# Comparative evaluation of salivary interleukin-18 in periodontitis patients with or without diabetes mellitus


## ABSTRACT

**Aim:** The study was conducted to compare the salivary concentrations of interleukin-18 in periodontitis patients with or without diabetes mellitus

**Materials and Methods:** The sample size in the study was 30 and categorized into 3 groups, in each group 10 individuals were included in the respective category. The three groups are as follows: Group a- patients with clinically healthy gingiva, Group b- patients with periodontitis and diabetes mellitus, Group c- patients with periodontitis only.

**Results:** Salivary Interleukin-18 levels were found to be significantly higher ($p<0.013$) in periodontitis with diabetes mellitus (110±7.0 ng/L) when compared with periodontitis only (70.06±3.5 ng/L) and also when compared with healthy controls (66±5.6 ng/L).

**Conclusion:** From the study, diabetic patients with periodontitis have increased IL-18 levels than non-diabetic counterparts. Thus salivary IL-18 is an effective non-invasive biomarker associating periodontitis and diabetes mellitus.

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*Keywords: ELISA, Innovative technology, Interleukin-18, Periodontitis, Saliva*




## 1. INTRODUCTION

Periodontitis is a multi-factorial, chronic inflammatory disease which affects the tissues surrounding the teeth with interactions between the host defense system and the pathogenic organisms. The close relationship between diabetes (DM) and periodontitis has long been known [1]. Patients with DM are at increased risk of developing periodontitis and those with untreated periodontitis have a negatively regulated glycemic status. Possible mechanical interactions between DM and periodontitis have been suggested, including altered polymorphonuclear cell (PMN) activation, increased adipokine production and altered apoptosis which could lead to increased cytokine production in both patients with periodontitis and DM [1]. Recent studies have identified inflammation as an important factor in the pathogenesis of DM [2, 3]. In clinical studies, it was found that elevated levels of several pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL) -1, IL-6, and IL-18 were associated with various types of diabetes [4 - 6].

Recent studies have reported conflicting results regarding the roles of IL-17A and IL-18 in periodontal disease. Awang et al. [7] reported significantly higher levels of IL-17A in GCF, saliva, and serum of subjects with periodontitis compared to those subjects with periodontal health. The study by Esfahrood et al. [8] did not show significant differences in both salivary and GCF levels of IL-18 between subjects with chronic periodontitis and those with a healthy periodontium. In contrast, Ozcaka et al. [9] found lower salivary IL-17A and elevated levels of IL-18 in subjects with chronic periodontitis compared with those without periodontitis. Therefore, it is still unclear whether periodontal inflammation is caused by increased levels of IL-18 and IL-17A.

In addition to the potential role of IL-17A and IL-18 in periodontitis, the systemic role of these cytokines and the interaction with DM have also been identified. In mice, DM has been shown to improve mRNA expression and IL-17 protein levels in several tissues [10,11]. In personality, however, Roohi et al. [12] revealed similar levels of IL-17A in the serum of both patients with type 1 DM and those with type 2 DM compared with healthy controls. Elevated levels of serum IL-18 have been reported in patients with metabolic syndrome and type 2 DM [13,14]. Human data on serum and salivary IL-17A and IL-18 in DM patients with and without periodontitis are still insufficient [15-25]. Our team has extensive knowledge and research experience that has translated into high quality publications [26-34]. The aim of this study is to evaluate the salivary interleukin-18 levels in periodontitis patients associated with or without diabetes mellitus.

## 2. MATERIALS AND METHODS

30 patients were recruited for the study and were categorized as periodontal health (Group a), patients with periodontitis and DM (Group b) and patients with periodontitis only (Group c). Unstimulated salivary samples were taken.

The patients with periodontitis were taken to the study by following the norms given below:
- not more than 2 teeth missing in each quadrant;
- greater than or equal to 30% of periodontal sites with periodontal pocket depth greater than or equal to 4 mm.
- greater than or equal to 20% of periodontal sites with interproximal clinical attachment loss >2 mm.
- greater than or equal to 30% of sites showing bleeding on probing.
- radiographic evidence of bone loss visible in posterior bitewing films.

The patients excluded had the following features:
- patients who have undergone periodontal therapy in the last 6 months
- patients under medications like antibiotics or anti-inflammatory drugs
- patients with history of alcoholism
- patients with a history of smoking or usage of tobacco in any form, betel nut users
- patients with history of known systemic diseases that would alter the healing response of the oral tissues
- acute periodontal conditions, such as periodontal abscess and acute necrotizing gingivitis
- detection of any obvious oral mucosal lesion.

Among the 30 patients in the present study, ten individuals with clinically healthy periodontium of similar age, race, ethnicity, and sex who had <10% of sites with bleeding on probing, no sites with periodontal pocket depth greater than or equal to 4 mm, no clinical attachment loss >2 mm, and no radiographic evidence of bone loss visible in posterior bitewing radiographs formed the control group (Group a).
Ten individuals with periodontitis along with diabetes mellitus (Group b) and ten individuals with periodontitis (Group c) were selected.

### 2.1. Saliva Collection

Participants were instructed to refrain from eating, drinking, and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all patients using expectoration into sterile bulbs. Collected samples were immediately transported to the laboratory, where it was centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at -70°C.

### 2.2 Salivary IL-18 analysis in saliva

Salivary IL-18 levels were measured in duplicate using a commercially available human IL-18 ELISA Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analyse the IL-18 levels using sandwich enzyme immunoassay technique. The samples were diluted with calibrator diluent provided with a ratio of 1:4 and the assay was performed according to the instructions. Standards were included and all results were read as the value of optical density set to 450 nm. The intra and inter assay coefficient variance (CV) was found to be ＜11% and <9%.

### 2.3. Statistical analysis

The triplicate analysis results of the experiments performed on control and test subjects were expressed as mean ± standard deviation. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

## 3. RESULTS AND DISCUSSION

The present study showed that salivary Interleukin- 18 levels were significantly higher ($p<0.013$) in periodontitis with diabetes mellitus (110±7.0 ng/L) when compared with periodontitis only (70.06±3.5 ng/L) and also when compared with healthy controls (66±5.6 ng/L) (Figure 1 and Table 1). IL-18 levels are associated with both diabetes mellitus and periodontitis. Although the role of IL-18 in periodontal disease has been observed in recent studies [35,36,37], our study did show significant differences in levels of salivary IL-18 between subjects with and without type 2 DM. Ozcaka et al. [38] showed significantly higher levels of salivary IL-18 in subjects with chronic periodontitis compared to short-term healthy subjects. In addition, no elevated levels of serum IL-18 in the chronic periodontitis group were reported. In another study, plasma samples were collected from 40 patients with chronic periodontitis and 20 healthy subjects. IL-18 measurements were made using commercially available ELISA kits. Patients with chronic periodontitis showed a 46% increase in plasma IL-18 levels compared with control subjects. Notably, elevated IL-18 levels reached> 5-fold in patients with chronic periodontitis compared with healthy individuals [40]. These conflicting results between these studies may be due to the choice of subjects and the various methods used to determine IL-18 levels.

Researchers have also found an association between salivary levels IL-18 and chronic inflammation. This finding can be explained by the effect of hyperglycemic spikes on the growing inflammatory cytokines described by Esposito et al. [41]. In their study, hyperglycemic risk due to high glucose injection can increase plasma levels and can lead to increased levels of IL-6, IL-18 and TNF-α in both healthy and diabetic subjects as well. Serum IL-18 levels are associated with HbA1C but not periodontitis. Elevated levels of serum IL-18 in type 2 DM and metabolic syndrome have been reported and suggested to contribute to microangiopathy in type 2 DM in recent studies [42,43,44]. Few studies also reported an interaction between serum IL-18 and HbA1C [45, 46].

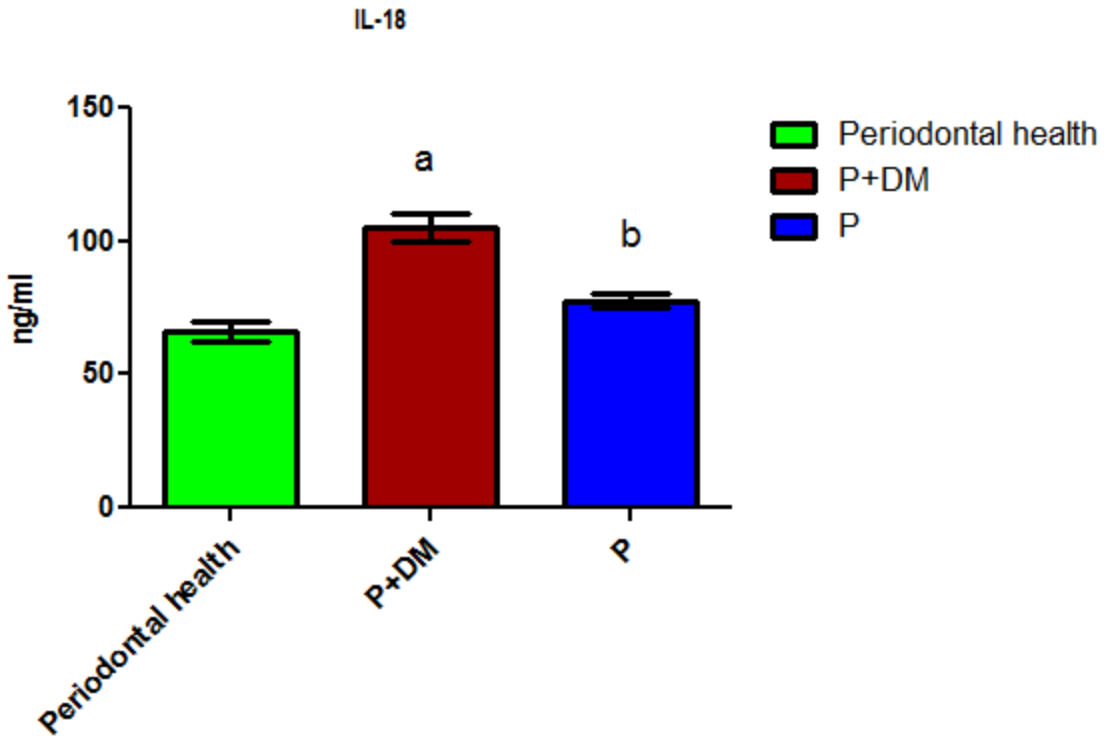


Figure 1: Assessment of salivary IL- 18 concentration among periodontal health, periodontitis and periodontitis with diabetes mellitus. The levels of salivary IL-18 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05, a- compared with the periodontal health group. b-compared with periodontitis with diabetes mellitus.

| GROUP | PERIODONTAL HEALTH | P+DM | P | P VALUE |
|---|---|---|---|---|
| IL-18(ng/L) | 66±5.6 | 110±7.0 | 70.06±3.5 | P<0.013 |

Table 1: Comparison of salivary interleukin 18 levels among 3 groups (periodontitis patients- P , patients with Periodontitis along with diabetes mellitus- P+DM and patients with periodontal health). The values are expressed in ng/L.



## 4. CONCLUSION

From the study, we conclude that IL-18 levels significantly increase in periodontitis patients with and without diabetes mellitus. In summary, diabetes mellitus and periodontal diseases are closely associated. Persistent hyperglycemia leading to exaggerated immune-inflammatory responses that are induced by periodontal pathogens is likely to be responsible for the greater risk and severity of periodontal disease in diabetic patients.

**COMPETING INTERESTS DISCLAIMER:**

**Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.**

**REFERENCES**


1. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, et al. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012;55(1): 21–31. Pmid:22057194
2. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2): 98–107. Pmid:21233852
3. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Investig. 2005;115(5): 1111–1119. Pmid:15864338
4. Jha JC, Jandeleit-Dahm KA, Cooper ME. New insights into the use of biomarkers of diabetic nephropathy. Adv Chronic Kidney Dis. 2014;21(3): 318–326. Pmid:24780461
5. Wong CK, Ho AWY, Tong PCY, Yeung CY, Kong APS, Lun SWM, et al. Aberrant activation profile of cytokines and mitogen-activated protein kinases in type 2 diabetic patients with nephropathy. Clin Exp Immunol. 2007;149(1): 123–131. Pmid:17425653
6. Nakamura A, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, et al. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. Diabetes Care. 2005;28(12): 2890–2895. Pmid:16306550
7. Awang RA, Lappin DF, MacPherson A, Riggio M, Robertson D, Hodge P, et al. Clinical associations between IL-17 family cytokines and periodontitis and potential differential roles for IL-17A and IL-17E in periodontal immunity. Inflamm Res. 2014;63(12): 1001–1012. Pmid:25369802
8. Esfahrood Z, Zare D, Reza J, Rahmanian F. Levels of interleukin-18 in saliva and gingival crevicular fluid in patients with chronic periodontitis and healthy subjects. ARRB. 2016;10(3): 1–6.
9. Ozcaka O, Nalbantsoy A, Buduneli N. Interleukin-17 and interleukin-18 levels in saliva and plasma of patients with chronic periodontitis. J Periodontal Res. 2011;46(5): 592–598. Pmid:21635252
10. Jain R, Tartar DM, Gregg RK, Divekar RD, Bell JJ, Lee H-H, et al. Innocuous IFNgamma induced by adjuvant-free antigen restores normoglycemia in NOD mice through inhibition of IL-17 production. J Exp Med. 2008;205(1): 207–218. Pmid:18195074
11. Emamaullee JA, Davis J, Merani S, Toso C, Elliott JF, Thiesen A, et al. Inhibition of Th17 cells regulates autoimmune diabetes in NOD mice. Diabetes. 2009;58(6): 1302–1311. Pmid:19289457
12. Roohi A, Tabrizi M, Abbasi F, Ataie-Jafari A, Nikbin B, Larijani B, et al. Serum IL-17, IL-23, and TGF-beta levels in type 1 and type 2 diabetic patients and age-matched healthy controls. Biomed Res Int. 2014;2014: 718946. Pmid:24995325
13. Zaharieva E, Kamenov Z, Velikova T, Tsakova A, El-Darawish Y, Okamura H. Interleukin-18 serum level is elevated in type 2 diabetes and latent autoimmune diabetes. Endocr Connect. 2018;7(1): 179–185. Pmid:29217651
14. Troseid M, Seljeflot I, Arnesen H. The role of interleukin-18 in the metabolic syndrome. Cardiovasc Diabetol. 2010;9: 11. Pmid:20331890
15. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241–8.
16. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.
17. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol

Macromol. 2018 Dec;120(Pt A):876–85.
18. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. Cochrane Database Syst Rev. 2018 Nov 26;11:CD011423.
19. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. Hypertens Res. 2020 Aug;43(8):851–3.
20. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol. 2019 Dec;16(12):935–6.
21. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. Comput Commun. 2019 Dec 15;148:176–84.
22. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing Relationship between Patient and Doctor in Public Dental Health using Particle Memetic Multivariable Logistic Regression Analysis Approach (MLRA2). J Med Syst. 2018 Aug 29;42(10):183.
23. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. J Dent Educ. 2019 Apr;83(4):445–50.
24. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, Characterization and Biological Applications of Biosynthesized Silver Nanoparticles with Chitosan-Fucoidan Coating. Molecules [Internet]. 2018 Jun 12;23(6). Available from: http://dx.doi.org/10.3390/molecules23061429
25. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of Different Concentrations of Three Root Canal Sealers on Human Mesenchymal Stem Cells. Biomolecules [Internet]. 2018 Aug 1;8(3). Available from: http://dx.doi.org/10.3390/biom8030068
26. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. Biotechnol Bioprocess Eng. 2018 Aug 1;23(4):383–93.
27. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. Measurement. 2019 Mar 1;135:672–7.
28. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. J Endod. 2021 Aug;47(8):1198–214.
29. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. Oral Dis [Internet]. 2021 Jun 21; Available from: http://dx.doi.org/10.1111/odi.13937
30. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. Oral Dis [Internet]. 2021 Feb 11; Available from: http://dx.doi.org/10.1111/odi.13798
31. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021 Jun 16;105390.
32. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol. 2021 Jun 14;105375.
33. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humaniz Comput [Internet]. 2018 Dec 14; Available from: https://doi.org/10.1007/s12652-018-1166-8
34. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. Risk Manag Healthc Policy. 2021 Jul 7;14:2851–61.
35. Kramer JM, Gaffen SL. Interleukin-17: A new paradigm in inflammation, autoimmunity, and therapy. J Periodontol. 2007;78:1083–93. [PubMed] [Google Scholar]
36. Nair V, Bandyopadhyay P, Kundu D, Das S. Estimation of interleukin-18 in the gingival crevicular fluid and serum of Bengali population with periodontal health and disease. J Indian Soc Periodontol. 2016;20:260–4. [PMC free article] [PubMed] [Google Scholar]

37. Chitrapriya MN, Rao SR, Lavu V. Interleukin-17 and interleukin-18 levels in different stages of inflammatory periodontal disease. J Indian Soc Periodontol. 2015;19:14–7. [PMC free article] [PubMed] [Google Scholar]
38. Ozçaka O, Nalbantsoy A, Buduneli N. Interleukin-17 and interleukin-18 levels in saliva and plasma of patients with chronic periodontitis. *J Periodontal Res.* 2011;46:592–8.
39. Isaza-Guzmán DM, Cardona-Vélez N, Gaviria-Correa DE, Martínez-Pabón MC, Castaño-Granada MC, Tobón-Arroyave SI. Association study between salivary levels of interferon (IFN)-gamma, interleukin (IL)-17, IL-21, and IL-22 with chronic periodontitis. Arch Oral Biol. 2015;60:91–9. [PubMed] [Google Scholar]
40. Yetkin Ay Z, Sütçü R, Uskun E, Bozkurt FY, Berker E. The impact of the IL-11: IL-17 ratio on the chronic periodontitis pathogenesis: A preliminary report. Oral Dis. 2009;15:93–9. [PubMed] [Google Scholar]
41. Inflammatory Cytokine Concentrations Are Acutely Increased by Hyperglycemia in Humans Role of Oxidative Stress Katherine Esposito, Francesco Nappo, Raffaele Marfella, Giovanni Giugliano, Francesco Giugliano, Myriam Ciotola, Lisa Quagliaro, Antonio Ceriello, and Dario Giugliano Originally published 30 Sep 2002.
42. Netea MG, Stuyt RJ, Kim SH, Van der Meer JW, Kullberg BJ, Dinarello CA. The role of endogenous interleukin (IL)-18, IL-12, IL-1beta, and tumor necrosis factor-alpha in the production of interferon-gamma induced by Candida albicans in human whole-blood cultures. J Infect Dis. 2002;185:963–70. [PubMed] [Google Scholar]
43. Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. Oral Microbiol Immunol. 2006;21:256–60. [PubMed] [Google Scholar]
44. Awang RA, Lappin DF, MacPherson A, Riggio M, Robertson D, Hodge P, et al. Clinical associations between IL-17 family cytokines and periodontitis and potential differential roles for IL-17A and IL-17E in periodontal immunity. Inflamm Res. 2014;63:1001–12. [PubMed] [Google Scholar]
45. Poorsattar Bejeh-Mir A, Parsian H, Akbari Khoram M, Ghasemi N, Bijani A, Khosravi-Samani M. Diagnostic role of salivary and GCF nitrite, nitrate and nitric oxide to distinguish healthy periodontium from gingivitis and periodontitis. Int J Mol Cell Med. 2014;3:138–45. [PMC free article] [PubMed] [Google Scholar]
46. Yang X, Li C, Pan Y. The influences of periodontal status and periodontal pathogen quantity on salivary 8-hydroxydeoxyguanosine and interleukin-17 levels. J Periodontol. 2016;87:591–600.