ABSTRACT

Aim: Melatonin catch more attention as adjunctive periodontal therapy. This study was conducted to assess the benefit of locally delivered melatonin gel as adjunctive to scaling and root planning (SRP) in the treatment of chronic periodontitis. Subjects and Methods: Forty chronic periodontitis selected patients divided into two groups. Group I: included 20 chronic periodontitis patients, treated by conventional periodontal therapy SRP combined with intra-pocket application of Melatonin gel once weekly for 1 month begin application at the second week after initial therapy. Group II: included 20 chronic periodontitis patients, treated by SRP combined with the injection of placebo, weekly for one month. The periodontal parameters were recorded at baseline, 1 week, 1 and 3 months. Gingival crevicular fluid (GCF) samples were collected and a quantitative measurement of receptor activator nuclear K ligand (RANKL) was carried out by using Enzyme-Linked Immunosorbent Assay (ELISA) at baseline, 1 week, 1 and 3 months. Results: We found noticeable significant reduction in all clinical parameter and in the amount of GCF RANKL in group I in all evaluation period at 1,3-month over group II. Conclusion: We found noticeable significant reduction in all clinical parameter and in the amount of GCF RANKL in group I in all evaluation period at 1,3-month over group II.

INTRODUCTION

Periodontitis is chronic diseases affect tissue surrounding teeth, caused by gram negative bacteria in dental plaque. Direct effect of bacteria and toxic product induce inflammatory response in host tissue. Pathogenesis of periodontal disease affected by multiple cytokines, which have direct effect on expression of osteoprotegrin and receptor activator of nuclear factor –k B ligand (RANKL).these are consider as critical factors in regulating the differentiation and maturation of osteoclasts as well as bone resorption. In pathological process of periodontal disease OPG/RANKL equilibrium is disrupted lead to increase bone resorption.
RANKL is a member of the Tumor Necrosis Factor (TNF) ligand superfamily, was identified as a cell membrane-bound factor responsible for stimulation of osteoclast differentiation and bone resorption. By activating its cognate RANK receptor on the surface of pre-osteoclasts, it triggers their fusion and differentiation into mature osteoclasts, thus activating bone resorption\(^{(2)}\).

A number of treatment options are available for the treatment of periodontal disease ranging from the traditional non-surgical periodontal therapy (mechanical hand instrumentation, ultrasonic debridement, local drug delivery (LDD), systemic antibiotic therapy and host modulation therapy to recent surgical treatment modalities\(^{(3)}\). LDD achieve a potential advantages compared to systemic therapy. Higher concentrations of the drug at site of action by usage of lower dose, thus reducing the side effects\(^{(4)}\).

Melatonin is an indoleamine synthesized in the pineal gland and other organs. This gland produces melatonin in a circadian manner, synchronizing a number of biologic processes in a 24-hour, day–night rhythm. Melatonin uptodate consider as one of host modulation material possesses a variety of essential properties such as anti-inflammatory, antioxidant, oncostatic and neuroprotective actions. Moreover, melatonin was recently found to promote osteoblastic differentiation and suppress osteoclastic formation through downregulation of the receptor activator of nuclear \(\kappa\)-B ligand (RANKL).\(^{(5,6,7)}\)

Accordingly, the present study was conducted to clarify the locally applied melatonin gel on enhance ment periodontal condition in chronic periodontitis.

**PATIENTS AND METHODS**

Forty chronic periodontitis patients (15 male and 25 female with age ranged from 23 to 39 years) selected from those attending at the outpatients clinic, Oral Medicine and Periodontology Department, Faculty of Dental Medicine, Al-Azhar University, Assiut branch.

**Inclusion criteria**

All patients were free from any systemic diseases according to the criteria of Cornell Medical Index and its modification\(^{(8,9)}\). Patients with 4 or more teeth per jaw, with a Pocket depth less than 6 mm and Clinical attachment loss \(\leq 4\) mm.

**Exclusion criteria**

Uncooperative and smokers patients. Patients subjected to previous periodontal therapy during at least 6 months. Pregnant and lactating female patients.

**Patients grouping and interventions:**

**Group I:** included 20 chronic periodontitis patients, treated by conventional periodontal therapy (scaling and root planning) combined with intra-pocket application of 2% Melatonin gel once weekly for 1 month). The injection of melatonin was applied at the second week after initial therapy.

**Group II:** included 20 chronic periodontitis patients, treated by SRP combined with the injection of placebo, weekly for one month started at the second week after initial therapy.

**Preparation of Melatonin Gel 2%:**

Melatonin was prepared in the department of pharmaceutics and industrial pharmacy, Faculty of Pharmacy Al-Azhar University at Assiut.\(^{(10)}\)

**Intra-pocket application of 2% Melatonin gel:** The application accomplished by inserting the needle to base of periodontal pocket first and then placing the gel while working the way up until gingival margin. All patients were instructed to avoid eating, drinking and spitting at least 1 hour after application as well as teeth brushing and flossing 4 hour after application. The injections were repeated once weekly for 1 month. Patients were instructed for plaque control regime and oral hygiene instruction were provided every appointment.
Clinical evaluations:

The periodontal status was examined clinically and recorded the diseased sites of each patient (Fig. -1) at baseline (at the same day and before initial phase therapy), 1 week, 1 and 3 months using the following clinical parameters:

- Plaque Index (PI)\(^{(11)}\)
- Gingival Index (GI)\(^{(12)}\)
- Probing Depth (PD)
- Clinical Attachment Level (CAL)\(^{(13)}\)

Biochemical Evaluation (RANKL levels in gingival crevicular fluid)

The RANKL levels in GCF was assessed using ELISA kits (Sinogeneclon Co., Ltd, China R) at baseline, 1 week, 1 and 3 months by sandwich ELISA assay.

Gingival crevicular fluid samples collection

GCF samples were taken from the deepest periodontal involved sites Figure 1(c) Prior to sampling supragingival deposits were removed with sterile cotton pellets without touching the marginal gingival, and the sample sites were isolated with cotton rolls and the crevicular site was then dried gently with a syringe. Standardized paper points (META BIOMED Co Ltd, Korea R) size #30\(^{(14)}\) were inserted into the crevice until mild resistance was felt. The paper points were left in pocket for 30 seconds\(^{(15)}\). Paper points which were contaminated with blood and saliva were discarded. The collected GCF samples were immediately pooled and diluted in phosphate buffer saline up to 600 μl (PBS; 137 mm NaCl, 10 mm Na2HPO4 and 2.7 mm KCl; pH 7.3) in Eppendorf tube and transported to the lab. Figure 2 (c). The samples were frozen at -80° C till they were assayed for RANKL evaluation.

Quantification of RANKL level:

The samples were assayed for RANKL levels using commercially available Human soluble receptor activator of nuclear factor –KB ligand and (sRANKL) ELISA Kit. (Sinogeneclon Co., Ltd, China R) CatalogNo: SG-10220, assay.

Statistical Analysis

The data were collected, tabulated and statistically analyzed by SPSS (Statistical Package for Social Sciences) version 24 that programmed to produce:

- Descriptive analysis. Paired t-test used for comparison between the baseline reading and the subsequent readings within the same group. Data are presented as the Mean ± standard deviation (SD). Continuous variables were compared by the Student t test (two-tailed) test for parametric data with Bonferroni post hoc test to detect differences between two groups. Independent t-test used for comparison between the two groups at the different interval.

Fig. (1)
Clinical photograph showing female 39 aged chronic periodontitis. (a) before treatment (b) clinical examination (c) GCF collection (d) Intra - pocket Melatonin application.)
RESULTS

A total of 40 patients (15 males and 25 females) were included and completed the study in adherence to the prescribed protocol. Demographic data, clinical parameters, and RANKL levels of the patients were summarized in (Table 1) At 1 week, 1, and 3 months in both group.

Table (1) show Mean and standard deviation (SD) of GI, PI, PPD, CAL, and RANKL levels in two groups at different intervals. At baseline, there were no statistically significant differences among patients of both groups in clinical parameters and RANKL levels. However, both group I and II showed significant improvement in clinical and biochemical parameters over the study period. The mean PD at baseline in the group I was (4.8±0.41) and in the group II was (4.9±0.31) which was not statistically significant (P=0.06). During the study, a statistically significant reductions in PD compared to baseline were seen in both groups at 1 week, 1, and 3 months (P<0.05). Comparison between the group I and II showed a greater reduction in mean PD in the group I than in the group II at 3 months (group I 3.23±0.64, group II 4.3±0.47) from baseline (P<0.05). Compared to baseline, the CAL also showed significant reductions at 1 week, 1 and 3 months (P<0.05) in both groups. Comparison between the group I and group II showed a greater reduction in CAL in the group I than in the group II at all intervals (group I 2.8±0.41, group II 2.9±0.31) at 3 months (group I 1.2±0.7, group II 2.3±0.47) from baseline (P<0.05). The mean BOP values of both TG and CG were significantly lower at 1 week, 1 and 3 months compared to baseline (P<0.05). The reduction was more significant in the group I compared to group II at 3 months. From baseline to 3 months RANKL levels were significantly reduced in both groups (P<0.05). However, RANKL levels in the group I was significantly lower than that of group II at all intervals (group I 13.89±2.13, group II 15.43±3.53) 3 months (group I 11.84±1.32, group II 13.35±1.32), (P<0.05).

The result in table (2) show correlating clinical and biochemical results, Spearman rank test showed a statistically significant positive correlation between PPD, CAL, and RANKL level at 1–3 months (P<0.05) especially in group I. This confirm higher RANKL level at baseline refer to periodontal disease activity and lower RANKL level at 1-3 months refer to periodontal healing which was clearly appear with high significant in group I.

Table (1) Means ± SD of Gingival index, Plaque index, Pocket depth, Attachment level, RANKL, paired t-test and P-values in the two groups:

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.8±0.41</td>
<td>2.6±0.5</td>
<td>1.378</td>
<td>0.109</td>
</tr>
<tr>
<td>1 week</td>
<td>1.6±0.5</td>
<td>1±0</td>
<td>5.339</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>1 month</td>
<td>0.7±0.47</td>
<td>1.1±0.31</td>
<td>3.183</td>
<td>0.003**</td>
</tr>
<tr>
<td>3 month</td>
<td>0.8±0.41</td>
<td>1.85±0.37</td>
<td>8.536</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>PI</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.5±0.51</td>
<td>2.6±0.5</td>
<td>0.623</td>
<td>0.205</td>
</tr>
<tr>
<td>1 week</td>
<td>1±0</td>
<td>1±0</td>
<td>6.332</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>1 month</td>
<td>1±0</td>
<td>1±0</td>
<td>6.332</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>3 month</td>
<td>1±0</td>
<td>1.9±0.55</td>
<td>7.285</td>
<td>&lt;0.0001***</td>
</tr>
</tbody>
</table>
Evaluation of The Efficacy of Topically Applied Melatonin Gel as Adjunctive Therapy in Chronic Periodontitis; Randomized Control Trail

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>t- test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.8±0.41</td>
<td>4.9±0.31</td>
<td>0.872</td>
<td>0.24</td>
</tr>
<tr>
<td>1 week</td>
<td>4.15±0.67</td>
<td>4.6±0.5</td>
<td>2.401</td>
<td>0.01*</td>
</tr>
<tr>
<td>1 month</td>
<td>2.55±0.51</td>
<td>3.7±0.47</td>
<td>7.411</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>3 month</td>
<td>3.25±0.64</td>
<td>4.3±0.47</td>
<td>5.921</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>Attachment level</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.8±0.41</td>
<td>2.9±0.31</td>
<td>0.872</td>
<td>0.24</td>
</tr>
<tr>
<td>1 week</td>
<td>2.15±0.67</td>
<td>2.6±0.5</td>
<td>2.401</td>
<td>0.01*</td>
</tr>
<tr>
<td>1 month</td>
<td>0.55±0.51</td>
<td>1.7±0.47</td>
<td>7.411</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>3 months</td>
<td>1.2±0.7</td>
<td>2.3±0.47</td>
<td>5.858</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>RANKL</td>
<td>Mean±SD</td>
<td>Mean±SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.89±2.13</td>
<td>15.43±3.53</td>
<td>1.661</td>
<td>0.105</td>
</tr>
<tr>
<td>1 week</td>
<td>10.56±1.63</td>
<td>12.61±1.96</td>
<td>3.593</td>
<td>0.001**</td>
</tr>
<tr>
<td>1 month</td>
<td>10.33±0.82</td>
<td>12.81±2.42</td>
<td>4.343</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>3 month</td>
<td>11.84±1.32</td>
<td>13.35±2.05</td>
<td>3.1</td>
<td>0.004**</td>
</tr>
</tbody>
</table>

Table (2) Correlation Coefficient (r) between RANKL levels & clinical parameter in both groups at different intervals

<table>
<thead>
<tr>
<th>RANKLE concentration</th>
<th>plaque index</th>
<th>gingival index</th>
<th>pocket depth</th>
<th>Attachment level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.7</td>
<td>0.315</td>
<td>-0.218</td>
<td>-0.218</td>
</tr>
<tr>
<td>1 week</td>
<td>0</td>
<td>0.249</td>
<td>-0.453</td>
<td>-0.453</td>
</tr>
<tr>
<td>1 month</td>
<td>0</td>
<td>0.199</td>
<td>0.148</td>
<td>0.148</td>
</tr>
<tr>
<td>3 month</td>
<td>0</td>
<td>0.528</td>
<td>0.051</td>
<td>0.107</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>-0.568</td>
<td>0</td>
<td>0.406</td>
<td>0.406</td>
</tr>
<tr>
<td>1 week</td>
<td>0</td>
<td>0</td>
<td>0.462</td>
<td>0.462</td>
</tr>
<tr>
<td>1 month</td>
<td>0</td>
<td>-0.467</td>
<td>0.535</td>
<td>0.535</td>
</tr>
<tr>
<td>3 month</td>
<td>0.449</td>
<td>-0.061</td>
<td>0.532</td>
<td>0.532</td>
</tr>
</tbody>
</table>

DISCUSSION

The present study was designed to evaluate the effectiveness of combining LDD as adjuncts to SRP in the treatment of chronic periodontitis.

The severity of chronic periodontitis was similar in group I and group II at the beginning of the present study. Significant improvements in clinical and biochemical parameters were observed after treatment in both groups, patients were evaluated every 1 week, 1, and 3 months and improvements were maintained throughout the study period.

Data from previous studies showed that the addition of LDD to SRP resulted in significant clinical improvement in CAL gain and PPD reduction over SRP alone in patients with periodontal disease following 1 and 3 months of therapy.

In the present study, the levels of RANKL in GCF were measured by using ELIZA test and high
levels of RANKL were reported at baseline in both study groups. The levels of RANKL showed marked reductions following treatment in both groups but the results were statistically significant for the group I rather than group II.

Melatonin is non-toxic when administered in local forms. It acts as an antioxidant (16).

The present study confirms the beneficial effect of topical application of melatonin on clinical parameter and a statistically significant decrease in gingival index, and pocket depth and a significant decrease in RANKL level. This suggest that; Melatonin may have a favorable effect on slowing osteoclastogenesis, and preventing progression of periodontal diseases and tooth loss. Melatonin seemed to enhance the periodontal pockets healing process. This results confirm the beneficial effect of melatonin as host modulatory agent that confirmed with other studies which administrate melatonin systemically; 3 mg of melatonin administered for 4 weeks as an adjunct to SRP in chronic periodontitis patients versus patients treated by SRP only were show significant reduction in salivary RANK levels following the initial therapy and topical application of melatonin in periodontitis patients, significantly reported a reduction in GI and PD in diabetic patients with periodontitis (17,18).

In addition, similar results were reported in other study that assessed the effects of 1mg melatonin administered for 1 month versus placebo on periodontal parameters in periodontitis patients who underwent NSPT (19).

Komama et al (20) show similar results were reported in other study that assessed the effects of daily intraperitoneal injection of melatonin for 1 month significantly reduce bone resorption parameter and decrease RANKL expression.

Correlation of gingival crevicular fluid RANKL levels with clinical parameters in the diseased sites in the present study was investigated; RANKL was positively correlated with Probing Depth, and Gingival index, all comparisons before and after treatment with Melatonin were statistically significant, with positive association between periodontal inflammation and RANKL levels in GCF.

In this regard, failed to report significant correlations between GCF RANKL level and clinical parameter of disease severity in terms of PD, CAL, and inflammation with regard to bleeding on probing in patients with chronic periodontitis (21). However, previous study reported a positive correlation between the GCF RANKL level and PD in patients with periodontitis, this indicate reduction of inflammation, is not necessarily associated with a reduced capacity for bone destruction which indicates different healing patterns (22).

**CONCLUSION**

- LDD of Melatonin gel 2% act as adjunctive therapy to initial periodontal therapy is more effective than initial therapy alone in treatment of chronic periodontitis.

- The RANKL level in GCF can be used as biochemical marker for diagnosis and treatment of chronic periodontitis.

- Melatonin may act as potential host modulatory agent for periodontal disease management.

- Some limitations of the study should be considered, including the small sample size and including severe periodontitis patients so the recommendations for further studies with adequate sample power and several categories of periodontal disease are required.

**ACKNOWLEDGEMENT**

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**Conflicts of interest:** There are no conflicts of interest.
REFERENCES


Evaluation of The Efficacy of Topically Applied Melatonin Gel as Adjunctive Therapy in Chronic Periodontitis; Randomized Control Trial

Shaymaa Hussein Rafat Kotb, Fathia El-Zeid, Hamad Mohamed

Objective:
To evaluate the efficacy of topically applied melatonin gel as an adjunctive therapy in chronic periodontitis.

Materials and methods:
20 patients with chronic periodontitis were randomly assigned to two groups. Group 1 received SRP treatment followed by melatonin gel application once a week for one month, starting from the second week of the initial treatment. Group 2 received placebo and SRP treatment once a week for one month. Clinical measurements and GCF RANKL levels were recorded.

Results:
Significant improvements were observed in the clinical parameters and GCF RANKL levels in Group 1 compared to Group 2.

Conclusion:
The use of topically applied melatonin gel as an adjunctive therapy in chronic periodontitis showed promising results.

Keywords:
Chronic periodontitis, melatonin gel, SRP, GCF, RANKL.