

## EVALUATION AND COMPARISON OF TOTAL ANTIOXIDANT CAPACITY OF SALIVA IN PATIENTS WITH AND WITHOUT CHRONIC PERIODONTITIS PATIENT

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*Submitted 18 July 2022; accepted 28 August 2022*

### ABSTRACT

**Background:** Saliva despite being rich in ions, proteins, and antibodies also contains antioxidants: ascorbic acid, uric acid, albumin, and enzymes, which play an important role against oxidative stresses (OS) that causes oral cavity diseases like periodontitis. The aim of study is to analyze and compare the total antioxidant capacity of saliva between chronic periodontitis patients (at baseline and after therapy) and healthy non-chronic periodontitis patients (control group) in addition to comparing periodontal measurements such as gingival index, pocket depth and clinical attachment loss among chronic periodontitis patients at baseline and after one month from treatment.

**Methods:** The methodology of this study is a clinical quantitative study, which consists of A total of 53 patients who visited Duhok dental polyclinic/ Kurdistan region of Iraq from April 2021 to November 2021 were enrolled in this study. Among them, 37 patients suffered from chronic periodontitis, while 16 individuals were healthy and regarded as control group, age group was between 30 and 55 years old, out of 52 patients, 23 were female and 30 were male. Unstimulated saliva samples were collected from patients at baseline and one month after therapy (non-surgical treatment) and from control group. All samples were analyzed using total Antioxidant Capacity Assay Kit (Sigma-Aldrich, USA) by EPOCH2 plate reader (BioTek, USA).

**Results:** The means of periodontal parameters (Gingival Index, Periodontal Pocket Depth, Clinical Attachment Loss) in chronic periodontitis patients were significantly decreased after non-surgical treatment, while the Total Antioxidant Capacity (TAC) of saliva was significantly increased in chronic periodontitis patients after one month of treatment but still significantly lowered when compared with the control group.

**Conclusions:** The Total Antioxidant Capacity (TAC) of saliva decreased in chronic periodontitis patients as the result of oxidative stress generated by bacterial pathogens but elevated by non-surgical treatment and good oral hygiene home care.

**Duhok Med J 2022; 16 (2): 1-10.**

**Keywords:** *Antioxidants, Saliva, Periodontitis, Total Antioxidant Capacity.*

**P**eriodontitis is an inflammatory disease caused by bacteria that ranges from mild to massive destruction of tooth supporting tissue<sup>1</sup>. The disease is always preceded by gingivitis and if left untreated it will progress down the root

surface, destructing the bone and periodontal ligament leading to the formation of pockets<sup>2</sup>. It can occur at any age and the progression of disease depends on many factors such as type of putative microorganisms, tooth type and position,

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<https://doi.org/10.31386/dmi.2022.16.2.1>

smoking, presence of systemic disease and type of treatment (surgical and non-surgical treatment)<sup>3</sup>.

Saliva is a biological body fluid secreted by major and minor salivary gland<sup>4</sup> play a prime role in sustaining the integrity of the oral cavity by acting as a calcium and phosphate ion reservoir, cleansing, Potential of Hydrogen (PH) buffer, containing Immunoglobulins (Ig), proteins, hormones, and antioxidants<sup>5</sup>.

Antioxidants in saliva comprise mainly of uric acid (70%) with a lesser amount of albumin, ascorbate, glutathione and trace elements of transferrin, lactoferrin and caeruloplasmin) which play as defense mechanisms against free radicals<sup>6</sup>. Any disproportion between antioxidants and free radicals will lead to formation of oxidative stress<sup>7</sup>.

The oxidative stress generated during bacterial and viral infection, hyperthermia ionizing, UV irradiation and environmental pollutant will lead to destruction of all biocompatible molecules such as DNA, protein, and lipid component of cell membrane leading to cell death<sup>8</sup>. Oxidative stress has been associated with diabetes, atherosclerosis, inflammatory conditions, hypertension, heart disease, neurodegenerative diseases, cancer, and periodontitis<sup>9</sup>.

The purpose of this study was to analyze the TAC of saliva in chronic periodontitis patients and to determine the effect of scaling and root planning on TAC value after one month of treatment, the results are compared (at baseline and after treatment) and those values are compared simultaneously with the control group.

## **2. MATERIALS AND METHODS**

Data was clinically studied at baseline and after treatment with control variable:

statistical package for social science version 24 has been used for analyzing the data after attaining the data from Enzyme Linked Immunosorbent Assay (ELISA) technique using total antioxidant capacity assay, catalog number MAK187 (sigma Aldrich, USA) and EPOCH 2 plate reader (Bio Teck, USA). while the type of data collection method is quantitative.

### **2.1. Patient Selection**

In this cross-sectional study, 53 patients (aged between 30-55 years old, out of 52 patients 23 were female and 30 were male.) who visited Duhok specialized dental poly clinic in Duhok city Kurdistan region of Iraq from April 2021 to November 2021 were participated. Among them 37 patients were suffered from chronic periodontitis and 16 individuals were clinical healthy (periodontitis free) who visited dental poly clinic for other purposes (decayed tooth, orthodontic purposes, etc.) regarded as control group. Ethical committee consent obtained from ministry of health (Duhok Directorate General of health). A special consent form was obtained from each patient after explaining the whole procedure to the patient with the purposes of this research. General information has obtained from each patient which include patients name, age, gender, phone number and date of sample taking. The consent form has been signed by the patient.

The studied population were divided into two groups:

#### **Treatment Group**

Unstimulated saliva samples were collected from 37 patients with chronic periodontitis at base line (before receiving any treatment) and after one month from receiving treatment (scaling and root planning with using chlorhexidine

gluconate 0.2% mouth wash for two weeks).

#### Control Group

Unstimulated saliva was collected from 16 healthy patients without chronic periodontitis.

The inclusion criteria are:

Clinical systemically Healthy patient with no medication.

Chronic Periodontitis patients, moderate to Severe periodontitis with attachment loss  $\geq$  5 mm in at least 30% of teeth (except for control group).

Age (30-55) years old patients.

Non-Smokers.

Non-Alcoholics.

Non-Pregnant women.

#### 2.2 Saliva samples:

Patients were instructed not to eat or drink one hour before saliva collection. Each patient sat in upright position on dental chair instructed not to swallow or spit for about five minutes then 15 ml of saliva was collected by spitting into plastic container labeled with the name of the patient and date of collection and kept at -200C for analysis<sup>10</sup>.

#### 2.3 Recording Periodontal Measurements

Periodontal measurements were recorded for both treatment group and control group by using WHO periodontal probe which include:

Gingival index (GI) scored as

Normal gingiva

Slight change in color and mild edema with slight change in texture but no bleeding

Redness, hypertrophy, edema and bleeding on probing

Marked redness, hypertrophy, edema with spontaneous bleeding<sup>11</sup>.

Probing pocket depth (PPD) periodontal probe is inserted in each tooth pocket individually and measurement taken from

gingival margin to the base of the pocket<sup>12</sup>. Clinical attachment loss (CAL) periodontal probe run along each tooth surface and measurement taken from cemento-enamel junction (CEJ) to the base of the pocket<sup>12</sup>.

#### 2.4 Laboratory method

All frozen Saliva samples were thawed at room temperature then centrifuged to separate debris and analyzed by Enzyme Linked Immunosorbent Assay (ELISA) technique using total antioxidant capacity assay, catalog number MAK187 (sigma Aldrich, USA) and EPOCH 2 plate reader (Bio Teck, USA). According to the instructions supplied with the kit, the antioxidant activity of saliva samples was expressed as nanomole Trolox equivalent per milligram (nmol TE/mg).

### 3. RESULTS:

#### 3.1. Multicollinearity Test

Before analyzing the data, multicollinearity test has been checked among the indices of this study. The results of multicollinearity test were in boundaries of statistical significance which indicated that data of the indices were different between before and after the treatment for all three indices. Therefore, all necessary statistical tests can be deployed for the collected 37 samples.

#### 3.2 Descriptive Statistics for Indices (GI, PD, CAL)

Below tables and figures show the effectiveness of non-surgical therapy and oral hygiene maintenance on decreasing gingival inflammation, pocket depth and clinical attachment loss. The results showed lowered GI, PD and CAL indices after treatment in comparison to before treatment.

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**Table 1. descriptive statistics for GI, PD, CAL indices**

Indices	N	Minimum	Maximum	Mean	Std. Deviation
Baseline GI	37	1.50	4.80	3.3486	0.78938
After GI	37	0.60	3.00	1.8676	0.60187
Baseline CAL	37	0.80	6.20	3.6973	1.40801
After CAL	37	0.60	5.30	2.9919	1.34089
Baseline PD	37	0.70	3.20	1.7257	0.47516
After PD	37	0.30	2.50	1.0378	0.44244

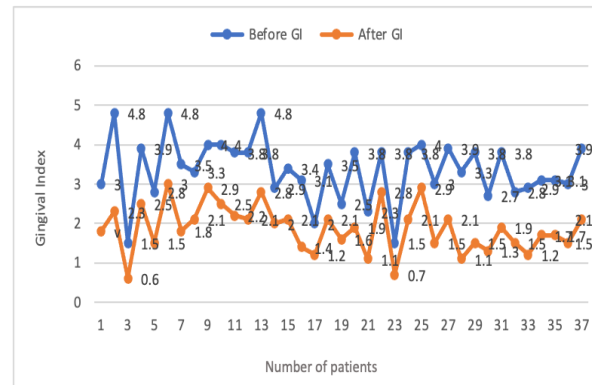
The means for all indices after treatment were decreased significantly compared to before treatment, which indicate improvement in the health of periodontium after treatment with non-surgical therapy. As robustness of the results, the standards deviation of the data is confirming again the improvement of the health of periodontium after the treatment with non-surgical therapy.

**Table 2. One Sample T. Test for GI, PD, CAL Indices**

Indices	t	df	Sig. 2 tailed P-Value	95% Confidence Interval of the Difference	
				Lower	Upper
Baseline GI	25.804	36	0.000	3.0855	3.6118
After GI	18.874	36	0.000	1.6669	2.0682
Baseline CAL	15.973	36	0.000	3.2278	4.1668
After CAL	13.572	36	0.000	2.5448	3.4390
Baseline PD	22.091	36	0.000	1.5672	1.8841
After PD	14.269	36	0.000	0.8903	1.1854

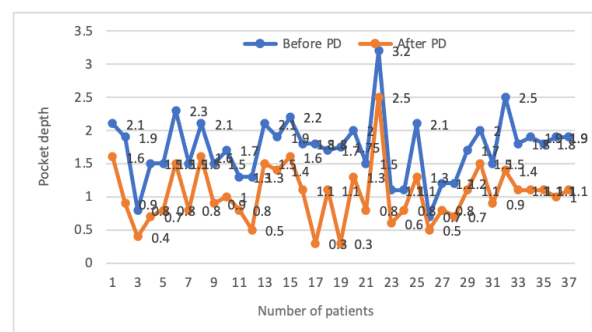
Table 2 illustrates that all samples were distributed normally for all indices as shown in the one sample t-test with statistical confidence of 95%. Concerning GI before and after treatment, there was significantly decreasing of indices after

treatment with non- surgical therapy compared with indices before treatment as shown in figure 1. indicating to the improvement of the health of periodontium after treatment with non-surgical therapy.



**Figure 1. comparison between baseline and after GI**

Concerning PPD at baseline and after treatment, there was significantly decreasing of indices after treatment with non- surgical therapy compared with indices before treatment as shown in figure 2. indicating to the improvement of the health of periodontium after treatment with non-surgical therapy.



**Figure 2. comparison between baseline and after PPD**

Although no significant differences was detected for CAL at baseline and after treatment as shown in figure 3. but there was a slight improvement in attaining some attachment which is beneficial for improving periodontal health.

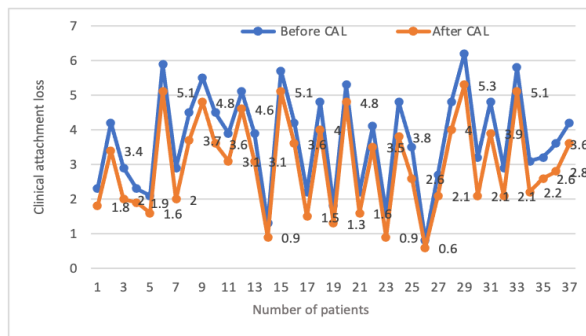


Figure 3. comparison between baseline and after CAL

### 3.3. Descriptive Statistics TAC

Table 3 shows the values of TAC in patients treated with non-surgical therapy and control group. The TAC of saliva of patients after treatment has been significantly increased but not differ significantly with those of control group.

Table 3. One sample test for TAC between patients and control group

Test Value = 0						
				Mean	95% Confidence Interval of the Difference	
	t	Df	Sig. (2-tailed)	Difference	Lower	Upper
Base Line	5.126	36	.000	.03376	.0204	.0471
After T	4.612	36	.000	.04681	.0262	.0674
Control	25.307	15	.000	.06038	.0278	.0329

The values of TAC in patients with periodontitis before and after treatment compared to TAC in control group as shown in figure 4. The TAC values were low before treatment and high after treatment but when compared to TAC in control group no significant differences were found.

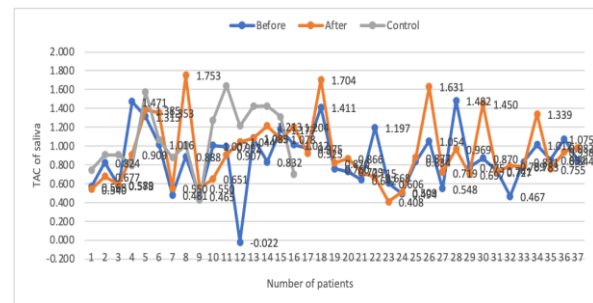


Figure 4. baseline & after treatment with control (TAC)

## 4. DISCUSSION

Although bacterial pathogens are the principal element in the initiation of periodontitis, host reaction to these pathogens modified by genetics, environmental, microbial pathogenicity and acquired risk factors<sup>13</sup>. Saliva contains enzymatic and nonenzymatic antioxidant which can be used as diagnostic biomarker as it acts as prime line defense against oxidative stress that released during disease<sup>14</sup>.

In the present study, the periodontal status GI, PD and CAL indices in chronic periodontitis patient had been recorded both at base line and one month after non-surgical treatment and the outcomes of the study showed improvement in all the indices after one month of treatment which included (scaling and root planning with using chlorhexidine gluconate 0.2% mouth wash for two weeks) and this indicate refinement in overall periodontal health.

The gingival index (GI) decreased significantly after treatment, and this indicate resolution of gingival inflammation by reversing the cardinal signs of inflammation which led to reduction of leukocyte infiltration into the area and decreasing vascular permeability and vasodilatation<sup>13</sup>.

The pocket depth reduced significantly after one month of treatment this shows



the capability of (Scaling and Root planning) in creation a favorable condition to initiate new attachment apparatus specially in shallow pockets  $\leq 5\text{mm}$  as elimination of the entire pockets in severe cases need more regenerative procedures (surgical)<sup>14</sup>. SRP had beneficial effect in attaining new attachment and this shown by reduction in clinical attachment loss |(CAL|) one month after treatment, however advanced clinical attachment loss needs progressive surgical procedures.

Medically compromised patients, alcoholic, pregnant women, and smokers are excluded in this research to know the exact effect of periodontitis on TAC without any contributing factors, as those conditions mentioned earlier are known to cause decreasing in TAC of saliva by generating oxidative stress<sup>15</sup>. Mild form of periodontitis had been excluded in this study; however, some studies show no difference in TAC of saliva in relation to severity of periodontitis<sup>16</sup>.

Salivary TAC values were evaluated in healthy and chronic periodontitis patients at base line and after treatment. The TAC in periodontitis patient both at base line and after treatment dropped in comparison with healthy group, however there was an elevation of TAC value in periodontitis patient after treatment when compared with the base line which indicate that non-surgical therapy has the power to diminish oxidative stress that is generated by bacterial pathogens. The study results indicated that periodontitis diseases induce generation of ROS which regress antioxidant capacity, and this will lead to more progressive destruction of periodontal tissue. Based on these results, the total antioxidant capacity of saliva can be raised with improvement of periodontal tissue parameters by nonsurgical therapy.

The results of the recent study agreed with the results of 17-18 who also found the periodontitis can lower TAC by inducing generation of ROS, while others<sup>17, 18, 20-22</sup> found elevation of TAC in circulatory system. A pragmatic correlation has been founded between TAC in saliva and periodontal status<sup>17, 20</sup> as the periodontal indices (GI, PPD, CAL) show improvement the TAC of saliva increased accordingly. meanwhile a controversially exist whether if periodontal therapy can influence TAC in saliva<sup>21, 22</sup>. More studies demanded to determine the effect of non-surgical periodontal treatment on salivary antioxidants.

In some research antioxidants (natural or synthetic) had been given directly to patients with chronic periodontitis in addition to nonsurgical therapy to increase defense mechanism of antioxidant against ROS generated due to tissue destruction by periodontitis<sup>23</sup>. Grapefruit administered to patients with periodontitis as a natural source of vitamin C showed reduced sulcus bleeding score<sup>24</sup>. While<sup>25</sup> used green tea consumption prove to be a promising adjunctive prophylactic and therapeutic modality in minimizing oxidative damage in saliva of periodontal patient. In another study by<sup>26</sup> showed reduced probing pocket depth within 8 months after administrating encapsulated fruits, vegetable and berry juice powder concentrates adjunct with SRP when compared with placebo<sup>26</sup> used green tea and showed increasing in TAC in saliva.

The restriction of this study was it didn't evaluate the amount of oxidative stress in health and periodontitis groups which is the major causative factor in reducing antioxidant levels. The study didn't show the effect of periodontitis severity on TAC, another limitation was the study didn't

evaluate the types of antioxidants present in saliva individually as the evaluation done in total, no categorization done according to gender or age.

### 5. CONCLUSION & RECOMMENDATIONS

Periodontitis results in reduction of total antioxidants as revealed in this study when compared with control group, promising results shown in this study as the TAC of saliva can be elevated with non-surgical treatment by improving the periodontal status which has been proven by showing the periodontal indices at baseline and after treatment. The researcher recommends patients for oral checkup every six months to early detection of periodontal disease in order to prevent progression of disease and prevent future tooth loss as the integrity of oral cavity may affect the health of the whole human body systems.

The government might have a principal role in raising the awareness of society from periodontal disease and its destructive consequences by increasing medical services in government hospitals with providing assistance and programs for individuals who do not have access to dental care.

This study recommends future research to expand the sample size of the patients, different age group, determining periodontitis severity effect on TAC and evaluate types of antioxidants present in saliva separately.

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## پوخته

نەرخاندنا و بەرھەمەدانانی ئۆکسیدانت ل تەقەبەتە فەیدای ل نەخۆشەکان دەروارە درێژخایەنەکان

### پێشەکی و ئارمانج:

سەرەرای کۆلیک یا دەولەمەندە ب نایۆن و پرۆتین و دژمەنەکان ھەروەسا دژە ئۆکسینەر تێدا ھەنە: ترشی نەسکۆریک، ترشی یوریک، ئەلبومین، و ئەنزیمەکان پۆلەکی گەرم دەگیرن ل دژی فشارا ئۆکسینەرەکان کە دبیته ئەگەر نەخۆشەکان بۆشایی دەفی وەک ھەوکردنا شانەین دەرووبەرین دانا. ئارمانجا قەوڵینی شیکردن و بەراوردکردن کۆی توانای دژە ئۆکسینەرە لیکی بوو دناق بەرا نەخۆشی ھەوکردنێ درێژخایەنێ دەرووبەرینددا (لە ھێلی بنەرەتی و دوا چارەسەرکردن) و نەخۆشین تەندروست باش (گروپی کۆنترۆلکری) جەدا ژ بەراوردکردن پێوانە دەرووبەرینددا وەک پێوەرین پووک، قوولی گیرفان و ژ دەستدانا لکاندنێ کلینیکی دناق بەرا نەخۆشین ھەوکردنا درێژخایەن دەرووبەرین دانا ل ھێلی بنەرەتی و پێشتی ھەیفەک لە چارەسەرکردن.

### رێبازا قەوڵینی:

کۆی گشتی ۵۳ نەخۆش کۆل نیسانا ۲۰۲۱ تا چریا دووی ۲۰۲۱ سەردانا کلینیکا دانا ل دەوک/ ھەرمی کوردستانی عێراقی کر بوو، دفی قەوڵینی دا ناڤین وان ھاتبوونە تۆمارکردن. دناق بەرا ۳۷ نەخۆشین تووشی ھەوکردنا درێژخایەنێ دەرووبەرین دانا بوون، دەمەکی دا ۱۶ کەس تەندروست بوون و وەک گروپی کۆنترۆل ھاتبوونە ھەوکردن. نموونە لیکی ھاتە وەرگرتن ل نەخۆشین ل ھێلا بنەرەتی و ھەیفەک پێشتی چارەسەرکردن (چارەسەریا بێ نیشترەگەری) و ل گروپی کۆنترۆلکری. ھەمی نموونە ھاتنە تاقیکردن بکار ھینانا کیتی تاقیکردن توانای کۆی گشتی دژە ئۆکسینەرە (Sigma-Aldrich, USA) ژلایی خوینەرە پلانی EPOCH2 ھاتنە شیکردن بریکا نامیرێ (بایۆتیک، ئەمریکا).

### ئەنجام:

مامناوەندی پارامیترەین دەرووبەرین دانا Gingival Index, Periodontal Pocket Depth, Clinical Attachment (Loss) دگەل نەخۆشین ھەوکردنا درێژخایەنێ دەرووبەریندانا ب شێواژەکی بەرچاڤ کیم کریە پێشتی چارەسەرکردن نەشتەرگەری بو ھاتنەکردن، دەمەکی دا کۆی گشتی دژە ئۆکسینەرە بێ لیکی ب شێواژەکی بەرچاڤ زیدە بوو ل نەخۆشین ھەوکردنێ درێژخایەن دەرووبەرین دانا پێشتی بێک ھەیف ژ چارەسەرکردن بێ نیشترەگەری بەلێ ھیشتا ب شێوھەکی بەرچاڤ دابەزییە دەمەکی دا ب بەراوردی دگەل گروپی کۆنترۆل کری.

### دەرەنجامەکان:

کۆی گشتی دژە ئۆکسینەرە بێ لیکی ل نەخۆشین ھەوکردنا درێژخایەن دەرووبەرین دانا کیم کریە ژ ئەنجامی فشارا ئۆکسینێ کە ژ لایەنێ ماددە نەخۆشین بەکتریا دروست دبیته بەلێ ژبەر چارەسەریا بێ نیشترەگەری و چاڤدیریا باش و پاکخاویێ دەم و دانا بەرزبوو.

## الخلاصة

تقييم ومقارنة السعة الكلية لمضادات الأكسدة في لعاب المرضى الذين يعانون من مريض التهاب الأسنان المزمن والذين لا يعانون من الالتهاب المزمن

### الخلفية والأهداف:

على الرغم من كون اللعاب غنياً بالأيونات والبروتينات والأجسام المضادة فإنه يحتوي أيضاً على مضادات الأكسدة: حمض الأسكوربيك وحمض البوليك والألبيومين والإنزيمات التي تلعب دوراً مهماً ضد الاجهاد التأكسدي التي تسبب أمراض تجويف الفم مثل التهاب دواعم الأسنان. الهدف من الدراسة هو تحليل ومقارنة السعة الكلية لمضادات الأكسدة في اللعاب بين مرضى التهاب دواعم السن المزمن (عند خط الأساس وبعد العلاج) ومرضى بدون التهاب دواعم السن المزمن (المجموعة الضابطة) بالإضافة إلى مقارنة قياسات دواعم السن مثل مؤشر اللثة وعمق الجيب وفقدان المرفق السريري بين مرضى التهاب دواعم السن المزمن عند خط الأساس وبعد شهر واحد من العلاج.

### منهجية البحث:

تم تسجيل مجموعة مكونة من 53 مريضاً في هذه الدراسة زاروا مجمع طب الأسنان في دهوك/ إقليم كردستان العراق في الفترة من نيسان 2021 إلى تشرين الثاني 2021. وكان من بينهم 37 مريضاً يعانون من التهاب دواعم السن المزمن، بينما كان 16 شخصاً يتمتعون بصحة جيدة ويعتبرون مجموعة ضابطة. تم جمع عينات اللعاب غير المحفزة من المرضى عند خط الأساس وبعد شهر واحد من العلاج (العلاج غير الجراحي) ومن المجموعة الضابطة. تم تحليل جميع العينات باستخدام طقم فحص لقياس سعة مضادات الأكسدة الكلية (Sigma-Aldrich، الولايات المتحدة الأمريكية) بواسطة قارئ لوحة EPOCH2 (BioTek، الولايات المتحدة الأمريكية).

### النتائج:

انخفضت مؤشرات دواعم الأسنان (مؤشر اللثة وعمق الجيب وفقدان المرفق السريري) بشكل ملحوظ في مرضى التهاب دواعم السن المزمن بعد العلاج غير الجراحي، بينما زاد سعة مضادات الأكسدة الكلية من اللعاب بشكل ملحوظ في مرضى التهاب دواعم السن المزمن بعد شهر واحد من العلاج ولكن لا يزال منخفضاً بشكل ملحوظ عند المقارنة مع المجموعة الضابطة.

### الاستنتاجات:

انخفض معدل سعة مضادات الأكسدة الكلية في مرضى التهاب دواعم السن المزمن نتيجة الإجهاد التأكسدي الناتج عن مسببات الأمراض البكتيرية ولكنه زاد عن طريق العلاج غير الجراحي والعناية المنزلية الجيدة بنظافة الفم.