AL-AZHAR
Assiut Dental Journal

The Official Publication of The Faculty of Dental Medicine, Al-Azhar Assiut University, Egypt

AADJ, Vol. 5, No. 1, April (2022) — PP. 61:68
ISSN 2682-2822

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

Shaymaa H. Kotb*[1], Fathey A. Abo Zaid[1], Ragaa H. Salama[2] , Gamal Mohamed Sultan Zayed[3]

Codex : 07/2022/04

Aadj@azhar.edu.eg

ABSTRACT

Aim: 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, 1week, 1and 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, 1week, 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:** The results proved the benefit of Melatonin as a promising adjunctive therapy in improving periodontal parameters. However, further long-term studies with large sample size are needed..

KEYWORDS

Chronic periodontitis , Gingival crevicular fluid, Melatonin , RANKL , ELISA

1. Department of Periodontology, Oral Diagnosis and Dental Radiology, Faculty of Dentistry, Al-Azhar University, Assuit, Egypt
2. Department of Medical Biochemistry, Faculty of medicine, Assuit University, Assuit, Egypt
3. Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt

* Corresponding Author e-mail: shaymarafat.dental @gmaill.com

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.(1)

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 resoprtion. 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 acheive 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 κ-B ligand (RANKL).[5,6,7]

Accordingly ,the present study was conducted to clarify the locally applied melatonin gel on enhancement 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 out-patients 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 ≤ 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 patients (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, 1and 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 1week ,1and 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 groupI 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 1week, 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 1week, 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 3months (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 1week, 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:*

	Group I	Group II	t- test	P-value
	Mean±SD	Mean±SD		
		GI		
Baseline	2.8±0.41	2.6±0.5	1.378	0.109
1week	1.6±0.5	1±0	5.339	<0.0001***
1month	0.7±0.47	1.1±0.31	3.183	0.003**
3month	0.8±0.41	1.85±0.37	8.536	<0.0001***
		PI		
Baseline	2.5±0.51	2.6±0.5	0.623	0.205
1week	1±0	1±0	6.332	<0.001**
1month	1±0	1±0	6.332	<0.001**
3month	1±0	1.9±0.55	7.285	<0.0001**
		PD		
Baseline	4.8±0.41	4.9±0.31	0.872	0.24
1week	4.15±0.67	4.6±0.5	2.401	0.01*
1month	2.55±0.51	3.7±0.47	7.411	<0.0001***
3 month	3.25±0.64	4.3±0.47	5.921	<0.0001***
	Attachment level Baseline			
	2.8±0.41	2.9±0.31	0.872	0.24
1week	2.15±0.67	2.6±0.5	2.401	0.01*
1month	0.55±0.51	1.7±0.47	7.411	<0.0001***
3months	1.2±0.7	2.3±0.47	5.858	<0.0001***
		RANKL		
Baseline	13.89±2.13	15.43±3.53	1.661	0.105
1week	10.56±1.63	12.61±1.96	3.593	0.001**
1month	10.33±0.82	12.81±2.42	4.343	<0.001***
3month	11.84±1.32	13.35±2.05	3.1	0.004**

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

RANKLE concentration	Plaque index	Gingival index	Pocket depth	Attachment level
		Group I		
Baseline	0.7	0.315	-0.218	-0.218
1 week	0	0.249	-0.453	-0.453
1month	0	0.199	0.148	0.148
3month	0	0.528	0.051	0.107
		Group II		
Baseline	-0.568	0	0.406	0.406
1 week	0	0	0.462	0.462
1month	0	-0.467	0.535	0.535
3month	0.449	-0.061	0.532	0.532

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 w were observed after treatment in both groups, patients were evaluated every 1week,1and 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 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 intraperiotoneal 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 sever periodontitis patients so the recommendations for further studies with adequate sample power and several categories of periodontal disease are required.
- **Acknowledgement :**The author would like to thank Medical Research center for their help and support.

Financial support and sponsorship: no external funding (self-funding)

Conflicts of interest: There are no conflicts of interest.

REFERENCES

1. Zhao L, Chen J, Cheng L, Wang X, Du J, Wang F, et al. Effects of Enterococcus faecalis lipoteichoic acid on receptor activator of nuclear factor-kappaB ligand and osteoprotegerin expression in periodontal ligament fibroblasts. Int Endod J. 2014;47:163–72.
2. Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: Involvement in the orchestration of pathophysiological bone remodeling. Cytokine & Growth Factor Reviews. 2004;15(6):457-75
3. Preshaw PM, Heasman L, Stacey F, Steen N, McCracken GI, Heasman PA. The effect of quitting smoking on chronic periodontitis. J Clin Periodontol. 2005; 32(8):869-79.
4. Pradeep, A.R.; Bajaj, P.; Agarwal, E.; Rao, N.S.; Naik, S.B.; Kalra, N.; Priyanaka, N. Local drug delivery of 0.5% azithromycin in the treatment of chronic periodontitis among smokers. Aust. Dent. J. 2013, 58, 34–40.
5. Cutando A., Gómez-Moreno G., Arana C., Muñoz F., Lopez-Peña M., Stephenson J., Reiter R.J. Melatonin stimulates osteointegration of dental implants. J. Pineal Res. 2008;45:174–9.
6. Girgert R., Hanf V., Emons G., Gründker C. Membrane-bound melatonin receptor MT1 down-regulates estrogen responsive genes in breast cancer cells. J. Pineal Res. 2009;47:23–31.
7. Tan DX, Manchester LC, Terron MP, Flores LJ and Reiter RJ: One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res 2007 , 42: 28-42 .
8. Abrumson G. The Cornell medical index as an epidemiological tool. A.M. J. Public health.1966; 65:287-98.
9. American Dental Association Council on dental practice, general guidelines for referring dental patients to specialists and other settings for dental care. Chicago: American Dental Association 1991
10. Edelby Y, Balaghi S and Senge B. Flow and Sol-Gel Behavior of Two Types of Methylcellulose at Various Concentrations. AIP Conf. Proc.2014 , 1593, 750-4.
11. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand.1964; 22:121-35
12. Löe H, Silness J. The gingival index, and the retention index system. J periodontol.1967; 38:610-6.

13. Ramfjord S. The periodontal disease index (PDI). J periodontol. 1967; 38:602-10.

14. 14. Lamster I. Evaluation of components of gingival crevicular fluid as diagnostic tests. Ann Periodontol.1997; 2:123-37.

15. Cimasoni G, Giannopoulou C. Can crevicular fluid component analysis assist in diagnosis and monitoring periodontal breakdown? In: Guggenheim B. editor. Periodontology Today, International Congress, Zurich. Basel: Karger, 1988; 260–70.

16. Cutando A, Montero J, Gomez-de Diego. R, Ferrera M.J, and Lopez-Valverde A, "Effect of topical application of melatonin on serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha) in patients with type 1 or type 2 diabetes and periodontal disease," J of Clin and Exp Dent,2015; 7; 628–33.

17. Cutando, A.; López-Valverde, A.; de Diego, R.G.; de Vicente, J.; Reiter, R, et al. Effect of topical application of melatonin to the gingiva on salivary osteoprotegerin, RANKL and melatonin levels in patients with diabetes and periodontal disease. Odontology 2014, 102, 290–6.

18. Marawar, AP; Marawar, PP, Nandal, DH; Kunkulol, RR. Evaluation of antioxidant potential of melatonin in periodontitis with a focus on vitamin C. Int. J. Basic Clin. Pharmacol. 2020; 9: 378-88

19. Tinto, M; Sartori, M; Pizzi, I; Verga, A; Longoni, S. Melatonin as host modulating agent supporting nonsurgical periodontal therapy in patients affected by untreated severe periodontitis: A preliminary randomized, triple-blind, placebo-controlled study. J. Periodontal Res. 2020; 55: 61–7.

20. H. Koyama, O. Nakade, Y. Takada, T. Kaku, H. Lau. Melatonin at pharmacological doses increases bone mass by suppressing resorption through down-regulation of the RANKL-mediate osteoclast formation and activation.J Bone Miner Res,2002 : 17 , 1219-29

21. Mogi, M, Otogoto J, Ota J, Togari A. Differntial experession of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis.J of Dental Res.2004;83:166-9.

22. Bostanci N, İlgenli T, Emingil G, Afacan B, Han B, et al., "Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. J of Clin Periodonto, 2007; 34: 370–6.

النشر الرسمي لكلية طب الأسنان
جامعة الأزهر أسيوط
مصر

الأزهـــر
مجلة أسيوط لطب الأسنان

AADJ, Vol. 5, No. 1, April (2022) — PP. 68

تقييم فعالية جل الميلاتونين المطبق موضعياً كعلاج مساعد في التهاب اللثة المزمن. مسار التحكم العشوائي

شيماء حسين رأفت قطب *1 ، فتحي عبد العظيم إبراهيم أبو زيد1 ، رجاء حمدي محمد سلامة2, جمال محمد سلطان زايد

1. 1قسم طب الفم وأمراض اللثة، والتشخيص والأشعة، كلية طب الأسنان، جامعة الازهر، أسيوط، مصر
2. 2قسم الكيمياء الحيوية الطبية _كلية الطب، جامعة أسيوط، اسيوط، مصر
3. 3 قسم الصيدلانيات والصيدله الصناعيه ، كليه الصيدله /بنات) ،جامعه الازهر ، اسيوط ، مصر

* البريد الإلكتروني: SHAYMARAFAT.DENTAL @GMAILL.COM

الملخص :

الهدف: أجريت هذه الدراسة لتقييم فائدة هلام الميلاتونين الذي يتم تسليمه محليًا كمساعد للتقشر وتخطيط الجذر (SRP) في علاج التهاب اللثة المزمن

المواد والاساليب: تم اختيار أربعين مريضاً من مرضى التهاب دواعم السن المزمن ، مقسمين إلى مجموعتين. المجموعة الأولى: تضمنت 20 مريضًا بالتهاب دواعم السن المزمن ، عولجوا بعلاج اللثة التقليدي SRP جنبًا إلى جنب مع تطبيق داخل الجيب من الميلاتونين جل مرة واحدة أسبوعيا لمدة شهر واحد يبدأ التطبيق في الأسبوع الثاني بعد العلاج الأولي. المجموعة الثانية: تضمنت 20 مريضًا بالتهاب دواعم السن المزمن ، تم علاجهم بواسطة SRP مع حقن الدواء الوهمي ، أسبوعياً لمدة شهر. تم تسجيل معلمات اللثة عند خط الأساس ، أسبوع واحد ، شهر و 3 أشهر. تم جمع عينات السائل اللثوي (GCF) وتم إجراء قياس كمي لمنشط مستقبلات(K LIGAND RANKL) باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) في خط الأساس ، 1 أسبوع ، 1 و 3 أشهر

النتائج: وجدنا انخفاضًا ملحوظًا في جميع المتغيرات السريرية وفي كمية GCF RANKL في المجموعة الأولى في جميع فترات التقييم في 1، 3 أشهر على المجموعة الثانية.

الخلاصة: أثبتت النتائج فائدة الميلاتونين كعلاج مساعد واعد في تحسين معايير اللثة. ومع ذلك ، هناك حاجة إلى مزيد من الدراسات طويلة الأجل مع حجم عينة كبير..

الكلمات المفتاحية: التهاب دواعم السن المزمن ، سائل شلل اللثة ، ميلاتونين ، رانكل ، إليسا.