for a follow-up visit.

### *Clinical outcome measurements*

Weight was determined using a specific bathroom scale, and height was determined using a height measuring tape [21]. BMI was calculated by dividing weight by height squared in kilograms per meter ($kg/m^2$). BMI scales (underweight <18.5, normal weight =18.5-24.9, and overweight = 25-29.9) In obese subjects, the BMI value was greater than 30 $kg/m^2$. Periodontal parameters included BOP demonstrated as mean percentages of score one (bleeding) and score zero (no bleeding), PPD and RAL examined at baseline and after one month of treatment. The acrylic stent was made with a vertical groove to measure RAL; the measurement was reported from the reference point marked on the lower edge of the groove of the stent to the base of the pocket by a manual periodontal probe that was pushed gently parallel to the tooth's long axis until resistance was observed.

### *Serum RANKL estimation*

Venous blood (approximately 8.0 mL) was taken into a gel tube and centrifuged for 10 minutes at 3000 rpm. In the Al-Diwaniyah General Medical Laboratory, serum

was collected in a simple tube and stored at -80°C. After a month, all participants, "except controls," were contacted to collect RANKL in the same manner. The kit's microtiter plate pre-coated with RANKL-specific antibodies was used to measure serum RANLL levels. The RANKL-specific biotin-conjugated antibody was placed in the microtiter plate wells designated for standards or samples. The avidin conjugated to horseradish peroxidase (HRP) was then incubated in each microplate well. After adding a tetramethylbenzidine (TMB) substrate solution, only the RANKL, biotin-conjugated antibody, and enzyme-conjugated avidin wells changed color. The color shift that occurred at 450 nm after sulfuric acid terminated the enzyme-substrate reaction was measured using spectrophotometry. The RANKL concentration was calculated by comparing the optical density of the samples to the standard curve.

### Statistical analysis

The statistical package for social science (SPSS) was used for the research statistics. The Shapiro-Wilk-Wilk was used to determine normality for both parameters and RANKL. The mean and standard deviation were expressed for parametric details. Baseline data were compared using the one-way variance analysis (ANOVA) test. The comparison between study groups at baseline and recall (2nd) visits was determined using paired and independent t-tests [22].

## RESULTS

All clinical periodontal variables, including PPD, RAL, BOP, and RANKL, were normally distributed using the Shapiro-Wilk test at $p>0.05$. Results in Table 1 clarified that there was no significant difference between groups in the baseline visit except for PPD. In the 2nd visit, results showed a significant difference, and with higher variability and effect size for BOP score 1 (1.892) than RAL (0.853) and BOP score 0 (1.003), except for PPD, where the result was not a significantly different.

**Table 1:** Differences in the clinical parameters (BOP, PPD, and RAL) among studied groups at each visit

| Groups | PPD (mm) | | RAL (mm) | | BOP (score 1) | | BOP (score 0) | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 2nd visit | Baseline | 2nd visit | Baseline | 2nd visit | Baseline | 2nd visit |
| Group II | 5.79±0.39 | 4.73±0.39 | 7.54±0.32 | 6.60±0.47 | 63.26±6.44 | 41.60±8.27 | 36.06±6.34 | 58.33±7.66 |
| Group III | 6.29±0.34 | 4.87±0.36 | 7.65±0.27 | 6.28±0.26 | 65.46±6.82 | 28.26±5.56 | 33.60±6.83 | 71.30±4.70 |
| *p*-value | 0.000 | 0.179 | 0.190 | 0.002 | 0.204 | 0.000 | 0.153 | 0.000 |
| Effect size | 1.380 | | | 0.853 | | 1.892 | | 1.003 |

Values are presented as mean±SD. PPD: probing pocket depth; RAL: relative attachment level; BOP: bleeding on probing.

Table 2 illustrates the changes of PPD, RAL and BOP from baseline to the 2nd visit, indicating that there was a decrease in all those parameters for each group except for BOP score 0, which was increased in each group with a significant difference and higher variability and effect size for group III than those for group II.

**Table 2:** Changes in the values of PPD, RAL and BOP between visits in each group

| Groups | Variables | | Paired t-test | *p*-value | Effect size |
|---|---|---|---|---|---|
| Group II | PPD (mm) | pre-post | 14.639 | 0.000 | 1.890 |
| | RAL (mm) | pre-post | 15.753 | 0.000 | 2.034 |
| | BOP (score1) | pre-post | 12.619 | 0.000 | 1.629 |
| | BOP (score 0) | pre-post | 13.508 | 0.000 | 1.744 |
| Group III | PPD (mm) | pre-post | 34.128 | 0.000 | 4.406 |
| | RAL (mm) | pre-post | 25.613 | 0.000 | 3.307 |
| | BOP (score1) | pre-post | 30.564 | 0.000 | 3.946 |
| | BOP (score 0) | pre-post | 29.887 | 0.000 | 3.858 |

PPD: probing pocket depth; RAL: relative attachment level; BOP: bleeding on probing.

Results in Table 3 showed that RANKL in the baseline was found to be highest in Group III (0.682), Group II follows (0.612), and the least number in Group I (0.336) regarding the 2nd visit. However, the RANKL in group III (0.566) is lower than in group II (0.584), but with a non-significant difference in the change in this inflammatory marker; in each study group, there was a decrease in that marker from baseline to the 2nd visit, with a significant difference and a larger effect size in group III (0.841) than group II (0.637).

**Table 3**: Serum RANKL levels (ng/ml) between different groups and at differents visits

| Groups | Baseline | 2nd visit | *p*-value |
|---|---|---|---|
| Group I | 0.34±0.04 | --- | --- |
| Group II | 0.62±0.14 | 0.58±0.14 | 0.000 |
| Group III | 0.68±0.14 | 0.57±0.13 | 0.000 |
| *p*-value | 0.000 | 0.615 | |

Values are presented as mean±SD. PPD: probing pocket depth; RAL: relative attachment level; BOP: bleeding on probing.

Table 4 showed a significant difference between each study group and the control group but no significant difference between the studied groups.

**Table 4:** Games-Howell post hoc comparisons of baseline serum RANKL levals (ng/ml).

| Groups | | Mean difference | *p*-value |
|---|---|---|---|
| Group I | Group II | -0.279 | 0.000 |
| | Group III | -0.346 | 0.000 |
| Group II | Group III | -0.068 | 0.154 |

Table 5 showed that only a weak negative and significant positive correlation was found between RANKL and BOP scores 0 and 1, respectively, in the 2nd visit in group II.

**Table 5**: Correlation between the clinical periodental parameters at the recall visit periodontal and serum RANKL levels

| Groups | | *r* | *p*-value |
|---|---|---|---|
| Group II | PPD (mm) | -0.338 | 0.068 |
| | RAL (mm) | -0.296 | 0.112 |
| | BOP (score1) | 0.479 | 0.007 |
| Group III | PPD (mm) | -0.154 | 0.417 |
| | RAL (mm) | -0.062 | 0.744 |
| | BOP (score1) | 0.051 | 0.787 |

RAL: relative attachment level; BOP: bleeding on probing; r: Pearsons' correlation coefficient.

## DISCUSSION

Clinical periodontal parameters (PPD, RAL, and BOP) were examined only with study groups (group II and group III). Statistically, there was no significant difference between study groups at baseline except for PPD, which has a significant difference according to the selection criteria of subjects. All these parameters changed from baseline to the recall visit, which showed a decrease in means except for BOP; group III had a significantly higher BOP score of zero than group II. SRP alone effectively increased RAL gain and decreased BOP score and PPD. Previous studies supported these findings by Wennstrom *et al.* (2005) and Santos *et al*. (2009) [23,24]. Cutando *et al*. found that topical melatonin reduced pro-inflammatory factors and bone loss in periodontitis patients, reducing PPD and RAL [25]. Additionally, Montero *et al*. found that topically applying melatonin (1 percent orabase cream formula) for 20 days reduces clinical periodontal parameters [26]. The RANKL from baseline to the recall visit differed between the control and studied groups at baseline ($p<0.05$). These results agreed with Kawai, Akira and War-Aswapati *et al*., who found that RANKL concentration increased in individuals suffering from periodontitis compared with the control group [27–29]. The highest level of RANKL is associated with an increased level of cytokines (IL-1 and TNF-α), which play an essential role in the activation of the RANKL molecule that stimulates osteoclastogenesis and bone resorption with the help of B and T lymphocytes [30]. Similarly, Liu *et al*. demonstrated that the upregulation of RANKL and the downregulation of OPG play a critical role in developing periodontal disease and bone destruction [31]. Regarding the recall visit, group III significantly decreased concentration with a large effect size compared to group II. These results were similar to those of Thelolere et al., who discovered that melatonin changed the actions of RANKL/OPG because it is directly connected to the coordination of the molecular trio "OPG/RANK/RANKL" [32]. Similarly, Koyama *et al*. demonstrated that treatment with adjunctive melatonin therapy besides SRP would stimulate the proliferation and differentiation of osteoblasts, decrease the expression of RANKL, and increase the production of OPG by osteoblasts [33]. Melatonin also changes things by binding directly to active sites of COX2, which produces prostaglandins (PGE2). These PGE2 molecules help break down bones by increasing the production of osteoblast RANKL. Thus, suppressing COX2 by melatonin will decrease RANKLE production [34]. Regarding the correlation between RANKL and periodontal parameters, the results revealed that there were non-significant correlations between the inflammatory marker and clinical periodontal parameters in recall visits in each study group, except for BOP score 0 and score 1 with RANKL in group II, where the results were a weak negative and positive significant correlation, respectively. Our results are consistent with many studies that reported the effect of scaling and sub-gingival root surface debridement that significantly reduced the bacterial population and reduced the level of gingival inflammation, leading to a decrease in the mean percentage of BOP score 1 and an increase in the mean percentage of BOP score 0 [35,36]. This study also agreed with the analysis of Lopez Roldan *et al*., who found that there was a negative correlation between BOP score 0 and RANKL in the concept of scaling, and root surface debridement significantly decreased the bleeding on the sites of active periodontitis from 47% to 6.7% and increased the level of RANKL from the pathological location to the level of a healthy location since SRP increased osteoclastogenesis and promoted osteoblast differentiation and activation [37].

## CONCLUSION

Daily use of a single dose of 5 mg melatonin improves periodontal health and lowers serum levels of RANKL.

### Conflict of interests

No conflict of interest was declared by the authors

### Funding source

The authors did not receive any source of fund.

### Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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