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THE EFFECT OF NON-SURGICAL PERIODONTAL THERAPY ON PERIODONTAL PARAMETERS, MALONDIALDEHYDE, TOTAL OXIDANT STATUS AND ANTIOXIDANT CAPACITY IN SMOKERS WITH CHRONIC PERIODONTITIS

LANA **B**. **A**LI[*] and **C**HENAR **A**. **M**OHAMMAD[**]
[*]College of Dentistry, Hawler Medical University, Erbil, Kurdistan Region-Iraq
[**]Dept. of Periodontics, College of Dentistry, Hawler Medical University, Erbil, Kurdistan, Region, Iraq

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ABSTRACT

BACKGROUND AND OBJECTIVE: Oxidative stress can be described as a state brought on by an unfavorable rise in the generation of reactive oxygen species (ROS), a growth in ROS production, or a decline in antioxidant defense. The study aimed to investigate the impact of non-surgical periodontal treatment on clinical periodontal parameters and serum malondialdehyde MDA, total antioxidant capacity TAOC, and total oxidant status TOS in smokers and non-smokers periodontitis patients.

METHODS: A clinical comparative research was carried out on 60 systemically healthy subjects with an average age of 30-50 years; 30 with chronic periodontitis (15 non-smoker periodontitis NSP and 15 smoker periodontitis SP) and 30 with healthy periodontium (15 non-smoker healthy NSH and 15 smoker healthy SH). Blood samples and clinical periodontal parameters were collected at baseline before scaling & root planing SRP and after six weeks of treatment to estimate MDA, TOS, and TAOC.

RESULTS: Clinical periodontal parameters (PI,GI,BOP,PPD&CAL) and oxidative stress markers (MDA and TOS) were significantly reduced after SRP for periodontitis patients groups(smoker periodontitis SP and non-smoker periodontitis NSP) with exception for the non-significant reduction of gingival recession GR and non-significant elevation of TAOC (P>0.05). However, no correlation was found between biochemical and clinical parameters at baseline and after therapy, except for the significant negative correlation between BOP and TAOC in SP and between TAOC & PPD in NSP before therapy.

CONCLUSION: Non-surgical periodontal therapy could significantly improve clinical periodontal parameters, reduce oxidative stress and improve the antioxidant defense system of the body.

KEYWORDS: Periodontitis, Oxidative Stress Markers, Antioxidant, Smoking, Scaling & Root Planing

1. INTRODUCTION

Periodontal disease (PD) is a disease of chronic inflammatory caused by bacterial microorganisms and includes extreme chronic inflammation that induces tooth-supporting structures to be damaged and can lead to tooth loss(Kassebaum *et al*,2014). Periodontitis develops when inflammation-related alterations reach the alveolar bone and periodontal ligament and are permanent and eventually destructive, resulting in tooth loss(Könönen *et al*,2019).

Several factors increase the risk of chronic periodontitis (CP). These risk factors, modifiable and non-modifiable, the most noteworthy major modifiable risk factor of PDs is tobacco smoking(Genco and Borgnakke, 2013). According to reports, around 50% of those who have periodontitis smoke(Heitz-Mayfield, 2005). Smoking has an impact on immunity by altering the production of inflammatory mediators, vascular factors, fibroblast activities, antibody production, and neutrophil function(Johnson and Guthmiller, 2007).

Mostly, evidence suggests that smokers have more serious disease of periodontal compared to non-smokers, with enhanced

attachment loss, bone loss, and gingival recession, as well as pocket formation(Nociti *et al*, 2015), For the treatment of CP, non-surgical therapies such as scaling and root planning (SRP) are determined to be effective. SRP has a severe impact on declined inflammation, gain in clinical attachment level (CAL), declined probing depth (PD), and therefore, increasing total clinical effectiveness (Akram *et al*, 2020).

It appears that smoking have a significant function in the periodontal diseases pathogenesis by an enhancement in oxidative stress, induction of an imbalance between antioxidant capacity and oxidative stress, and dysregulation of the adaptive and innate immune system. Oxidative stress is defined as a condition caused by an unfavorable rise in the generation of reactive oxygen species (ROS) (Guentsch *et al*, 2008). A rise in free radicals induces MDA overproduction in patients with periodontitis(Kumar *et al*, 2019). To evaluate the body's overall oxidation state, total oxidant status (TOS) is frequently utilized (Erel, 2005). It has been reported to have greater level of TOS in serum of smokers with chronic periodontitis (Ahmadkhaniha *et al*, 2021). Total antioxidant capacity (TAOC) is an integrative metric which captures the cumulative effect of mostly non-enzymatic antioxidants found in body fluids and plasma (Sies, 2007). Measuring TAOC might reveal information about the balance between antioxidant and oxidants systems (Collins, 2005). Measurement of MDA, TAOC, and TOS in serum appears to be a valid approach that can offer a new and useful tool to assess the level of oxidative stress in periodontitis patients before non surgical periodontal therapy (scaling and root planing SRP) and after therapy, for that the current study aimed to evaluate the effect of SRP on clinical periodontal parameters (PI, GI, BOP, PD, CAL & GR), biochemical oxidant (MDA&TOS) and antioxidant (TAOC) markers in periodontitis patients groups (non-smokers and smokers) after 6 weeks of therapy, and correlate between clinical and biochemical parameters at base line and after therapy.

2. METHODS

2.1 Setting, time, and design of the study

A clinical comparative study was carried out in Erbil city, Rizgari Teaching Hospital and periodontic department/College of Dentistry/Hawler Medical University from October 2021 to February 2022.

2.2 Ethical considerations

The study was reviewed and approved by the institutional ethical committee of the College of Dentistry/ Hawler Medical University in Erbil City/Kurdistan Region of Iraq (Ethical approval number:71). The study was explained to all volunteers before the conduction of the study and informed written consent was approved and signed by all participants before the procedure was carried out.

2.3 Sample groups

Sixty systemically healthy male subjects with an average age of 30- 50 years: 30 with chronic periodontitis CP (mild to moderate) and 30 with healthy periodontium. The 30 subjects with periodontitis were divided randomly into two groups: group (A), included 15 smokers with periodontitis SP and group (B), included 15 non-smokers with periodontitis NSP.While the rest 30 subjects with healthy periodontium were divided randomly into two groups: group (C) included 15 smokers with healthy periodontium SH and group (D), included 15 non-smokers with healthy periodontium NSH.

The inclusion criteria for all participants were: systemically healthy subjects with the presence of at least 20 teeth. For smoker patients the inclusion criteria were: smoking of at least ten cigarette / day and the duration of smoking was at least of five years, while for non-smoker subjects the inclusion criteria were never smoking and for subjects with healthy periodontium, the inclusion criteria were: no presence of gingival inflammation or periodontitis(GI<0.6, BOP=0, PPD< 3mm, CAL=0). For chronic periodontitis patients, the inclusion criteria was mild to moderate periodontitis, for mild periodontitis (≥2 interproximal sites with CAL ≥ 3mm & PD ≥ 4mm or PD ≥ 5mm (not in the same teeth) or one site PD ≥ 5mm, and for Moderate: ≥2 interproximal sites with CAL

≥4mm or PD≥5mm (not in same teeth) according to page and Eke, 2007.

The exclusion criteria for all participants were: patients with systemic diseases, alcoholic patients, female gender, periodontal therapy or vitamin supplementation within the previous 3 months and immune suppressive agents or antibiotic intake in preceding 6 months.

2.4 Data collection:

All the data concerning age, gender, occupation, past dental and medical history, smoking status, duration of smoking (< 6years, 6-12 years and >12 years), and the number of cigarettes smoked per day (10 cigarettes, > 10-20, and > 20 cigarettes) were recorded in questionnaire sheet before clinical periodontal parameters assessments.

2.5 Clinical periodontal assessment:

All participants, clinical periodontal examination was cassessedat baseline before SRP by single trained examiner, using dental mirror, Williams periodontal probe and dental explorer. Plaque thickness was measured according to plaque index PI by Silness and Loe, 1964, gingival inflammation (extent and severity) was measured according to gingival index (GI) according by Loe and Silness, 1963 and the presence (1) or absence of bleeding (0) on probing according BOP by Ainamo and Bay, 1975. Periodontal pocket depth (PPD), clinical attachment loss (CAL) and gingival recession (GR) measurements were recorded in millimeters according to Lindhe et al, 2008 and Newman *et al*, 2014. For GI and PI , the measurements were conducted at four sites for each tooth (mesio-labial/buccal, mid-labial/buccal, destio-labial/buccal and mid lingual/palatal), and for BOP, PD, CAL and GR the measurements were recorded at six sits for each tooth (three sites for labial surface and three sites for lingual surface). Non-surgical periodontal therapy (SRP) was conducted for periodontitis patients (SP and NSP). Then clinical periodontal parameters were assessing again after six weeks of therapy.

2.6 Blood sample collection:

4 ml of fasting venous blood sample was collected from each patient, and stored in gel tube for 30 minutes, centrifuged at 3,000 rpm for 20 minutes to obtain clear supernatant, then aspirated by a plastic pipette and transferred into an epindrope (each epindrope labeled with a patient number and full name) and immediately stored at -70°C for later subsequent estimation of MDA, TOS and TAOC. Blood samples were collected at baseline and after 6 weeks of therapy in periodontitis groups (SP and NSP groups), and at baseline only for healthy subjects groups (SH& NSH groups). . Elisa Kits: Elabscience, USA, Elabscience, USA and SUNLONG, China were used for MDA, TOS and TAOC estimation, respectively.

2.7 Statistical Analysis

Using the Statistical Package for Social Sciences (SPSS, version 25), data were analyzed. Normality of data was checked using Shapiro-Wilk test, accordingly non-parametric tests were used when indicated. One way analysis of variance (ANOVA) and its Kruskal Wallis test of non-parametric equivalent were used for comparing the means and mean ranks of three or more groups. Unpaired T-test (T-test for two samples of independent) and its Mann-Whitney test of non-parametric equivalent were used for comparing the means and mean ranks of two groups. Paired T-test and its non-parametric equivalent Wilcoxon signed ranks test were utilized for comparing the means and medians of the same sample but at two different time periods. Statistical significance was set*P* value of <0.05.

3.RESULTS AND DISCUSSION

Sixty patients were involved in the research. Their mean age (SD) was 40.9 (6.6) years, the age range was 30-50 years, and the median was 41.5 years. Figure 1 shows that the mean age of the non-smoker healthy and smoker healthy groups (39.3 and 36.5 years respectively) were less than those of the smoker with periodontitis (43.2 years) and non-smoker with periodontitis (44.5 years) (p= 0.002)

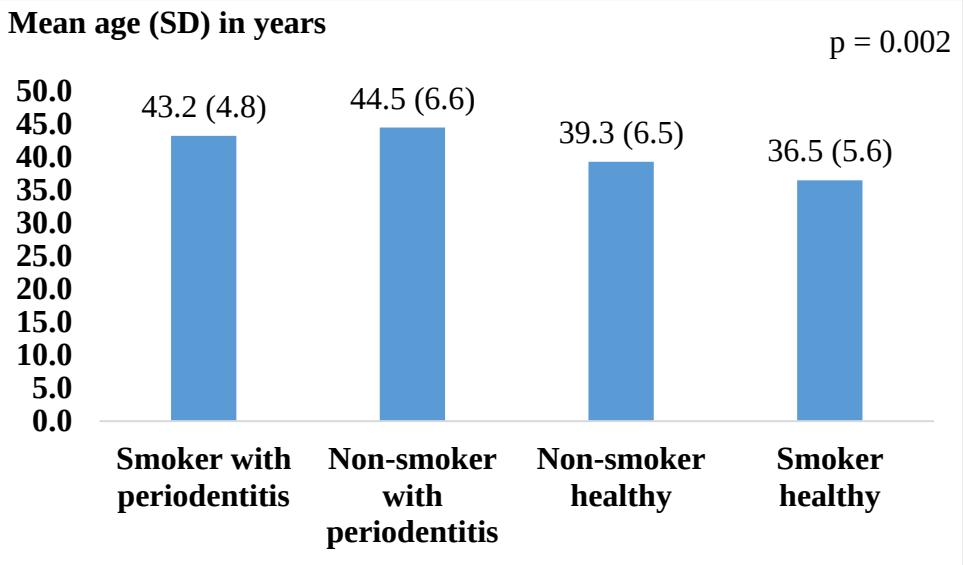

Fig. (1): mean age of study group

3.1 Clinical periodontal parameters in healthy and periodontitis patients groups at baseline:

The result showed that the mean value of PI in periodontitis patient groups (SP and NSP) were 1.057±0.268 and 0.937±0.225, respectively, with non-significant differences between both groups. While in control healthy groups (SH and NSH) the mean value of PI were 0.931±0.399 and 0.943±0.479, respectively, with non-significant differences between both and in comparison between control groups (SH and NSH) and periodontitis groups (SP and NSP) non-significant differences were found. Also non-significant differences were found between four groups (P=0.737).

The non-significant increase in PI in SP may be due to that smokers were less motivated for keeping oral hygiene of high-quality (Bergström *et al*, 1988; Luzzi *et al*, 2007) and also habits of tooth brushing in non-smokers tend to be more favorable comparing to smokers. Smokers might spend significantly less time for cleaning and brushing their teeth which might lead to more plaque persistence on their teeth after brushing comparing to non-smokers (Amarasena *et al*, 2002; Pejčić *et al*, 2007). The result of the current study was in agreement with other studies, reported a slight rise in the mean value of PI in smoker comparing to non-smoker with non-significant difference(Haffajee and Socransky, 2001a; Gloria Calsina *et al*, 2002; Sreedevi *et al*, 2012). In contrary, other studies reported significant less plaque in smoker periodontitis as compared to non-smokers periodontitis (Markkanen *et al*, 1985; Machuca *et al*, 2000).

For GI, BOP, PD, CAL, and GR the mean value in smoker periodontitis SP were: 0.879±0.174, 1.521±1.292%, 4.426±0.340 mm, 3.596±0.441 mm,1.241±1.147 mm, respectively, and for non-smokers periodontitis the mean values for GI, BOP%, PD, CAL, and GR were: 0.918±0.213,7.267±5.007 %, 4.310±0.338 mm, 3.623±0.282 mm, and 1.319±1.357 mm, respectively, with non-significant differences between both groups (SP and NSP).While in the smoker healthy SH, the mean values of GI, BOP, PD, CAL, and GR were: 0.430 ± 0.223, 0.001±0.004 %, 1.743±0.287 mm, and 0.000±0.000 mm, respectively, and for non-smokers healthy NSP the mean values were: 0.537 ± 0.203, 0.001±0.004 %, 1.687±0.326 mm, and 0.000 ± 0.000 mm, respectively, with non-significant differences between both groups in regard to GI (P=0.155), BOP, PD, CAL, and GR (P=1.000). For the comparison between each of periodontitis groups (SP and NSP) and healthy control groups (SH and NSH) significant differences were found, also significant differences were reported between the four studied groups (P<0.001) as shown in table 1.

Regarding GI and BOP, the present study showed that smoker patients (SP and NSP) at base line before therapy had reduced GI and BOP comparing with non-smokers (NSP and NSH). The highest mean

of GI was found in NSP followed by SP, NSH and the least mean value was found in SH. Regarding BOP, the highest mean reduction of BOP was found in SP followed by NSP and the least mean values were found in healthy control groups (SH and NSH), but with non-significant differences between SP & NSP and between SH&NSH. The reduction in gingival inflammation and BOP in smoker periodontitis may be due to the vasoconstrictor effects of nicotine, leading to a decline in the blood flow and resulting in local inflammation (Sreedevi et al, 2012; Luzzi *et al*, 2007). This can be supported by some studies reported that tobacco might be related to less expressive symptoms and signs of periodontal inflammation, including edema, erythema, and gingival bleeding, referring to a suppressive impact on the inflammatory response (Haffajee and Socransky, 2001b; Luzzi *et al*, 2007; Gautam *et al*, 2011; Sa, 2014).

The finding of the present study was in agreement with studies, reported that the difference in the mean values of GI between SP and NSP was not significant (Sa, 2014; Smiley *et al*, 2015). In contrast, other studies reported that GI was significantly increase in NSP as compared to SP (Preber and Bergström, 1986; Dietrich *et al*, 2004). Regarding BOP, the present result was in agreement with a study reported an overall tendency to more bleeding sites in the NSP with no statistically significant differences as compared to SP (Luzzi *et al*, 2007). In contrast, another studies reported that SP patients exhibited statistically significant decrease of BOP than did in NSP (Machuca *et al*, 2000; Bergström and Boström, 2001; Sreedevi*et al*, 2012; Şentürk *et al*, 2018).

Regarding PD, the present study showed that at baseline, the highest mean of PD were found in SP followed by NSP, SH and the least mean value was found in NSH. The results of the current study emphasized that in smokers, periodontal tissue was more affected than non-smokers but with non-significant difference. A tendency for higher PD mean was observed in all sites analyzed in SP as compared to NSP. This deterioration in the periodontal health of smokers can be attributed to the detrimental impact of tobacco which alters the host`s immune response, halts the proliferation of gingival fibroblasts, enhances the inflammatory response and causes bone destruction and connective tissue attachment loss (Zhang *et al*, 2019). The current study revealed that the mean values of CAL and GR in non-smokers and smokers periodontitis were relatively the same with non-significant differences. This was in agreement with another study, showed similar CAL in both periodontitis of non-smoker and smoker (Visvanathan *et al*, 2014). The shift in the host response, the subgingival bacteria virulence, and the changes in the composition of subgingival plaque which promote the bone resorption and periodontium destruction, may all be contributing factors to the rise in CAL in periodontitis (Haffajee and Socransky, 2001b). While smoking tobacco may harm collagen tissues, collagenase synthesis is increased, gingival fibroblast development is suppressed, and fibronectin & collagen production are also increased (Zhou *et al*, 2007). Regarding GR, our study showed NSP had almost similar gingival recession as that of SP. A study by Muller et al.(2002) was in agreement with the current study and reported no significant difference in the mean of GR between SP and NSP, and did not support the hypothesis that those who smoke had higher gingival recession. Whereas other studies have been reported an increase in the gingival recession in smokers(Nandhana *et al*, 2019).

Table (3.1): Clinical periodontal parameters at baseline in periodontitis and healthy groups .

Parameters	**Periodontitis/**Mean±SD		**Healthy/**Mean±SD		**Groupvsgrpup**		**P††(post-hoc)**	
	SP / (A)	**NSP/(B)**	**SH/ (C)**	**NSH/ (D)**				
PI	1.05±0.26	0.93±0.22	0.94±0.47	0.93±0.39	AXB AXD BXD	AXC BXC CXD	0.36 0.34 0.96	0.38 0.96 0.93
P value	0.737*							
GI	0.87±0.17	0.91±0.21	0.43±0.22	0.53±0.20	AXB AXD BXD	AXC BXC CXD	0.60 <0.001 <0.001	<0.001 <0.001 0.15
P value			<0.001*					
BOP (%)	1.52±1.29	7.26±5.00	0.00±0.00	0.00±0.00	AXB AXD BXD	AXC BXC CXD	0.22 0.001 <0.001	0.001 <0.001 1.00
P value	<0.001†							
PD (mm)	4.42±0.34	4.31±0.33	1.74±0.28	1.68±0.32	AXB AXD BXD	AXC BXC CXD	1.00 <0.001 <0.001	<0.001 <0.001 1.00
P value	<0.001†							
CAL (mm)	3.59±0.44	3.62±0.28	0.00±0.00	0.00±0.00	AXB AXD BXD	AXC BXC CXD	1.00 <0.001 <0.001	<0.001 <0.001 1.00
P value	<0.001†							
GR (mm)	1.24±1.14	1.31±1.35	0.00±0.00	0.00±0.00	AXB AXD BXD	AXC BXC CXD	1.00 0.005 0.005	0.005 0.005 1.00
P value	<0.001†							

*By ANOVA; †By Kruskal Wallis test; ††: LSD was used after ANOVA, and Dunn-Bonferroni test was used after Kruskal Wallis test.

3.2 Biochemical markers in healthy and periodontitis patients groups at baseline:

Table 3.2 Shows that the mean level of MDA at baseline, in periodontitis patients groups were highest in SP (602.7ng/mL) followed by NSP (551.9ng/mL) with non-significant differences between both groups (P=1,000). While the lowest mean of MDA level were found in control healthy group, SH (277.2ng/mL) followed by NSH (218.8ng/mL), with non-significant differences between both groups (P=1.000). All the differences between periodontitis patients groups (SP and NSP) and healthy groups (SH and NSH) were significant and also between the four studied groups (P < 0.001). The same table shows non-significant elevation in the mean levels of TOS in SP group comparing with NSP and in SH comparing with NSH, with non-significant differences between control and periodontitis patients groups and among the four studied group (P=0.286). Regarding TAOC, the result showed non-significant reduction in the mean levels of TAOC in SP as compared to NSP and in SH as compared to NSH. With non-significant differences between the four studied groups in regard to TOS (P=0.286) and TAOC (P=0.056).

Due to the habit of cigarettes smoking, the mean level of MDA in SP has been increased in SP as compared to NSP, but with non signficant differences.This has an impact on the formation of free radicals that

damages the lipids in cell membranes. According to Reejamol and Swaminathan (2013), the rise in free radicals may be caused by cigarette smoke, which is a rich source of free radicals on its own, as well as by the activation of polymorphonuclear neutrotrophils by cigarette smoke, which boosts the body's free radical activity (Lobo *et al*, 2010). And for NSP, the increase of MDA level may be also due to the increase of ROS which cause tissue damage by relatively different mechanisms: significant enzymes oxidation, protein damage, and Lipid peroxidation (Taba *et al*, 2005).The present research can be supported by a study reported an increase in gingival tissue`s MDA levels in periodontitis patients, particularly when they are smokers (Garg *et al*, 2006). Other studies reported an increase of MDA levels in inflamed periodontal tissues and demonstrated MDA function in the periodontitis destructive processes (Chapple *et al*, 2002; Tsai *et al*, 2005; Garg *et al*, 2006).

Regarding TOS, the mean values of TOS were non-significantly increased in SP as compared to NSP and in SH as compared to NSH. Furthermore, in smokers there was a non-significant reduction of TAOC (SP and SH) comparing with non-smokers (NSP and NSH). Periodontal pathogenesis is probably affected by an imbalance between antioxidant AO defense mechanisms and ROS generation. This imbalance might be brought on by inhibited AO mechanisms and enhanced ROS production or by diminished AO capability in periodontitis (Rudrakshi C et al, 2017; Brock *et al*, 2004). In addition, smoking might decrease the function of the body's AO defense system and increase oxidative stress. These results make it evident that smokers' decreased AO capacity is linked to their higher generation of free radicals and oxidants. The large TAOC decrease in smokers might be caused by the elevated ROS that smoking causes.

The present result was in agreement with the finding of a study by Aslan et al, 2014, reported that the difference between SP and NSP in regard to mean levels of TOS and TAOC was not significant (Aslan *et al*, 2014). Another studies reported non-significant reduction of TAOC level in serum of NSP (Brock *et al*, 2004; cited by Konuganti *et al*, 2012).In contrary, studies reported that SP was associated with statistically significant high levels of TOS(Erel, 2005; Baltacıoğlu *et al*, 2014), and another studies reported statistically low levels of TAOC in SP as compared to NSP(Chapple*et al*, 2002; Guentsch *et al*, 2008; Bansal *et al*, 2018).

Table (3.2): Oxidant and antioxidant biomarkers at baseline in healthy and periodontitis patients groups .

Markers	Periodontitis groups Mean±S.D		Healthy groups Mean±S.D		Group vs group		P††(post-hoc)		P†
	SP A group	**NSP B group**	**SH C group**	**NSH D group**					
MDA ng/mL	602.79± 151.86	551.93± ±103.24	277.21± 121.12	218.78± 86.73	AXB AXD BXD	AXC BXC CXD	1.00 <0.001 <0.001	<0.001 <0.001 1.00	<0.001
TOS µmol/L	81.04± 18.67	72.87± ±25.88	90.44± 17.31	88.62±10. 86	AXB AXD BXD	AXC BXC CXD	NA	NA	0.286
TAOC U/ml	1.91±1. 36	2.61±4.78	2.36±0. 73	2.62±1.31	AXB AXD BXD	AXC BXC CXD	NA	NA	0.056

†By Kruskal Wallis test; ††Dunn-Bonferroni test was used after Kruskal Wallis test (post-hoc test); NA: Not applicable (because the P- value by the Kruskal Wallis test was not significant).

3.3 Clinical periodontal and biochemical markers after therapy

Table 3.3 shows that the mean values of clinical periodontal (CAL, PD, GI, BOP, and PI) and oxidative stress (TOS and MDA) parameters were significantly reduced after SRP in SP and NSP groups, with the exception for the non-significant increase of TAOC in SP (P=0.281) & NSP (P=0.156), and non-significant reduction of GR in SP (P=0.050)& NSP (P=0.068) after therapy as compared to baseline.

The improvement of clinical parameters after scaling and root planning that reduce periodontitis-induced bacteremia/endotoxemia may be due to the fact of decreasing the intraoral bacterial bio-burden, which may be positively affect the status of systemic inflammatory (Taylor and Borgnakke, 2008). This confirms that tooth deposits such as calculus and plaque are the main cause of periodontal disease and that removing them with SRP may enhance the health of periodontium(Haffajee and Socransky, 2001).

This decrease of the mean values of PI,GI and BOP after NSPT in SP and NSP groups as compared to baseline, are in agreement with other studies(Dietrich *et al*, 2004; Akpinar *et al*, 2013). The current study also showed that the mean values of PD and CAL were significantly reduced after SRP in SP & NSP, and the differences between non-smokers and smokers periodontitis were statistically significant after six weeks of therapy. Such findings are compatible with other studies (Söder *et al*, 1999; Akpinar *et al*, 2013).

Regarding GR, the mean values of GR was non significantly reduced after therapy in SP& NSP comparing with healthy control groups, this findings is in agreement with the findings of other studies reported that the location of the mucogingival junction was essentially consistent(Batenhorst *et al*, 1974; Talari and Ainamo, 1976). They reported that after SRP for all teeth, both interproximal and midline regions were examined and found that there was no discernible change in where the mucogingival junction was located. The current study results are also consistent with other studies, have shown that the certain kind of underlying connective tissue defines the features of the overlying mucosa genetically. The subepithelial connective tissue in routine SRP is left intact, so it is not to be expected that the mucogingival junction location will change (Karring *et al*, 1975; Hughes and Caffesse, 1978). In contrast to our study, there was slightly increase in GR after non-surgical therapy (Badersten *et al*, 1984; cited by Kaufmann *et al*, 2020). Similar effects of improvement in clinical parameters after mechanical periodontal therapy have been also reported by another studies(Al-Katma *et al*, 2007)(Grant *et al*, 2010; Marcaccini *et al*, 2010). In contrast, poorer treatment outcomes can be noticeable in SP than NSP(Theodoro*et al*, 2018).

Regarding the changes in serum levels of MDA, TOS and TAOC after non-surgical therapy for SP & NSP. There was significant reduction in the mean levels of MDA and TOS after SRP for both SP & NSP with the exception for the non-significant elevation of TAOC levels in both SP & NSP after therapy. The significant decrease of MDA and TOS levels in periodontitis patient groups (SP and NSP) after therapy might be due to the oxidative stress reduction (Bansal *et al*, 2018). The noxious stimuli are removed after therapy, and there will be a less production of ROS. The current study is in accordance with the results of other studies reported decline in oxidative stress markers (MDA and TOS) after therapy (Aziz *et al*, 2013; Önder *et al*, 2017). Also other studies reported that periodontitis was related to high level of TOS which was reduced by periodontal treatment (Erel, 2005; Akalin *et al*, 2007; Wei *et al*, 2010; Akpinar *et al*, 2013; Baltacıoğlu *et al*, 2014).

The non-significant increase in TAOC after SRP in SP and NSP appears to be occurred because of oxidative stress markers reduction. The balance shifts

toward a coherent AO system with decreased ROS generation, which promotes a periodontal health-friendly environment. The present study supported by Zhang et al, 2016 and Chapple *et al*, 2007 showed that after nonsurgical therapy there was no significant change in serum TAOC levels (Chapple *et al*, 2007; Zhang *et al*, 2016).Unlike the current findings, studies showed significant increase in serum TAOC level restoring to a level comparable to control (Aziz *et al*, 2013; Thomas *et al*, 2014; SIVARAMAN HRISHI *et al*, 2016; Bansal *et al*, 2017). Such a disagreement may be due to the distinct intervals utilized to measure oxidative markers and clinical parameters among the studies (Chapple *et al*, 2007).

Table (3.2) Means, standard deviation and the medians of the studied parameters before and after treatment of periodontitis patients groups (SP and NSP).

		Before treatment		After treatment		
Groups	Parameters	Mean± S.D	Median	Mean±S.D	Median	P-value
	PI	1.06± 0.27	0.98	0.52±0.16	0.49	< 0.001*
	GI	0.88±0.17	0.88	0.44±0.12	0.48	< 0.001*
SP	BOP %	1.52±1.29	1.00	0.52±0.31	0.60	0.003†
	PD mm	4.43±0.34	4.37	3.63±0.50	3.50	0.001†
	CALmm	3.60±0.44	3.80	3.26±0.36	3.30	0.017†
	GR mm	1.24±1.15	1.60	1.11±1.01	1.50	0.050†
	TOS	81.04±18.68	86.40	51.78±4.95	50.60	0.001†
	MDA	602.80±151.86	568.13	450.94±91.18	448.50	0.001†
	TAOC	1.91±1.37	1.59	1.98±3.24	1.07	0.281†
	PI	0.94±0.22	1.00	0.54±0.17	0.49	< 0.001*
NSP	GI	0.92±0.21	0.95	0.54±0.19	0.57	< 0.001*
	BOP %	7.27±5.01	6.00	2.35±1.67	2.00	0.001†
	PD mm	4.31±0.34	4.20	3.38±0.34	3.40	< 0.001†
	CALmm	3.62±0.28	3.70	3.17±0.32	3.13	0.001†
	GR mm	1.32±1.36	1.29	1.17±1.24	1.09	0.068†
	TOS	72.87±25.89	72.20	53.81±10.64	50.10	0.023†
	MDA	551.94±103.24	541.80	390.15±90.40	388.55	0.001†
	TAOC	2.62±4.78	0.64	3.83±5.81	1.91	0.156†

*By Paired t test; †By Wilcoxon Signed Ranks test.

3.4 The mean differences between SP and NSP in regard to clinical and biochemical parameters before and after therapy.

Table 3.4 shows that the mean differences between the SP and NSP were not significant in regard to PI, GI, PD, CAL, GR, TOS, and MDA parameters. While the mean differences of BOP was significant highest in NSP 4.913±3.738% than SP 1.001±1.202% (P<0.001), and TAOC readings after SRP were higher than those before treatment, so the difference was in a negative sign. The mean difference was -1.2 vs. -0.068 in the NSP and SP respectively (p = 0.041).

The present result showed that the mean differences between SP and NSP after therapy in regard to the improvement of clinical and biochemical parameters were not significant with the exception for the BOP and TAOC.

Table (3.3): Comparing the mean differences (before-after treatment) between non-smoker periodontitisNSPand smoker periodontitisSP.

Parameters	**SP**	**NSP**	
Mean difference (B-A treatment)	**Mean ±S.D**	**Mean ± S.D**	**P value**
PI	0.537±0.256	0.401±0.162	0.094†
GI	0.436±0.207	0.381±0.166	0.431†
BOP%	1.001±1.202	4.913±3.738	< 0.001*
PD mm	0.796±0.299	0.930±0.233	0.217*
CALmm	0.334±0.416	0.458± ±0.191	0.412*
GRmm	0.133 ±0.219	0.147 ±0.297	0.653*
MDAng/mL	151.855±132.661	161.785 ±45.542	0.786†
TOSμmol/L	29.260 ±19.845	19.060 ±27.094	0.249†
TAOCU/ml	-0.068±3.565	-1.213 ±3.517	0.041*

†By unpaired t test;*By Mann Whitney test.

3.5The correlation between periodontal parameters and biochemical markers

Table 3.5 shows that in SP group, all the correlations between the clinical and biochemical markers were not significant at baseline before therapy, except for the significant negative correlation between BOP and TAOC (rho = -0.547, p = 0.035), and after treatment, all the correlations between biochemical and clinical parameters were not significant. Table 3.6 demonstrates the correlation between clinical and biochemical markers in NSP group at baseline. All the correlations were not significant except for the significant negative correlation between PD and TAOC (rho = -0.554, p = 0.032), and after treatment, all the correlations were also not significant.

This negative significant correlation between TAOC & PD in NSP and between TAOC & BOP in SP at baseline before therapy indicated that TAOC were significantly decrease with increased gingival inflammation and periodontal tissue destruction. The findings of the present study are in accordance with the findings of a study by Akalin *et al*, 2007 on non-smokers, reported a weak correlation between TOS level and clinical parameters before non-surgical therapy and no marked correlation were assess between MDA and clinical parameters (Akalin *et al*, 2007). Although A substantial negative correlation between PD and TAOC was discovered in NSP and between TAOC & BOP% in SP, the findings reported by Brock et al. and Baser et al. are congruent with the outcomes of our results (Brock *et al*, 2004; Baser *et al*, 2015).

Table (3.4): Correlation between the oxidative stress and clinical parameters at base line before therapy and after treatment, among smoker periodontitis patients groups SP(Spearman(rho) correlation).

Time	Clincal parameters	Oxidative stress parameters					
		TOS		MDA		TAOC	
		rho	(P)	rho	(P)	rho	(P)
Before	PI	0.407	(0.132)	0.036	(0.899)	0.061	(0.830)
	GI	0.046	(0.869)	0.318	(0.248)	-0.368	(0.177)
	BOP %	-0.289	(0.296)	0.351	(0.200)	-0.547	(0.035)
	PD mm	0.031	(0.913)	0.109	(0.700)	0.082	(0.773)
	CAL mm	0.226	(0.418)	0.298	(0.281)	0.314	(0.254)
	GR mm	0.467	(0.080)	-0.114	(0.685)	0.413	(0.126)
After	PI	0.260	(0.350)	-0.088	(0.756)	-0.429	(0.111)
	GI	0.433	(0.107)	-0.197	(0.482)	-0.204	(0.466)
	BOP %	-0.298	(0.280)	-0.101	(0.721)	-0.268	(0.334)
	PD mm	-0.497	(0.060)	0.165	(0.556)	0.111	(0.693)
	CAL mm	0.194	(0.489)	-0.158	(0.575)	-0.281	(0.311)
	GR mm	0.067	(0.811)	-0.484	(0.068)	-0.262	(0.346)

Table (3.5): Correlations between clinical and oxidative stress parameters before and after treatment, among non-smoker periodontitispatients groups NSP(Spearman(rho) correlation).

Time	Clinical parameters	TOS		MDA		TAOC	
		rho	(P)	rho	(P)	rho	(P)
Before	PI	-0.048	(0.864)	-0.020	(0.945)	-0.168	(0.549)
	GI	-0.331	(0.229)	-0.166	(0.554)	0.032	(0.909)
	BOP %	-0.187	(0.505)	-0.259	(0.352)	0.016	(0.954)
	PD mm	0.192	(0.493)	0.354	(0.195)	-0.554	(0.032)
	CALmm	0.079	(0.779)	-0.034	(0.904)	0.014	(0.959)
	GR mm	-0.107	(0.703)	-0.177	(0.528)	0.405	(0.134)
After	PI	-0.009	(0.975)	-0.023	(0.934)	-0.061	(0.830)
	GI	-0.050	(0.859)	-0.048	(0.864)	0.134	(0.634)
	BOP %	-0.085	(0.763)	-0.172	(0.540)	-0.212	(0.449)
	PD mm	0.324	(0.239)	0.027	(0.923)	-0.053	(0.851)
	CALmm	-0.469	(0.078)	-0.167	(0.552)	0.323	(0.240)
	GR mm	-0.015	(0.958)	-0.053	(0.852)	0.094	(0.739)

3.7 Level of biochemical markers with duration and number of cigarettes/day.

Table 3.7 shows that at baseline, and in SP patients, there was no significant association between the mean levels of TOS (P = 0.268) and duration of smoking, MDA (P = 0.730), and TAOC (P = 0.806). The same can be applied for NSP patients group, where it is evident that there were not a significant association between MDA (p = 0.700), the mean levels of TOS (p = 0.410), and TAOC (p = 0.469) and duration of smoking. Table 3.8 also shows at baseline in SP, there was no significant association between the number of cigarettes smoked per a day with TOS (p = 0.784), MDA (P = 0.593), and TAOC (P = 0.552). In addition in SH group, the relationship between the number of cigarettes with TOS (P = 0.607), MDA (P = 0.088), and TAOC (P = 0.272) was not significant.

The present study, demonstrated that all duration and number of cigarette smoking did not have any significant association with oxidative markers (MDA, TOS, and TAOC). Although the mean values of MDA and TOS were highest in periodontitis patients smoked 10-20 cigarettes/day for more than 12 years, and the mean values of MDA and TOS were highest in healthy subjects smoked 10-20 cigarettes/days for more than 12 years. Regarding MDA and TOS the present result support the hypothesis that oxidative damage in smokers was due to the number of hours of active exposing to cigarette smoking. It makes sense to assert that the harmful effect of smoking on the AO system are basically depend on daily dose. Studies reported that smoking was a significant risk factor for periodontal disease acceleration, and its impact was dose-dependent (Ho and Chang, 2006; Rao *et al*, 2016). Although, another

study, reported that people who smoked regularly had lower free radical levels than those who smoked less often (Kurku *et al*, 2015). The present study is in agreement with studies reported no correlation between serum MDA and the number of cigarettes smoked(Altuntaş*et al*, 2002)(Kahnamoei *et al*, 2014). However, the current findings are in contrast with a study reported that MDA levels in healthy smokers who smoked >10 sticks of cigarette per day for >10 years were substantially lower than those who smoked<10 sticks of cigarette per day for <10 years (Nsonwu-Anyanwu *et al*, 2018).

The current research also showed a non-significant association between the number of smoking cigarettes/day and TAOC. Regarding TAOC in SP group, the mean reduction was highest in patients smoked <10 cigarettes/day for < 6 years followed by patients smoked >20 cigarettes/day for >12 years and the least reduction in patients smoked 10-20 cigarettes/day for 6-12 years. And in healthy smokers the mean reduction of TAOC was highest in subjects smoked 10-20 cigarettes/day for duration <6 years followed by patients smoked >20 cigarettes/day for duration of 6-12 years; nevertheless, there was no significant association between the number of cigarettes smoked /day and TAOC. This finding is compatible with a research reported no correlation between TAOC and number of cigarettes smoked per day, when the frequency was 11-20 cigarettes/day, 6-10 cigarettes/day or 1-5 cigarettes/day (Kaufman and Lamster, 2000). A study demonstrated the correlation of serum TAOC level and number of smoked cigarette/day, supported our results, and reported that there was no correlation between the TAOC and the number of cigarettes smoked (Kahnamoei *et al*, 2014). Another study performed on SH with smoking history of minimum 10 cigarettes a day for 5 years, showed that there was no correlation between TAOC level and the number of cigarettes smoked (Gadham and Prasanna, 2017). In contrast a study reported a significant decrease in saliva level of TAOC among smokers with a rate of 10 cigarettes a day (Pham-Huy *et al*, 2008).

Also the present study shows that the association between different duration of smoking and TAOC level in smokers groups was not significant (SH and SP). A study by Shirzaiy et al, on saliva of smoker periodontitis patient who have the same habit duration as the current study, reported that relationship between duration of smoking habit and TAOC was not statistically significant, however, different body fluids were evaluated in these two studies (Shirzaiy *et al*, 2017). Also another study reported non signficant correlation between the duration of smoking and TAOC level, and this may be due to many confounding variables including food consumption, air pollutant, and other confounding variables, which have not been controlled(Pham-Huy *et al*, 2008).

Such conflicting findings of studies might be associated with a different in sample sizes, various AO elements assessment in different studies, different body fluids assessments, different laboratory techniques or kits, and various methods of the studies.

Table 3.6. Comparison between markers of oxidative stress, and the duration of smoking in smokersperiodontitis SP and smokers healthy SH at baseline.

Markers	Duration	N	Mean	± SD	P-value
Smoker periodontitis SP					
TOSμmol/L	< 6 years	1	53.30		
	6-12 years	3	82.87	±19.17	0.268
	> 12 years	11	83.57	±13.86	
	Total	15	81.04	±18.68	
MDAng/mL	< 6 years	1	482.30		
	6-12 years	3	591.75	±194.99	0.730
	> 12 years	11	616.77	±151.59	
	Total	15	602.80	±151.86	
TAOCU/ml	< 6 years	1	1.40		
	6-12 years	3	2.33	±3.10	0.806
	> 12 years	11	1.84	±0.78	

	Total	15	1.91	±1.37	
Smoker healthySH					
TOSμmol/L	< 6 years	1	83.55		
	6-12 years	6	83.70	±18.86	0.410
	> 12 years	8	96.46	±16.04	
	Total	15	90.44	±17.32	
MDAng/mL	< 6 years	1	233.40		
	6-12 years	6	251.51	±94.14	0.700
	> 12 years	8	318.78	±158.62	
	Total	15	277.21	±121.13	
TAOCU/ml	< 6 years	1	1.84		
	6-12 years	6	2.15	±0.96	0.469
	> 12 years	8	2.59	±0.53	
	Total	15	2.36	±0.74	

*By Kruskal Wallis test.

Table (3.7): Oxidative stress markers at baseline and number of cigarettes smoked per day in smokersperiodontitis and smokers healthy (SP and SH).

Markers	No. of cigarettes	N	Mean	±SD	P value
Smoker periodontitis SP					
TOSμmol/L	< 10 cig/day	3	78.70	±23.15	
	10-20 cig/day	2	91.50	±7.21	0.784*
	> 20 cig / day	10	79.65	±19.74	
	Total	15	81.04	±18.68	
MDAng/mL	< 10 cig/day	3	587.64	±198.10	
	10-20 cig/day	2	682.15	±201.88	0.593*
	> 20 cig / day	10	591.48	±144.94	
	Total	15	602.80	±151.86	
TAOCU/ml	< 10 cig/day	3	1.24	±1.08	
	10-20 cig/day	2	2.11	±0.48	0.552*
	> 20 cig / day	10	2.07	±1.56	
	Total	15	1.91	±1.37	
Smoker healthy SH					
TOSμmol/L	10-20 cig/day	9	91.15	±20.20	
	> 20 cig / day	6	89.38	±13.57	0.607**
	Total	15	90.44	±17.32	
MDAng/mL	10-20 cig/day	9	367.82	±139.97	
	> 20 cig / day	6	216.81	±56.29	0.088**
	Total	15	277.21	±121.13	
TAOCU/ml	10-20 cig/day	9	1.97	±0.97	
	> 20 cig / day	6	2.62	±0.41	0.272**
	Total	15	2.36	±0.74	

*By Kruskal Wallis test. ** By Mann Whitney test.

CONCLUSION

Non-surgical periodontal therapy improved clinical periodontal parameters and oxidative stress biomarkers (MDA and TOS) in both SP and NSP and enhanced the antioxidant defense system of periodontitis patients (SP&NSP)

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