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# Potential Antioxidant Effect of Systemic Melatonin Therapy on Obese Periodontitis Patient

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## ABSTRACT

*Background: Periodontitis and obesity are two common chronic inflammatory diseases leading to increased systemic inflammation, metabolic dysregulation and dyslipidemia, It has been reported that obesity may be associated to periodontitis through the increased production of reactive oxygen species (ROS).To address this aspect, the adjunctive use of melatonin as antioxidant, anti-inflammatory, immunomodulatory medication in order to improve periodontal condition associated with obesity.*

*Aim of the study: To evaluate the effectiveness of adjunctive systemic administration of melatonin therapy to mechanical non- surgical periodontal therapy in periodontitis patients associated with obesity.*

*Material and methods: Eighty subjects included in the study were divided into the following groups:*

*Group I: 20 subjects with healthy periodontium and normal weight (controls), this group exposed for estimation of clinical periodontal parameters (plaque index (PLI), gingival index (GI)) and total antioxidant capacity (TAO-C) at base line visit only.*

*Group II: 30 latest sleep obese patients suffering from advanced periodontitis treated with non-surgical scaling and root planning only (SRP).*

*Group III: 30 latest sleep obese patients suffering from advanced periodontitis treated with SRP and supplemented with 5mg melatonin tablet (NOW-USA).*

*The latest two groups exposed for estimation of clinical periodontal parameters (PLI, GI, bleeding on probing (BOP)) and TAO-C at base line and after one month recall visit.*

*Results: There were highly significant difference in base line visit between control and study groups in both clinical periodontal parameters and TAO-C, regarding the second visit, PLI, GI, BOP score 1 significantly decreased in both groups, while BOP score 0 and TAO-C showed increasing after treatment, with greater effect size and more variability in with melatonin group than that without melatonin group. There were only weak significant correlation found between BOP score 1 and TAO-C.*

*Conclusion: Daily supplementation with 5mg melatonin therapy as adjunct to SRP significantly improved clinical periodontal parameters and increased total antioxidant capacity by exerting its anti-inflammatory and antioxidant properties.*

**Keywords:** Periodontitis, Obesity, Chronic inflammatory diseases (CID), Melatonin, Antibiotics, immunosuppressant

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## INTRODUCTION

Periodontitis defined as destruction of periodontal tissue caused by specific

microorganisms resulting of pocket formation, recession and mobility [1]. Obesity known to increase the host tolerance by influencing the immune and inflammatory mechanisms in a way that inflammatory tissue destruction is predisposed and leave a person at high risk of developing periodontitis [2]. The relationship between periodontitis and obesity has been reported in several epidemiological and experimental studies [3]. Obesity can be a higher trigger of chronic stress , stress and how a person copes with stress has been shown to increase the risk of periodontitis [4]. Several studies have shown that preserving of normal weight by maintaining regular physical exercise is linked with a lower incidence of periodontitis [5,6].

As a matter of truth , overweight and obese individuals are more than twice as likely to have periodontitis compared to normal healthy persons [7]. It has been suggested that obesity decreased the blood flow to periodontal tissue, enabling the development of periodontal disorders [8]. The blood vessels of obese subjects demonstrate a thickening in the inner wall of arteries that reduce the flow of blood into periodontium [9]. The adipocytes produce various active molecules called adipokines involving (TNF- α, IL-6, leptin, adiponectin and others). These compound secrete various molecules of reactive oxygen species (ROS) that lowered antioxidant enzymes activity like (superoxide dismutase, catalase, glutathione and others). Thus modulating the effect of adipokines can lower the pathogenicity of periodontitis by reducing the effect of oxidative stress [10, 11]. Oxidative stress appear to be the main link between obesity and periodontitis and can aggravates pro-inflammatory pathways frequent in both pathologies [12]. Regarding the significance of oxidative stress in pathologies of both periodontitis and obesity, several antioxidants play an amazing role as a preventive and therapeutic measures for both diseases [13]. Multiple studies had a greater evidence toward melatonin which have an active antioxidant properties [14], which is an indolamine produced mainly by pinealocytes [15]. It has been documented that salivary melatonin were significantly decreased in patients with periodontal disease, indicated that melatonin may act as a biomarker for periodontal diagnosis and can be used as a possible therapeutic agent in various periodontal diseases [16]. It was demonstrated that daily dietary supplementation with 3mg melatonin tablet for 4 weeks along with scaling and root planing significantly reduced the oxidative stress in periodontitis patients [17]. Reduction in antioxidant capacity may be either due to direct causal factor of periodontal disease or due to reduction in scavenging antioxidants produced due to increased ROS. Determining the local antioxidant status has an importance to know the disease progression and susceptibility [18]. Similarly, significant reduction in gingival inflammation when melatonin administrated locally as adjunctive measure to standard periodontal therapy [19].

## MATERIAL AND METHODS

### Study population

This study were conducted in AL-Diwanyah dental specialized center at department of periodontology and approved by ethical committee /Baghdad University /College of Dentistry at Reference no.128619 in 28/11/2019 following the roles of Tokyo and Helsinki for human. All samples were selected from December 2019 till March 2020.

Individuals were enrolled voluntary to the study after signed the informed consent sheet to participate in research and submitted to questionnaire including their name, age, gender, medical history, dental history, sleep nature, BMI, diet regimen, smoking following by complete examination of periodontal parameter (PLI,GI,BOP). Exclusion criteria involved pregnant and lactating women, patient wearing removable prosthesis and orthodontic appliances, patients taking anti-inflammatory, antibiotics, immunosuppressant drugs, alcoholic and smoker patients, Endo-perio lesions and individuals had night work shifts.

### Trial design

Eighty subjects were selected to participate in this study, twenty individuals selected as healthy controls subjects. Sixty patients diagnosed to have obesity and periodontitis were included in this study, 30 patients of them received only accurate SRP by using ultrasonic scaler and hand periodontal curette. Another 30 patients receiving SRP with daily dietary supplementation of 5mg melatonin for 1 month once daily before bed time at the same day of SRP. For all patients

intensive oral hygiene instructions were given. Both study groups signed to recall visit after one month of treatment.

**Anthropometrics measurements**

Weight was measured by using specific Bathroom scale, while measurement of height was recorded by using height measurement tape [20]. BMI was calculated by dividing the body weight on the square of height kg/$m^2$.

BMI ranges:

Underweight <18.5

Normal weight=18.5-24.9

Overweight=25-29.9

In obesity BMI ranges 30 and greater [21].

**Periodontal records**

Periodontal parameters included PLI and GI indices were recorded at base line visit in all groups and after 4 weeks from treatment in both study groups .BOP that expressed as mean percentages in score 1 (presence of bleeding) and score 0 (absence of bleeding).

**Serum collection**

Blood sample (8ml) were drawn from antecubital vein by using disposable syringe size 10mm and pushed directly into gel tube, then the tube will centrifuged (3000 rpm at 10min) to obtain serum. the extracted serum putted into plain tube by using micropipette and put in Colling box to send into the laboratory were stored in -80°C in deep freeze in AL-Diwanyah general medical laboratory, after one month all individuals (except controls) were returned to performed the same manner of collection for assessment of TAO-C.

**Biochemical assessment**

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Total antioxidant capacity (TAO-C). TAO-C were added to wells that were pre-coated with TAO-C monoclonal antibody and then incubation done. After incubation, anti-TAO-C antibodies labeled with biotin were added to unit with streptavidin-HRP, which forms the immune complex. Removing the unbound enzymes after incubation and washing, the substrate A and B were added. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of TAO-C were positively correlated.

**Statistical analysis**

Statistical analysis were done by using statistical package for social sciences (SPSS). Shapiro-Wilk test used for testing normality among groups for both parameters and TAO-C. Parametric details were demonstrated as mean ± SD, Base line data were compared by using ANOVA (Analysis of variance) test. Paired and independent T test were used to compare between study groups at base line and recall visits. Games-Howell post hoc test was used to express significant variation among groups. Pearson correlation test was used to evaluate the correlation between serum TAO-C level and clinical periodontal parameters.

## RESULTS

All variables includes clinical periodontal parameters (PLI, GI, BOP) and TAO-C were normally distributed used Shapiro-Wilk test at P value greater than 0.05.

Findings in Tables 1 and 2 illustrated that both PLI and GI found to be higher in with melatonin group than other groups followed by without melatonin group with least in the control one with highly significant difference between them and more effect size to GI than that in PLI. Further analysis of multiple comparisons indicated that there was no significant difference between the two study groups while when compared each one with control, results found to be highly significant difference. Regarding the second visit, both PLI and GI are higher in without melatonin group than that of with melatonin one with highly significant difference between them and more effect size or greater variability found in GI than that of PLI, when compared from base line to the 2nd visit, there was decrease in PLI (2.491 ± 1.777) and (2.556 ± 1.564) and GI (2.4171 ± 0.673) and (2.499 ± 1.439) ) in without melatonin and in with melatonin group respectively with highly significant difference and more effect size and variability in with melatonin group other than that of without melatonin one.

Findings in Tables 3 and 4 illustrated that in base line visits BOP found to be higher in with melatonin group than those without melatonin group with no significant difference between them, regarding the second visit , all results demonstrated highly significant differences with

more variability and effect size toward melatonin group (1.892) than without melatonin group (1.003). The changes of BOP from base line to recall visits indicated that there was decreased for BOP score 1 and increased in BOP score 0 with more effect size and variability for with melatonin group (BOP1 -3.946/BOP0-3.858) than that without melatonin group (BOP1-1.629/BOP0-1.744).

Findings in Tables 5 and 6 demonstrated that in base line visit, TAO-C found higher in control group followed by with melatonin group while the least in the without melatonin group with means of (0.761 ,0.324 ,0.381) respectively. In the second visit, TAO-C was higher in with melatonin group (0.613) than without melatonin group (0.401).lastly about changes from base line to the second visit, there was increasing in its level with high significant difference and more effect size toward melatonin group (1.03) than without melatonin group (0.669). Further analysis of multiple comparison clarifies that when compared between each study groups, the results were not significant, while when compared with control one, the results were highly significant.

The listing Table 7 illustrated that there were weak with either positive or negative not significant correlation at base line and in recall visit, except the weak negative significant

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

| | | PLI | | | | | GI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Groups** | | **Base line 1 visit** | **Recall visit** | **Paired test** | **P value** | **ES** | **Base line (1 visit)** | **Recall visit** | **Paired test** | **P value** | **ES** |
| Control | Mean | 0.455 | | | | | 0.481 | | | | |
| | ± SD | 0.048 | | | | | 0.041 | | | | |
| Without melatonin | Mean | 2.491 | 1.777 | 25.89 | 0.000** | 3.342 | 2.417 | 1.673 | 21.953 | 0.000** | 2.834 |
| | ±SD | 0.237 | 0.229 | | | | 0.177 | 0.182 | | | |
| With melatonin | Mean | 2.556 | 1.564 | 30.233 | 0.000** | 3.903 | 2.499 | 1.439 | 40.077 | 0.000** | 5.174 |
| | ±SD | 0.171 | 0.173 | | | | 0.092 | 0.12 | | | |
| Statistics ( F or T) | | 978.536 | 4.074 | | | | 1911.323 | 5.872 | | | |
| df | | 2 | 58 | | | | 2 | 58 | | | |
| P value | | 0.000 HS | 0.000 HS | | | | 0.000 HS | 0.000 HS | | | |
| ES | | 0.962 | 1.05 | | | | 0.98 | 1.52 | | | |

**Table 2: Multiple comparisons of PLI and GI in the base line visits between groups using games-howell.**

| Dependent Variable | (I) groups | (J) groups | Mean Difference (I-J) | Sig. |
|---|---|---|---|---|
| PLI | Control | Without melatonin | -2.036 | 0.000 ** |
| | | With melatonin | -2.101 | 0.000 ** |
| | Without melatonin | With melatonin | -0.065 | 0.449 |
| GI | Control | Without melatonin | -1.936 | 0.000 ** |
| | | With melatonin | -2.018 | 0.000 ** |
| | Without melatonin | With melatonin | -0.082 | 0.072 |

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

| Groups | | BOP1, baseline | BOP1, recall visit | BOP0, baseline | BOP0, recall visit |
|---|---|---|---|---|---|
| Without melatonin | Mean | 63.267 | 41.6 | 36.067 | 58.333 |
| | ± SD | 6.443 | 8.27 | 6.341 | 7.662 |
| With melatonin | Mean | 65.467 | 28.267 | 33.6 | 71.3 |
| | ± SD | 6.822 | 5.564 | 6.831 | 4.706 |
| T | | 1.284 | 7.327 | 1.45 | 7.898 |
| Df | | 58 | 58 | 58 | 58 |
| P value | | 0.204 | 0.000 HS | 0.153 | 0.000 HS |
| ES | | | 1.892 | | 1.003 |

**Table 4: Statistical test of BOP changes between visits in each group and visits.**

| Groups | | Paired t test | df | P value | ES |
|---|---|---|---|---|---|
| Without melatonin | BOP1pre-BOP1post | 12.619 | 29 | 0.000 ** | 1.629 |
| | BOP0pre-BOP0post | 13.508 | 29 | 0.000** | 1.744 |
| With melatonin | BOP1pre-BOP1post | 30.564 | 29 | 0.000** | 3.946 |
| | BOP0pre-BOP0post | 29.887 | 29 | 0.000** | 3.858 |

**Table 5: Intra and inter comparisons of descriptive and statistical test of total anti-oxidant tao-c among groups and visits using one way analysis of variance ANOVA, paired sample T test and independent sample t test.**

| Groups | | Base line | Second visit | Paired T test | df | P value | Effect size |
|---|---|---|---|---|---|---|---|
| Control | Mean | 0.761 | | | | | |
| | ± SD | 0.046 | | | | | |
| Without melatonin | Mean | 0.324 | 0.401 | 5.182 | 29 | 0.000 HS | 0.669 |
| | ± SD | 0.228 | 0.243 | | | | |
| With melatonin | Mean | 0.381 | 0.613 | 7.973 | 29 | 0.000 HS | 1.03 |
| | ± SD | 0.194 | 0.24 | | | | |
| Statistics F or T | | 37.146 | 3.402 | | | | |
| df | | 2 | 58 | | | | |
| P value | | 0.000 HS | 0.001 HS | | | | |
| ES | | 0.491 | 0.878 | | | | |

**Table 6: Multiple Comparisons of TAO-C in the base line using Games-Howell post hoc test.**

| Multiple comparisons of TAO in the base line using Games-Howell post hoc test | | | |
|---|---|---|---|
| **Groups** | **Groups** | **Mean Difference** | **Sig.** |
| Control | Without melatonin | 0.437 | 0.000 HS |
| | With melatonin | 0.38 | 0.000 HS |
| Without melatonin | With melatonin | -0.057 | 0.555 NS |

**Table 7: Correlation between periodontal parameters and TAO-C in 2nd visit and in each group.**

| Groups | | TAO-C | |
|---|---|---|---|
| | | r | p value |
| Without melatonin | PLI | 0.313 | 0.093 |
| | GI | 0.348 | 0.06 |
| | BOP0 | -0.314 | 0.091 |
| | BOP1 | 0.216 | 0.253 |
| With melatonin | PLI | 0.114 | 0.549 |
| | GI | -0.074 | 0.696 |
| | BOP0 | 0.317 | 0.088 |
| | BOP1 | -0.436 | 0.016 |

correlation between BOP score 1 and TAO-C at recall visit in with melatonin group.

## DISCUSSION

Regarding the PLI and GI At base line visit, there was a highly significant difference between control and study groups due to the fact that control subjects had healthy periodontium and good plaque control by performing good oral hygiene measures, and also by the fact of selection criteria of subjects in all groups.

Another fact of difference is the accumulation of microbial biofilm on the teeth of study groups which consider the primary and major etiological factor for progression of periodontal disease [22]. Increased invasion of microbial biofilm will cause disruption of epithelial barrier and entry of huge number of immune cells that enhance the dilation of arterioles and venules which lead to increase permeability of microvascular bed that cause inflammation of gingiva and supporting tissue of periodontium [23]. These findings agree with the results of Rai et al. [24]. There was a highly significant difference between PLI and GI between the study groups at recall visit when compared with a baseline visit (p value ≤ 0.05) with more variability and effect size in with melatonin group than that without melatonin. Instructions and motivation with the aids of scaling with root planing results in significant reduction in the means of both PLI and GI in both study groups, but the effect of melatonin had a greater variability and significance when compared with the groups that treated with scaling and root planing only. These findings agreed with the study confirmed by cutando et.al which showed that gingival index and probing depth were reduced significantly [25]. Similar finding agreed with these results confirmed by Almughrabi et al. which showed that consumption of melatonin reduce the formation of bacterial biofilm, but this reduction was not significant compared with control group [26], similarly syrinath et al. suggested that melatonin had the activity against streptococcus mutans,

prevotella intermedia and porphyromonus gingivalis which consider the major cause for progression of periodontal diseases [16]. Regarding the bleeding on probing, it was examined only with study groups (without melatonin, with melatonin). Statistically there was non-significant difference between study groups at base line visit. BOP score1were decreased from base line to the recall visit, while BOP score 0 was increased with high significant difference and variability for with melatonin group than that without melatonin group. SRP alone (without melatonin group) was effective in decreasing BOP score 1, these findings were agreed with previous studies of Santos et.al and Wennstron et al. [27,28],while melatonin group exhibited more increasing in BOP 0.

Similar findings were reported by cutando et.al that reported that topical application of melatonin in diabetic patients will significantly reduce bleeding and probing in active periodontitis through the down regulation in pro-inflammatory factors and decreasing the rate of bone loss [19]. Another study by Montero et al. [29] was agree with 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.

**Total antioxidant capacity (TAO-C) from base line to recall visit**

At base line visit, the data of results showed a highly significant difference between control and study groups that showed a significant lowering in TAO-C in patients with periodontitis than healthy subjects. These results were consistent with Brocks et.al who found that the mean of TAO-C was lowered in patients with periodontal disease than healthy [30]. Chapple et al. found that GCF glutathione and TAOC were reduced in patients with chronic periodontitis , this represents the systemically increased ROS and decreased defenses of antioxidants in periodontal disease patients and might be clarified by the idea that induced ROS is published by PMNs to contribute with development in free radicals mediated periodontal injury [31].

Regarding the second visit, it showed that there was increased in the means level of TAO-C from base line in both study groups with highly significant differences and large effect size of this increasing to with melatonin group than without melatonin group. Similar findings reported by Javid AZ et al. [32] who found that consumption of melatonin for 8 weeks in adjunct to SRP significantly increase serum level of TAOC. Melatonin is proposed to be one of the most effective free radical scavenger that is working as a direct scavenger that neutralize many free radicals including superoxide anion radical, hydroxyl radical, singlet oxygen and others. Melatonin also defend against oxidative stress by enhancing mitochondrial function and stimulate antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) [33]. Also in the present study , the result of increasing in TAO-C in without melatonin group were consistent with results of Akpinar et.al who found that non-surgical periodontal treatment (SRP) only, decrease the oxidative stress and increase TAO-C [34]. Similarly Brock et.al indicated that treatment with SRP only with some enhancement in clinical periodontal parameters can improve the antioxidant protection in serum and GCF in chronic periodontal patients [30]. Another study found that there was increasing significantly in GPx and TAOC of saliva and not significant decreasing in SOD activity after SRP in chronic periodontal patients [35], decreasing in oxidative stress was explained by reduction of inflammation and circulating cytokines as a result of debridement of dominant bacteria by the effect of scaling and root planning.

There was only weak negative significant correlation found between BOP score 1 and TAO-C in with melatonin group. Results of the study agreed with studies of Javid et al. and Akpolat et al. [32,36] which demonstrated the positive effect of melatonin on improving periodontal indices when used as adjuvant to scaling and root planning , since melatonin significantly reduce sub-gingival micro-organisms and periodontal inflammation including BOP score 1, in addition to effect of SRP , furthermore melatonin significantly increase serum level of TAO-C by exerting its antioxidant effect in addition to SRP in decreasing the oxidative stress through reducing the virulence of invading microorganisms which consider the major source of producing free radicals.

## CONCLUSION

Daily supplementation with 5mg melatonin therapy as adjunct to SRP significantly improved clinical periodontal parameters and increased total antioxidant capacity by exerting its anti-inflammatory and antioxidant properties.

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