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Effects of topical doxycycline on serum prooxidant marker levels in periodontal disease: A pilot study

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Abstract

The aim of this study is to determine the changes in the serum levels of malondialdehyde and 8-hydroxy-2'-deoxyguanosine, as indicators of oxidative stress, in response to periodontal treatment adyuvanted with topical doxycycline.

Methods: Thirty four patients with periodontal disease were studied (DOX group n=16; Control group n=18), determining the serum levels of malondialdehyde and 8-hydroxy-2'-deoxyguanosine in urine at baseline and three months after non-surgical periodontal treatment.

Results: We observed a slight trend to decrease in 8-OHdG urine levels after periodontal treatment, being this decrease greater in the DOX group (t1: 9.64 ± 7.18 µg/g; t0: 9.84 ± 3.01 µg/g) than in the control group (t1: 8.79 ± 4.08 µg/g; t0: 8.82 ± 4.01 µg/g). However, these changes were not statistically significant. No changes in serum levels of MDA were observed after periodontal treatment. BOP was positively correlated with 8-OHdG, in the DOX group ($r = 0.563$, $p = 0.018$).

Conclusion: Periodontitis, as a local inflammatory disease, can cause an increase in oxidative stress, thus opening the way to new therapeutic strategies, while exploring novel strategies for modulating the host response.

Keywords: Periodontal diseases; 8-hydroxy-2′-deoxyguanosine; Malondialdehyde; Oxidative stress; Topical doxycycline

1. Introduction

The cells obtain energy through coupled oxidation-reduction (redox) reactions, within oxygen-to-water (H_2O) reduction processes during aerobic respiration. In this way, O_2 is responsible for the formation of the so-called reactive oxygen species or ROS, which are molecules of high reactivity by having a missing electron [1]. Molecular O_2 is reduced to O_2^- by capturing an electron, resulting in an unstable radical and a short half-life, which is reduced to hydrogen peroxide (H_2O_2), because of the action of superoxide dismutase (SOD) by capturing another electron and two hydrogens. If the H_2O_2 in turn captures another electron, it forms the very reactive hydroxyl radical (OH^-), which is rapidly reduced to

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H_2O. ROS can be divided into two groups: free radicals (with missing electrons) very reactive with a short half-life such as anion superoxide (O_2^-), hydroxyl radical (OH^-) and nitric oxide (NO), and more stable non-radical species with a longer half-life such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), lipid hydroperoxides (LOOH) [1] and peroxynitrile ($ONOO^-$). The main enzyme responsible to produce ROS against the attack of pathogens is NADPH oxidase primarily, they are also important xanthine oxidase (XO) and decoupled endothelial nitric oxide synthase (eNOS) [1] which promote ROS production and are involved in the development of vascular damage.

Inflammatory periodontal lesions present an important infiltrate of monocytes and macrophages, that has the purpose of containing the infectious process [2-8]. These defensive mechanisms will become an aggression for the periodontal tissues because of the production of free radicals.

Free radicals are oxidized when they react with a molecule, they oxide it. If these molecules are the fatty acids of the cellular lipid membranes [9], they are transformed into fatty acid radicals that in turn will have the capacity to oxidize another fatty acid molecule. This phenomenon is known as lipid peroxidation (LPO) [10-12], moreover during this process malondialdehyde (MDA) is generated as an indicator of tissue damage [12].

Another molecule damaged by the free radicals is DNA, because of the arrival of the ROS inside the cell nucleus, the action of the radical OH^- can originate more than 20 modifications in the nitrogenous bases. We can highlight 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is produced because of the interaction with guanine. It is premutagenic since it is capable of pairing with adenosine instead of cytosine, and as result it can be used like a marker of oxidative damage [13-21].

Therefore, serum concentrations of MDA and 8-OHdG are increased in patients with periodontal disease as an expression of increased oxidative stress in the etiology of the lesions and their quantification, consequently, it will allow us to assess the prooxidant state.

Previously named articles have evaluated oxidative stress through a global burden of ROS and guanine-derived biomarkers in gingival crevicular fluid, however, in this article we will evaluate the fluctuation in serum and urine levels of the two markers which are more commonly expressed in the oxidative damage associated with periodontal disease such as MDA and 8-OHdG.

2. Material and methods

2.1. Study Population

Study participants aged between 26 and 72 years, were consecutively recruited at our private dental clinic (Paterna, Valencia, Spain) between June 2019 and March 2020 for this interventional study. Participants were diagnosed with periodontitis when an interdental clinical attachment loss (CAL) in ≥2 non-adjacent teeth or a buccal or oral CAL ≥3 mm with pocketing >3 mm in ≥2 teeth is detected according to the 2017 World Workshop definition [22].

Patients diagnosed with periodontitis were selected for the present study with a therapeutic objective. The individuals underwent to periodontal treatment and topical doxycycline was applied as an adjuvant to optimize the results. The work is structured in three parts.

The first part with analytical purposes consisted in a periodontal study, which were performed at the time of diagnosis, as well as a blood test to determine the serum level of MDA and 8-OH-dG in urine.

The second part with an interventionist character, in which all patients underwent to non-surgical periodontal treatment with topical doxycycline application will in lesions ≥ 5 mm.

The third part consisted at a reevaluation periodontal three months after the treatment, and the serum levels of MDA and of 8-OH-dG in urine were determined again.

2.2. Selection of patients

This was an interventional case–control study conducted at the AndreuDental stomatology clinic located in the city of Paterna, Valencia (Spain). Pacients between the ages of 18 and 75 years were recruited at our clinic.

2.3. Inclusion criteria

Patients with a Probing Depth > 3 mm in at least one probing site in two or more teeth, and/or loss of interproximal clinical insertion ≥ 3 mm as definition to chronic periodontitis [22-25] according to criteria of the AAP-EFP 2018 [22].

Exclusion criteria were less than fourteen teeth, aggressive periodontitis, infectious or other inflammatory diseases, periodontal treatment in the last 6 months or antibiotics in the last 3 months, treatment with systemic anti-inflammatory drugs (NSAIDs), pregnancy or lactation and any medical condition requiring antibiotic treatment before the dental intervention.

It is a human observational study structured according to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines were conducted in accordance with the Helsinki Declaration-based Ethical Principles for Medical Research Involving Human Subjects, and the Oviedo convention. All procedures were approved by Arnau de Vilanova-Lliria hospital's Ethics Committee, and written informed consent was obtained from all subjects.

2.4. Clinical Periodontal Determinations

Periodontal examinations were conducted by an experienced dentist (R. Andreu). Periodontal assessments included measurements of probing depth (PD), CAL, number of sites with PD ≥4 mm, percentage of sites with PD 1–3 mm, 4–5 mm, and ≥6 mm, gingival bleeding on probing (BOP), and plaque index, which were recorded using a manual periodontal probe PCP UNC-15 (Hu-Friedy, Chicago, IL, USA). PD, CAL, and BOP were measured at six sites per tooth for all teeth, excluding third molars. PD was measured as the distance between the gingival margin and the clinical pocket base, CAL was recorded as the distance between the cement–enamel junction and the clinical pocket base, with both values expressed in millimeters, and the BOP percentage was calculated by dividing the number of sites with BOP by the number of sites explored and multiplying this value by 100. We assessed the Silness and Löe simplified Plaque Index and scored it in six representative Ramfjörd teeth: upper right first molar, upper left central incisor, upper left first premolar, lower left first molar, lower right central incisor, and lower right first premolar. Participants were classified according to three periodontitis stages according to the 2017 World Workshop definition [22]. Subjects were classified as having stage II when interdental CAL at site of greatest loss was 3 to 4 mm and maximum PD ≤ 5 mm, stage III when CAL ≥ 5 mm, PD ≥ 6 mm, and tooth loss due to periodontitis of ≤4 teeth, and stage IV when CAL ≥ 5 mm, PD ≥ 6 mm, and tooth loss due to periodontitis of ≥5 teeth.

2.5. Biochemical Determinations

Venous blood and urine samples were analyzed at the Analclinic Laboratory (Mislata- Valencia, Spain). Serum samples were obtained to measure MDA levels (µmol/L) were obtained by the Asakawa-Matsushita method by reacting MDA with 2-thiobarbituric acid, giving a colored MDA-TBA complex that is quantified using a Uvikon-810 spectrophotometer (Kontron Instruments, Augsburg, Germany). 8-OHdG levels (µg/g creatinine) were tested from urine samples using high-performance liquid chromatography (Analytical HPLC 1200 Series, Agilent Technologies, Santa Clara, CA, USA), which is also an oxidative stress marker, hsCRP and fibrinogen levels as inflammatory markers and acute phase reactants, and malondialdehyde (MDA) levels as oxidative stress markers. Serum hsCRP levels (mg/dL) were quantified using an immunonephelometric assay (Dade Behring, Marburg, Germany), fibrinogen levels (mg/dL) were obtained through the Coagulometry-Thrombin-Clauss time technique using a Solea 100 automatic analyzer (Biolabo diagnostics, Maizy, France).

2.6. Statistical Analysis

Statistical analyses were performed using SPSS software (IBM Co., Armonk, NY, USA). Continuous variables were expressed as mean and standard deviation for parametric data, qualitative data were expressed as percentages, and proportions were compared using a chi- square test. Continuous variables were compared among groups using an unpaired Student t-test and the changes of each variable were compared by a paired Student t-test within each group. Spearman's correlation coefficient was used to evaluate the strength of the association between periodontal and oxidative stress variables. A confidence interval of 95% was determined for all tests and a p-value <0.05 was considered statistically significant.

3. Results

This pilot study analyzed 34 subjects with chronic periodontitis (12 men and 22 women; mean age: 57.4 ± 8 years). All participants underwent non-surgical periodontal treatment and they were randomized into two groups: those with application of topic doxycycline (DOX group, n=16) and those without topical doxycycline application (Control group, n=18).

Demographic and descriptive parameters of the study population are shown in Table 1. No significant differences were found between groups regarding sex. However, respect to age the DOX group had a slightly older age (an average of 5 years) than the control group showing significant differences between groups (p=0.025). Most patients in this study did not smoke (70.6%) and no differences were detected in the smoking rate between the two groups when compared by a Chi-square test (p=0.072). Moreover, only three patients in the study were diabetic. Participants were classified into four periodontitis stages according to the 2017 World Workshop definition [22]: 2 subjects were classified as having stage I (mild periodontitis), 3 as having stage II (moderate periodontitis), 14 as having stage III (severe periodontitis), and 15 as having stage IV (advanced periodontitis). In both groups, most of the patients had stage III and IV of periodontitis, and no differences were observed between groups according to periodontitis staging (p=0.375).

Table 1 Descriptive parameters of the study population according to DOX group vs. control group

	Group			
	Total	**Control**	**DOX**	**p-valor**
n (% females)	34 (64.7)	18 (66.7)	16 (62.5)	0.800
Age (years)	57.4 ± 8.00	54.5 ± 6.2	60.6 ± 8.7	0.025
Diabetes Mellitus % (n)	8.8 (3)	11.1 (2)	6.3 (1)	0.618
Smoking habit % (n)				
Non smokers	70.6 (24)	61.1 (11)	81.3 (13)	
Smokers ≤ 10 cig/day	14.7 (5)	27.8 (5)	0	0.072
Smokers > 10 cig/day	14.7 (5)	11.1 (2)	18.8 (3)	
Stage of Periodontitis % (n)				
I	5.9 (2)	0	11.1 (2)	
II	8.8 (3)	6.3 (1)	11.1 (2)	0.375
III	41.2 (14)	37.5 (6)	44.4 (8)	
IV	44.1 (15)	56.3 (9)	33.3 (6)	

Data are presented as mean ± standard deviation for continuous variables or as percentage (n) for categorical variables. p ≤0.05 represents significant differences between groups when data were compared by an unpaired Student t-test or by a Chi-square test

Regarding periodontal clinical parameters, we observed that three months after non-surgical periodontal treatment all periodontal parameters improved in both groups (Table 2). However, this improvement was more statistically significant in the DOX group. In the control group, although we observed a significant improvement in the number of sites with PD ≥6mm (p= 0.025) and in the BOP (p= 0.022), the changes in PD and CAL, among others, were not significant. Instead, in the DOX group we observed a significant improvement in practically all the periodontal parameters analyzed. It is also true, that the DOX group started with slightly worse periodontal parameters, observing some differences between the groups at baseline and probably for this reason no differences were observed when compared the periodontal changes (t1-t0) between the groups.

To detect the influence of topical doxycycline application with the periodontal treatment in oxidative stress we determined levels of prooxidant parameters, such as MDA serum levels and 8-OHdG levels in urine (Figure 1). We observed a slight trend to decrease in 8-OHdG urine levels after periodontal treatment, being this decrease greater in the DOX group (t1: 9.64 ± 7.18 μg/g; t0: 9.84 ± 3.01 μg/g) than in the control group (t1: 8.79 ± 4.08 μg/g; t0: 8.82 ± 4.01 μg/g). On the other hand, we observed a slight increase in serum levels of MDA after periodontal treatment (DOX group, t1: 0.91 ± 0.47 μmol/L; t0: 0.74 ± 0.26 μmol/L and control group, t1: 1.24 ± 1.64 μmol/L; t0: 0.75 ± 0.21 μmol/L). However, these changes in prooxidant parameters were not statistically significant and the sample analyzed was very small.

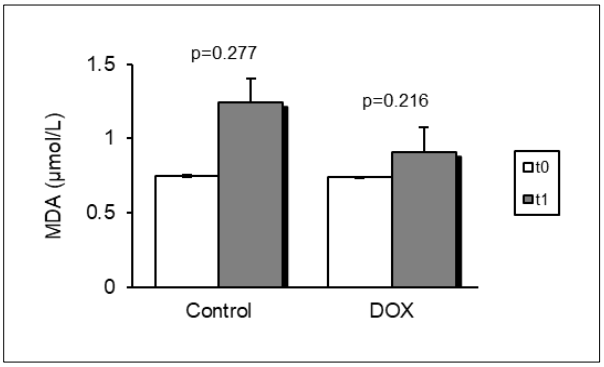
Correlation coefficients between pro-oxidant and periodontal parameters from control and DOX group are presented in Table 3. PD and CAL, which are periodontal clinical parameters that indicate disease and periodontitis severity, they weren't positively correlated with MDA (r = 0.201, p = 0.255; and r = 0.204, p = 0.252, respectively) and 8-OHdG levels (r = -0.100, p = 0.379; and r = -0.130, p = 0.344, respectively). However, BOP was positively correlated with 8-OHdG, in

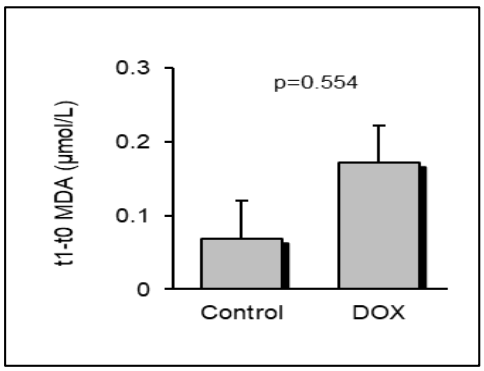
the DOX group (r = 0.563, *p = 0.018*); but not with MDA (r = 0.058, *p* = 0.422). No correlations were observed between the others clinical periodontal parameters and serum MDA and urine 8-OHdG levels.

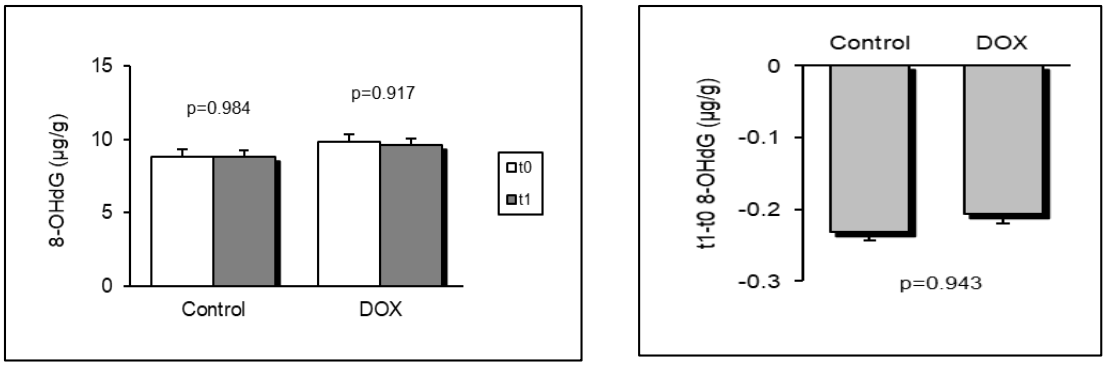
Table 2 Clinical periodontal parameters at baseline (t0), three months after non-surgical periodontal treatment (t1) and the difference t1-t0 in DOX group vs. Control group

	Group	**Baseline (t0)**	**3 months (t1)**	**Dif. t1-t0**	**p-value t1-t0**	**p-value t1-t0 between groups**
PD (mm)	Control	3.18 ± 0.82	3.11 ± 0.62	-0.07 ± 0.56	0.326	0.580
	DOX	3.74 ± 0.68	3.45 ± 0.72	-0.28 ± 0.41	**0.019**	
CAL (mm)	Control	3.36 ± 0.85	3.29 ± 0.64	-0.07 ± 0.55	0.352	0.762
	DOX	4.00 ± 0.70	3.76 ± 0.71	-0.25 ± 0.43	**0.047**	
Sites PD ≥4mm (n)	Control	44.0 ± 29.8	43.0 ± 26.8	-1.0 ± 15.3	0.794	0.108
	DOX	61.2 ± 23.2	50.0 ± 24.4	-11.2 ± 13.9	**0.008**	
Sites PD ≥6mm (n)	Control	8.33 ± 8.65	4.83 ± 7.45	-3.5 ± 6.06	**0.025**	0.532
	DOX	15.1 ± 12.9	9.20 ± 8.93	-5.9 ± 8.5	**0.017**	
Sites PD ≥4mm (%)	Control	31.6 ± 24.6	32.2 ± 22.5	0.62 ± 9.3	0.758	0.052
	DOX	47.5 ± 19.2	40.3 ± 20.5	-7.2 ± 10.4	**0.017**	
Sites PD 1-3mm (%)	Control	67.2 ± 24.8	62.5 ± 26.0	-4.7 ± 20.4	0.360	0.097
	DOX	52.5 ± 19.2	54.9 ± 23.5	2.39 ± 22.2	0.683	
Sites PD 4-5mm (%)	Control	24.5 ± 18.0	27.5 ± 19.6	3.05 ± 9.43	0.188	0.190
	DOX	34.5 ± 12.2	30.7 ± 12.6	-3.85 ± 7.71	0.074	
Sites PD ≥6mm (%)	Control	7.08 ± 8.40	4.64 ± 6.94	-2.45 ± 6.18	0.072	0.656
	DOX	12.9 ± 10.4	9.68 ± 9.88	-3.24 ± 4.80	**0.020**	
Plaque Index (A.U)	Control	0.61 ± 0.61	1.45 ± 0.86	0.84 ± 1.14	**0.006**	0.155
	DOX	0.99 ± 0.94	1.13 ± 0.88	0.14 ± 1.31	0.690	
BOP (%)	Control	27.5 ± 16.7	19.3 ± 21.9	-8.16 ± 23.8	**0.022**	0.373
	DOX	48.9 ± 32.4	26.6 ± 10.2	-22.3 ± 27.2	**0.012**	

Data are shown as mean ± standard deviation. p-value < 0.05 represents significant differences when data T1-T0 were compared by a paired Student t-test within each group. The changes obtained in each periodontal variable were compared between the groups by an unpaired Student t-test







Data are represented as mean + standard error. The differences between T1-T0 were compared by a paired Student t-test within each group and the changes obtained in MDA and 8-OHdG (t1-t0) were compared between the groups DOX and control by an unpaired Student t-test.

Figure 1 Levels of prooxidant parameters at baseline (t0), three months after non-surgical periodontal treatment (t1) in DOX group vs. Control group. Malondialdehyde serum levels (MDA) and 8-hydroxy-2'-deoxyguanosine urine levels (8-OHdG)

Table 3 Spearman's correlation coefficients between periodontal and oxidative stress parameters.

A. Control group				
	MDA	8-OHdG		
	r	p-valor	r	p-valor
PD (mm)	0.201	0.255	0.100	0.379
CAL (mm)	0.204	0.252	0.130	0.344
Number of sites PD≥4mm	0.156	0.306	0.158	0.312
Number of sites PD≥6mm	-0.189	0.269	-0.031	0.462
% sites PD≥4mm	0.048	0.438	0.193	0.274
% sites PD 1-3mm	-0.232	0.223	-0.487	*0.054*
% sites PD 4-5mm	0.105	0.367	0.123	0.352
% sites PD≥6mm	-0.101	0.372	0.070	0.414
Plaque Index	0.197	0.260	0.388	0.107
BOP (%)	0.0	0.489	0.372	0.117

Notes: PD, probing depth; CAL, clinical attachment loss; BOP, bleeding of probing; MDA, malondialdehyde; and 8-hydroxy-2´-sesoxyguanosine. Values in bold represent statistically significant correlations (p <0.05). r: Spearman´s correlation coefficient

B. DOX group				
	MDA		8-OHdG	
	r	p-valor	r	p-valor
PD (mm)	-0.068	0.409	-0.280	0.166
CAL (mm)	-0.111	0.352	-0.176	0.274
Number of sites PD≥4mm	-0.048	0.435	-0.243	0.202
Number of sites PD≥6mm	0.135	0.323	-0.309	0.142
% sites PD≥4mm	-0.132	0.327	-0.260	0.185

% sites PD 1-3mm	0.292	0.155	0.150	0.305
% sites PD 4-5mm	-0.058	0.422	-0.268	0.177
% sites PD≥6mm	0.047	0.437	-0.172	0.279
Plaque Index	-0.055	0.426	0.309	0.141
BOP (%)	0.058	0.422	0.563	0.018

Notes: PD, probing depth; CAL, clinical attachment loss; BOP, bleeding of probing; MDA, malondialdehyde; and 8-hydroxy-2´-sesoxyguanosine. Values in bold represent statistically significant correlations (p <0.05). r: Spearman´s correlation coefficient

4. Discussion

Clemens et al. (2018), Yagan et al. (2014) [23,24] revealed in their research how doxycycline inhibits the formation of by-products, the result of fatty acid oxidation and lipid peroxidation. It is important to highlight that MDA adducts are highly immunogenic and initiate inflammatory responses and, therefore, fuel the cycle of inflammation and oxidative stress, thereby inducing chronicity. In this way, reducing the formation of MDA adducts can ameliorate inflammation leading to ROS production and thus break the self-sustaining cycle of oxidative stress and inflammation. Therefore, it is possible that the poorly recognized antioxidant properties of these drugs may be a mechanism that offers additional benefit in the treatment of chronic inflammatory diseases.

More recently, Yagan et al. (2018) [24] used sub-antimicrobial doses of doxycycline in periodontal treatment, given its characteristics as an enzyme inhibitor and related anti-inflammatory properties, whereby doxycycline helps prevent periodontal tissue degradation by inhibiting local oxidative stress. Sulijaya et al. (2019) [25], Altoé et al. (2021) [26], showed that oxidative stress is one of the main causes of tissue destruction. Therefore, the concept of PE has changed, and our treatment approach must be adjusted to this latest paradigm. The modulation of inflammation and oxidative stress should be considered a primary objective, therefore, the targeted strategy through anti-inflammatory and antioxidant treatments serves as an excellent therapeutic approach to achieve the desired level of clinical benefit.

In the present study, on the one hand, no significant differences were observed in the levels of MDA and 8-OHdG depending on the different stages. On the other hand, very interesting correlations were observed between the levels of 8-OHdG in urine and the BOP index (p=0.018) in the group of patients treated with doxycycline. This finding means that, at a higher rate of post-treatment bleeding, that is, at greater inflammation, higher levels of the pro-oxidant marker 8-OHdG are observed in urine, because of DNA damage repair mechanisms. In other words, local inflammatory processes appeared to be related to systemic oxidative stress and therefore to the chronicity of pathologies related to low-grade systemic inflammation.

No significant improvement in periodontal parameters is observed after treatment, given the small sample size. On the other hand, when comparing the changes in the periodontal parameters between groups, no significant differences were observed either, therefore, to corroborate the benefit of doxycycline at a topical level, future studies of longer duration and with a larger sample size would be necessary.

5. Conclusion

It can be inferred that periodontitis, as a local inflammatory disease, can also cause an increase in oxidative stress at the systemic level and be involved in the development and perpetuation of the inflammatory diseases, thus opening the way to new therapeutic strategies. Future projects should include larger and longer studies, with the aim of more precisely objectifying the influence of periodontitis with low-grade systemic inflammation, while exploring novel strategies for modulating the host response.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of the Arnau de Vilanova-Lliria (Valencia, Spain) hospital (protocol RAM-LIG-2019-01).

Statement of informed consent

Informed consent was obtained from all subjects involved in the study.

Author Contributions

Conceptualization, S.S.-d.-R. and F.P.; data curation, R.A.; formal analysis, R. A. and M. M-H.; investigation, R.A.; methodology, R.A.; project administration, S.S.-d.-R. and F.P.; resources, R.A.; software, M. M-H.; supervision, S.S.-d.-R. and F.P.; validation, S.S.-d.-R. and L. D-L.; visualization, S.S.-d.-R. and J. M. C-V; writing—original draft, R.A.; writing—review and editing, S.S.-d.-R. and F.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

These data will be treated in accordance with the provisions of Regulation (EU) 2016/679 of April 27 (GDPR) and Organic Law 3/2018 of April 5 December (LOPDGDD), for which the following treatment information is provided. Purposes of the treatment: -Participation of the interested party in the Study on the relationship between periodontal disease and serum levels of MDA and 8-OHdG. -Statistical and/or scientific purposes. Data conservation criteria: they will be kept for no longer than necessary to maintain the end of the treatment and when it is no longer necessary for that purpose, they will be deleted with adequate security measures to guarantee the pseudonymization of the data or the destruction of the same. Communication of the data: the data will not be communicated to third parties, except legal obligation. Rights that assist the Interested Party: -Right to withdraw consent at any time. -Right of access, rectification, portability and deletion of your data and the limitation or opposition to its treatment. -Right to file a claim with the Control Authority (www.aepd.es) if you consider that the treatment does not comply with current regulations.

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