Research Article

Effect of melatonin supplementation on the gingival health and lipid profiles in obese periodontitis patients

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Abstract: Background: Obesity increases the host's susceptibility by modulating the immune and inflammatory systems in a manner that predisposes to inflammatory tissue destruction and leaves an individual at greater risk of periodontitis. Melatonin is a pineal secretory product involved in numerous actions, such as regulation of internal biological clocks and energy metabolism, and it functions as an antioxidant and antiinflammatory agent. There exists a substantial amount of evidence supporting the beneficial effect of melatonin supplementation on obesity and its complications. Aim of the study: To investigate the effects of systemic melatonin intake on periodontal health status and lipid profiles in obese periodontitis patients. Subjects and methods: Subjects included in the study were distributed into the following groups: Group I, 20 subjects with normal weight and healthy periodontium (controls) not subjected to any treatment. Group II: 30 obese periodontitis patients subjected to scaling and root planing (SRP) only. Group III: 30 obese periodontitis patients subjected to SRP and supplemented with 5mg melatonin tablets for 1 month. Study groups subjected to estimation of plaque index (PLI), bleeding on probing (BOP), cholesterol (chol), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) at baseline and after 4 weeks recall visit. Results: Regarding the clinical parameters, the second visit exhibited decreasing in all parameters in both study groups except BOP score 0 were it increased significantly. Regarding lipid profiles, the second visit showed decreasing in all profiles except HDL where it increased in both study groups with a significant difference. All correlations between lipid profiles in recall visit in both study groups exhibited a positive significant correlation between chol and TG, chol and LDL, LDL and TG in group III. In group II all results exhibited a positive significant correlation, whereas the only strong negative correlation was found between chol and HDL, HDL and LDL. Conclusion: Daily supplementation with 5mg melatonin tab significantly improved periodontal health and reduced chol, TG, LDL with increasing

Keywords: melatonin, lipid profile, obese periodontitis patients.

Introduction

Periodontitis is defined as—an inflammatory disease of the supporting tissues of the teeth. The inflammatory condition is induced by microbial dysbiosis, resulting in progressive destruction of the periodontal ligament and alveolar bone (1). Obesity increases the host's susceptibility by modulating the immune and inflammatory systems in a manner that predisposes to inflammatory tissue destruction and leaves an individual at greater risk of periodontitis (2). Obesity has been postulated to reduce blood flow to the periodontal tissues and promoting the development of periodontal disease (3). Periodontal blood vessels

in obese persons show a plickening in their innermost membrane, which indicates diminished blood flow in the periodontium ⁽⁴⁾. One of the reasons for the association between obesity and periodontal disease include the social stigma associated with obesity in yearnger adults. A greater source of chronic stress found in obese young individuals than in older adults is considered more acceptable. Stress and how an individual copes with stress have been shown to increase a person's risk for periodontal disease ⁽⁵⁾.

Using the classification and regression tree (CART) method, it has been suggested that obesity is cond only to smoking as the strongest risk factor for inflammatory periodontal tissue destruction (6). A study of the Fourth Korean National Health and Nutrition Example nation Survey found that abdominal obesity is significantly correlated with periodontitis (7). Despite these common inflammatory-mediated pechanisms, the specific underlying biological pathways linking both diseases are not well characterized. Some authors have suggested the possible influence of alterations in the circadian cycle since significant reductions in the melatonin hormone levels were reported in experimental studies in both, obesity and periodontitis (8). In fact, when melatonin was administered in obese subjects, significant reductions in systemic pro-inflammatory biomarkers together with reductions in body weight and adipose tissue deposits were reported (9). Melatonin participated in homeostasis and metabolism of energy through activation of brown adipose tissue and enhance energy expenditure (10). Multiple studies reported that melatonin significantly increases HDL level and decrease TG and HDL level in addition to increased cholesterol catabolism (11).

Materials and methods

This study was conducted in AL-Diwanyah dental specialized center at the Department of Periodontics, all subjects were collected from December 2019 to March 2020. This study approved by the ethical committee at the College of Dentistry, University of Baghdad, follow the roles of Tokyo and Helsinki for human (Reference no.128619 in 28/11/2019). The study sample consisted of 80 males and females individuals with age ranged between 24-55 years, all subjects enrolled voluntarily to the study after signing an informed consent sheet to participate in research. All subjects were submitted to a questionnaire including their name, age, total medical history, dental history, their BMI, if they were subjected to any diet regimen, sleep nature, smoking, drinking alcohol, followed by a complete examination of clinical periodontal parameters (PLI, BOP). The sample was divided into 3 groups according to their BMI, periodontal health status, and melatonin supplementation. Group I (20 subjects) (controls) was the normal weight group and healthy periodontium, their BMI was (18.50 - 24.99) this group presents at the baseline only for examination of clinical periodontal parameters (PLI, BOP), lipid profile (chol, TG, HDL, LDL), don't exposed to any periodontal therapy., Group II (30 patients) have obesity, (BMI was ≥30.00) (12) designed to have latest sleep, generalized stage III periodontitis don't follow any diet regimen. This group presents at baseline and after a one-month recall visit for determination of clinical periodontal parameters (PLI, BOP), lipid profiles (chol, TG, HDL, and LDL) in pre and post periodontal treatment with scaling and root planing only without melatonin supplementation. The third group (30 patients) have obesity (BMI was ≥30.00) designed to have latest sleep, generalized stage III periodontitis don't follow any diet regimen. This group presents at baseline and after a one-month recall visit for determination of clinical periodontal parameters (PLI, BOP), lipid profiles (chol, TG, HDL, LDL) in pre and post periodontal treatment with scaling and root planing with melatonin supplementation 5mg tablet for 1 month (NOW, USA). All individuals were instructed not to eat for at least 12 hr. before taking the sample. Blood samples (8ml) were drowned from antecubital vein by using disposable syringe size 10mm and pushed directly into the gel tube. The tube was centrifuged at 3000 rpm for 10min to obtain serum. The extracted serum was divided by using micropipette into plain tubes and put in Colling box to send into the laboratory were stored in -80c in a deep freeze in AL-Diwanyah general medical laboratory. Following one month, all individuals (except controls) were returned to perform the same manner of collection. The second visit was determined after one month and involved only group II and group III. Clinical periodontal parameters involving (PLI, BOP,) was recorded and the same manner for blood collection and storing was performed for all patients.

After completion of the sample, the frozen serum was thawed at room temperature before analysis for determination of lipid profiles by using a spectrophotometer (Cecil instrument limited CE 7200, England).

The participants were examined intraorally to determine the plaque index (PLI) and (13) Bleeding on probing (BOP) (14).

Results

All variables including clinical periodontal parameters (PLI, BOP) were tested for normality using the Shapiro-Wilk test at P value greater than 0.05 and they were normally distributed.

Table 1: Intra- and Inter-comparisons of PLI among groups and visits using one way Analysis of Variance ANOVA. Paired sample t-test and Independent Sample t-test.

			PLI			
Groups		Base line 1 visit	Recall visit	Paired test	P value	ES
Control						
	Mean	0.455				
	±SD	0.048				
Group II	Mean	2.491	1.777	25.890	0.000**	3.342
	±SD	0.237	0.229			
Group III	Mean	2.556	1.564	30.233	0.000**	3.903
	±SD	0.171	0.173			
Statistics (I	F or T)	978.536	4.074			
df		2	58			
P value		0.000 HS	0.000 HS	_		
ES		0.962	1.050			

Table 2: Multiple Comparisons of PLI in the baseline visits between groups using Games-Howell.

Dependent Variable	(I) groups	(J) groups	Mean Difference (I-J)	Sig.
PLI	control	Without melatonin	-2.036	.000 **
		With melatonin	-2.101	.000 **
	Without melatonin	With melatonin	065	0.449

The findings in tables 1 and 2 illustrated that PLI was found to be higher in group III than other groups followed by group II with least in the control one. Further analysis of multiple comparisons indicated that there is no significant difference between the two study groups while when compared each one with control, results were found to be a highly significant difference. Regarding the second visit, PLI appeared to be higher in group II than that of group III. There was a decrease in PLI (1.564±0.1) and (1.777±0.2) in group III and in group II respectively with a highly significant difference with more effect size and variability in group III than that of group II.

Table 3: Statistical test of BOP among groups and in each visit using independent sample T.

Groups		BOP1, baseline	BOP1, recall visit	BOP1 pre-BOP1 post , paired t-test	df	ES
Without	Mean	63.267	41.60	12.619	29	1.629
melatonin	±SD	6.443	8.27			
With melatonin	Mean	65.467	28.267	30.564	29	3.946
	±SD	6.822	5.564			
T		1.284	7.327			
P value		0.204	0.000 HS			

Findings in table 3 illustrated that in baseline visits BOP was found to be higher in group III than that of group II with no significant difference. Regarding the second visit, all results demonstrated highly significant differences between the two groups. The changes of BOP from baseline to recall visits indicated

that there was decrease for BOP score 1 with more effect size and variability for group III (BOP1: 3.946) than that group II (BOP1: 1.629).

Table 4: Statistical test of lipid profile among groups and visits (Inter and Intra comparisons) using One Way ANOVA, Independent and dependent sample t-test.

		Oric	. , , ay .	1110 111, 11	acpende	111 011101	acpeni	acric sampr	e coot.		
		Н	DL			LI	DL				
		3 pre	post	Paired t-test	P value	pre	post	Paired t-test	P value		
		70.475				113.055					
		8.095				6.833					
		22.000	24.0			135.0	132.4				
		35.000	36.0	6.440		162.0	162.0	6.142	0.000 ES=		
		29.127	30.167		0.831	142.64	140.930		0.793		
	-	4.320	4.134			6.525	6.863				
		20.500	35.2			133.2	111.6				
		36.000	57.6	28.008	0.000 HS ES=	167.0	146.2	21.976	0.000 HS ES=		
		29.547	43.94		3.616	145.72	126.96	_	2.837		
		4.219	5.364			9.372	7.41				
		2	58			2	58				
	-	0.000	0.000			0.000	0.000				
		HS	HS			HS	HS				
		0.917	2.88			0.551	1.956				
		_	Cho	olesterol	_		_	Triglyc	eride		
Group	s		3 pre	post	Paired t-test	P	value			Paired t-test	P value
Control	Me	an	165.935	i				166.520			
Control	±Sl	D	14.553					11.486			
								245.0	240.0		.000
Without					- 0.751	0	.459	377.0	375.0	4.946	ES=
melatonin	Me	an	280.923	280.44		ES=	=0.097	305.63	302.973	4.946	0.639
	±Sl	D	36.623	36.761				36.775	37.042		
						0	.000	254.0	243.8		0.000
With					_ 7.904	1	HS	399.0	381.0	15.140	HS
melatonin	Me	an	293.843	287.777	- 7.804 -	1	ES=	314.82	297.617	15.140	ES=
	±Sl	D	39.961	40.115		1	.007	38.718	37.372		1.955
Df			2	58				2	58		
P valu	e		0.000	0.463				0.000	0.579		
			HS	NS				HS	NS		
ES			0.715					0.785			

The results in table 4 demonstrated that in baseline visit, all lipid profiles were found to be highest in group III followed by group II with the least value in the control group. HDL level showed an exception of that, in which the control group has the highest value other than other groups followed by group III and the least value found in group II. In the second visit, the cholesterol and HDL levels were found to be higher in group II than in group III. The opposite findings in Triglyceride and LDL levels were found to be higher in group II (302.973)-than in group III (297.617). All lipid profiles were found to be lowered between visits in each group with significant differences except for HDL were it showed to be higher with significant

differences in each group with greater effect size and variability for group III. Table 5 clarified further analysis in the multiple comparisons of lipid profile in the baseline between groups. There was no significant difference in lipid profile between group III and II while when compared each one with the control group, the results were highly significant difference.

Table 5: Multiple comparisons of lipid profile in the baseline between groups by using Games-Howell test

Depende	ent Variable	Groups	Groups	Mean Difference	P value
		Company 1	Without melatonin	-114.988	0.000**
cholesterol	Games-Howell	Control	With melatonin	-127.908	0.000**
		Without melatonin	With melatonin	-12.920	0.398
		Control	Without melatonin	-139.110	0.000**
Triglyceride	Games-Howell	Control	With melatonin	-148.300	0.000**
		Without melatonin	With melatonin	-9.190	0.616
	Games-Howell	Control	Without melatonin	41.348	0.000**
HDL		Control	With melatonin	40.928	0.000**
		Without melatonin	With melatonin	420	0.923
	Games-Howell	Control	Without melatonin	-29.585	0.000**
LDL		Control	With melatonin	-32.665	0.000**
		Without melatonin	With melatonin	-3.080	0.338

Table 6: Correlation between lipid profiles in recall visit in each group

Groups			Trig	HDL	LDL
Without	Cholesterol	r	.925	274	.668
melatonin		p value	.000	.143	.000
	Trig	r		150	.702
		p value		.430	.000
	HDL	r			253
		p value			.177
With melatonin	Cholesterol	r	.972	696	.765
		p value	.000	.000-	.000
	Trig	r		700	.757
		p value		000	.000
	HDL	r			478
		p value			008

Results of table 6 showed that correlations between lipid profiles in group II are the same as for baseline visits in the same group. There were significant correlations between cholesterol/triglyceride, cholesterol/LDL and between triglyceride and LDL while others had no correlations. The same results were found in group III, in which all results were significantly correlated with each other. Negative correlations were found between cholesterol/HDL, HDL/LDL, and Triglyceride/ HDL.

Discussion

Regarding periodontal parameters, baseline visit showed a significant difference between control and study groups due to the fact that control subjects had healthy periodontium and good plaque control,

which may attribute to their performance of good oral hygiene measures, and also by the fact of selection criteria of subjects in all groups.

There was a highly significant difference of PLI between the study groups at recall visit when compared with a baseline visit (p value ≤0.05) with more variability and effect size in group III than group II. Instructions and motivation with the aid of scaling with root planing may results in a significant reduction in the means of PLI in both study groups. In addition, when melatonin was used, it showed greater variability and significance when compared with the groups that were treated with scaling and root planning only. These findings agreed with the study confirmed by Cutando et. al (15). Similar finding agreed with these results confirmed by Almughrabi et. al 2013 which showed that consumption of melatonin reduces the formation of bacterial biofilm, but this reduction was not significant compared with the control group. Similarly, Syrinath et. al showed that melatonin had the activity against *Streptococcus mutans*, *Prevotella intermedia* and *Porphyromonus gingivalis*, which play a key role for biofilm formation and progression of periodontal diseases (16). BOP score 1 was decreased from baseline to the recall visit. SRP alone (without melatonin group) was effective in decreasing BOP score 1₇ which agreed with previous studies (17, 18). While melatonin group exhibited more increasing in BOP 0, indicating there was an increase in the percentage of gingival health.

Similar findings were reported by Cutando et.al that topical application of melatonin in diabetic patients will significantly reduce bleeding and probing in active periodontitis through the down-regulation in pro-inflammatory mediators, and decreasing the rate of bone loss (19). Another study by Montero et.al (20) where agreed with the present study, they found that topically applied melatonin (1% orabase cream formula) for 20 days will significantly reduce clinical periodontal parameters involving BOP score 1. The free radicals scavenging action of melatonin decrease gingival inflammation by its antioxidant effect. In addition, its efficacy on reduction of lipid uptake of microorganisms, regulation of duplication of bacteria and its effect to bind with iron, may explain this effect.

Regarding lipid profiles; at baseline visit there was highly significant differences between the control and study groups, which may attributed to their chosen criteria as obese subjects compared to systemically healthy control counterpart.

According to F family test of statistical test (ANOVA. Repeated measures, between factors) of the G-power program, the complete sample size determined to be taken was 78 at 0.80 power on α error probability 0.05. So, 100 subjects decided to be included in this study to compensate for any anticipated dropout that described study sample that consisted of 80 males and females individuals with age ranged between 24-55 years, all subjects enrolled voluntarily to the study after signed informed consent sheet to participate in research.

Regarding the comparison between both study groups from baseline visit to recall visit, it demonstrated that there was a highly significant difference and variability about changes in lipid profiles in melatonin group than without melatonin group. The findings of the present study results were agreed with the previous study that showed administration of 5mg of melatonin for two months significantly decrease the LDL level in patient don't exposed for a hypolipidemic diet for 3 months (11). Another study reported that supplementation of melatonin significantly increase HDL level in peri-and postmenopausal women (27). The mechanism of promoting the effect of melatonin on lipid profile is attributed by stimulating Brown adipose tissue activity (28). Another mechanisms of melatonin on obesity are the enhancement of cholesterol catabolism by bile acids and increase the receptors of LDL to inhibit cholesterol synthesis (29, 30).

Regarding the correlation of recall visit, demonstrated that group III showed a significant correlation between cholesterol/HDL, triglyceride/HDL and LDL/HDL, in which it agreed with the results of Sun H et. al (32) (33)—that reported the significant association of melatonin on reducing the level of cholesterol, triglyceride and LDL and increasing the level of HDL. However, our results have disagreed with others that showed no significant correlation of melatonin with HDL (28) (29). This discrepancy may be due to the diversity

of dose and duration of melatonin therapy and the study population. Therefore, more research is needed on larger study groups to investigate the precise effect of melatonin on anthropometric indices. According to the current research design, certain limitations have been addressed which need to be determined and recommended for future work.

These may include: using different doses and duration of intake of melatonin supplements, larger sample size for statistical values to avoid the outlier effect, depending on more objective and reliable method for melatonin detection close to periodontal tissue other than serum such as gingival crevicular fluid, and using other designs such as parallel blinding or crossover clinical trial with local delivery to overcome the bias concerns.

Conclusion

Daily supplementation with 5mg melatonin tab may significantly improve periodontal health and reduced chol, TG, LDL with increasing HDL. This may be designed to use as an adjunctive in the treatment of periodontal diseases.

Conflict of interest: None.

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العنوان: تأثير مكملات الميلاتونين على صحة اللثة وملامح الدهون في مرضى التهاب دواعم السن والسمنة البادون: مسامي إسماعيل , مها شكري محمود البادون:

الخلفية العلمية. تزيد السمنة من قابلية المضيف للإصابة عن طريق تعديل جهاز المناعة والاتهابات بطريقة تودي إلى تدمير الأنسجة الالتهابية وتترك الفرد أكثر عرضة للإصابة بالتهاب دواعم السن. المعلية بالتهاب دواعم السن المصابين بالسمنة. المداك الدراسة الحالية إلى التحقيق في قار إعطاء الميلاتونين الجهازي على الحالة الصحية اللغوية وخصائص الدوران في الدراسة الحالية إلى التحقيق في قار إعطاء الميلاتونين الجهازي على الحالة الصحية اللغوية وخصائص التهاب دواعم السن المصابين بالسمنة. المواد وطرق العمل: تم توزيع الأشخاص والمرضى المشمولين في الدراسة على المجموعة الثانية: المجموعة الأولى ، 20 شخصا بوزن طبيعي ولثة صحية (المجموعة الضابطة) ولم يخضعوا المواد وطرق العمل: 30 مرضى التهاب دواعم السن المشمولين في الدراسة على المجموعة الثوبة، وهورة المجموعة الشابطة) ولم يخضعوا لأي علاج. المجموعة الثقية، 30 مريضا بعادون من السمنة المفرطة مع التهاب دواعم السن وتم خضوعهم التقفير وكشط الجزر (SRP) فقط. المجموعة الجرثومية (PLI) والنزيف عند المعاملة في المجموعة البرائية الموادي والمسلوب وحد 4 اسابيع . والنزيف عند المعاملة المعاملة المعاملة والمورة المعاملة على الكثافة (DHD) والبروتين الدهني منخفض الكثافة (DDD) عند خط الأسلس وبعد 4 اسابيع . المجموعة التنافية في المجموعة الشائمة المعاملة بين ملف الدهون في مجموعتي الدراسة معاختيل معنوي، لظهرت الزيارة الشعرة المعاملة بين ملف الدهون في مجموعتي الدراسة ما ختلاف معنوي، الظهرت جميع النتائج بمناط البها إيجابيًا قويًا معنويًا بين ال CDL و CDL و CDL و LDL و CDL و TDL و تودد ارتباط سلبي في فقط بين ال CDL و CDL و LDL و LDL و د CDL و LDL و لله الموروعة الثائمة في المجموعة الثائمة ، أظهرت جميع النتائج ارتباطًا إلى المحلك المدورة المقبطة على وفقط بين الكراك و LDL و LDL و LDL و حود ارتباط سلبي وفقط بين الكراك الكراك

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