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ORIGINAL ARTICLE



# The effect of menopause on the relationship between hyperlipidemia and periodontal disease via salivary 8-hydroxy-2′-deoxyguanosine and myeloperoxidase levels

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**ABSTRACT**

**Objective:** Impairment of the lipid metabolism could affect the periodontal disease; increased oxidative stress may have a role in this relationship. The aim of the present study was to evaluate the role of menopause in the relationship between hyperlipidemia and periodontal disease via oxidative stress markers in saliva.

**Materials and methods:** Sixty-seven women were enrolled in the study and divided into four groups as systemically healthy and premenopause (C) ($n = 18$), hyperlipidemia and premenopause (H) ($n = 16$), systemically healthy and postmenopause (M) ($n = 17$), and hyperlipidemia and postmenopause (MH) ($n = 16$). Sociodemographics, periodontal and metabolic parameters, and saliva oxidative markers (myeloperoxidase [MPO] and 8-hydroxy-2′-deoxyguanosine [8-OHdG]) were evaluated.

**Results:** Menopause and/or hyperlipidemia were associated with an increase in all evaluated periodontal parameters. Saliva 8-OHdG and MPO levels were higher in menopausal groups (M and MH). Multivariate linear regression analyses revealed that hyperlipidemia was related to an increase in periodontal parameters. Salivary oxidative stress markers and periodontal parameters were also positively associated with menopause and hyperlipidemia.

**Conclusion:** Saliva 8-OHdG and MPO levels may indicate that the relationship between periodontal disease and hyperlipidemia is aggravated by menopause.

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periodontal disease; menopause; hyperlipidemia; oxidative stress; saliva

## Introduction

Periodontitis, which causes destruction in periodontal tissues as a result of the host's immune response against microbial pathogens in dental plaque, is a chronic, inflammatory disease [1]. An altered lipid profile was shown to have a role in the pathogenesis of periodontal disease via proinflammatory cytokines [2,3]. It has been demonstrated that periodontal health is worsened in hyperlipidemic patients [4,5], and a disruption of the lipid metabolism may be important in the relationship between periodontitis and cardiovascular disease (CVD) [6].

Though Gram negative anaerobes and facultative bacteria are primary etiological agents in periodontal disease, it is believed that a loss of homeostatic balance between antioxidant defense systems and reactive oxygen species is responsible for the periodontal destruction [7]. Increased oxidative stress, which causes damage to lipids, carbohydrates, deoxyribonucleic acid (DNA), and proteins [8,9], may play an important role in both periodontal disease pathogenesis [7,10] and in the relationship between periodontal disease and various systemic conditions such as hyperlipidemia [9,11], postpartum period [12] and polycystic over syndrome [13]. Furthermore 8-hydroxy-2′-deoxyguanosine (8-OHdG), which was suggested to be a reliable indicator of oxidative DNA damage [14], was reported to have increased in patients with periodontal disease [10,12,15,16]. It has been reported that the 8-OHdG levels may increase as a result of a harmful oxidative status in association with hyperlipidemia and periodontitis [17].

Myeloperoxidase (MPO) is a specific peroxidase released by activated neutrophils, and its overexpression depending on the pathologic conditions may cause oxidative damage [18]. Increased MPO levels in otherwise healthy people were indicated to be associated with the future risk of CVD by causing lipid oxidation [19]. High total cholesterol (TC) levels may lead to an increase in MPO [20], causing increased oxidative damage, which is shown to be involved in the pathogenesis of CVD by participating in the formation of atherosclerosis [21]. Increased MPO in periodontal diseases was reported in several studies [13,22,23]; thus, MPO has been considered a promising marker of periodontal disease activity [24].

Estrogen deficiency is a risk factor for various systemic diseases [25], and an altered lipid profile [26] and increased

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oxidative stress [27,28] related to menopause may have a role in the pathogenesis. Estrogen has an antioxidant effect by inhibiting DNA guanine 8-hydroxilation; however, decreased estrogen levels can damage genetic material via its prooxidant effect [29]. Estrogen deficiency may contribute to bone loss and intense gingival inflammation during periodontitis [30]. Menopause is related to an increase in bone loss; thus, it can be concluded that bone loss related diseases, like periodontitis or tooth loss, may be affected by menopause [31].

We hypothesized that menopause combined with hyperlipidemia may have synergistic effects on the worsening of the periodontal disease via oxidative stress. The aim of our study was to evaluate the periodontal parameters and salivary oxidative markers in women with menopause and/or hyperlipidemia and to compare them with systemically healthy premenopausal controls.

## Material and methods

### Study population

Süleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee, Isparta, Turkey (11.02.2015/33), approved the study in accordance with the Declaration of Helsinki, which was revised in 2013.

From September 2013 to March 2015, three hundred forty volunteers who consulted with Süleyman Demirel University Faculty of Dentistry Department of Periodontology were invited to take part in the study. Among them, thirty-four premenopausal (Pre/M) (aged 28–43 years; median age: 32.5) and thirty three postmenopausal (Post/M) women (aged 45–56 years; median age: 48) who were eligible for inclusion were enrolled in the study. Four groups were created according to their systemic and menopausal status: 18 systemically healthy controls with premenopause (C) (aged 30–45 years; median age: 31), 16 hyperlipidemia with premenopause (H) (aged 28–43 years; median age: 29), 17 systemically healthy with postmenopause (M) (aged 47–55; median age: 48), and 16 hyperlipidemia and postmenopause (MH) (aged 45–56; median age: 46). Written consent forms were obtained, and information regarding sociodemographics (age, education level, monthly income, oral hygiene habits) was collected via questionnaire.

The patients were excluded if they were pregnant or lactating, had a history of systemic disease other than hyperlipidemia, had osteoporosis or had received antilipemic therapy, had undergone surgical menopause and/or hormone replacement therapy, had received periodontal treatment within the 6 months prior to the study, had aggressive periodontitis, were current or former smokers, had a BMI $>30\,kg/m^2$, had $<8$ teeth ($<2$ teeth in each quadrant), or had any antimicrobial and/or anti-inflammatory agents within 1 month of the study.

Pre/M is defined as having regular menstrual cycles in the last year and Post/M is defined as not having menstrual cycles monthly in the last $\geq 1$ year. Women whose last menstrual cycle was more than five years prior were also excluded.

### Periodontal parameters

Periodontal parameters of all teeth except third molars were measured by two calibrated dentists (E.D. and B.D.). Patients who were newly diagnosed with healthy, gingivitis or chronic periodontitis were included the study [32]. Inter-examiner and intra-examiner correlation coefficients were shown as .85 and .90 for probing pocket depth (PD) and .83 and .89 for clinical attachment level (CAL). Weighted k values (–1 mm) ranged from .84 to .92 for PD and .82 to .90 for CAL, respectively.

PD and CAL were measured at six sites (buccal and lingual aspects, each with mesial, median, and distal points), and plaque index (PI) [33] and gingival index (GI) [34] were evaluated at four sites (mesio-buccal, mid-buccal, disto-buccal, and mid-lingual) using a periodontal probe (Williams periodontal probe, Hu-Friedy, Chicago, IL). The number of missed teeth (MT) was also recorded.

### Metabolic parameters and saliva oxidative markers

An endocrinologist (B.K.) evaluated detailed medical history and biochemical tests to specify any previously mentioned diseases. Participants were instructed not to eat or drink at least 8 hours prior to sampling. Lipid parameters were measured and pathologic limits were described as triglyceride (TG) $>200$ mg/dL, TC $>200$ mg/dL, low-density lipoprotein cholesterol (LDL) $>130$, and high-density lipoprotein cholesterol (HDL) $<35$ mg/dL.

Patients were seated and were instructed to spit into a cup one time every 60 seconds over the course of 10 minutes [35]. Samples were centrifuged at 4000 g for 10 min. at 4 °C, and supernatants were stored in Eppendorf tubes at −80 °C until analysis. Enzyme-linked immunosorbent assay kits were used to measure saliva MPO (Human MPO ELISA Kit, Sunred Biotechnology, Shanghai, China) and 8-OHdG (Human 8-Hydroxy-desoxyguanosine ELISA Kit, Sunred Biotechnology, Shanghai, China) levels. The kits, whose sensitivity and assay range is 0.116 ng/mL and 0.3–30 ng/mL for MPO and 0.558 ng/ml and 1–100 ng/ml for 8-OHdG respectively, were prepared according to the manufacturer's instructions based on the principle of double-antibody sandwich technique. Color changes were measured spectrophotometrically at 450 nm. The results were expressed as ng/ml, according to the manufacturer's manual.

### Statistical analyses

Statistical analyses were performed by a package programme (SPSS, v.21.0 for Windows, IBM, Chicago, IL). A power analysis programme (G*power, v.3.1.9.2 for Windows, University of Kiel, Kiel, Germany) indicated a power of >80% at the $\alpha = .05$ level for periodontal (GI, PD, CAL) and oxidative (8-OHdG, MPO) parameters among the study groups.

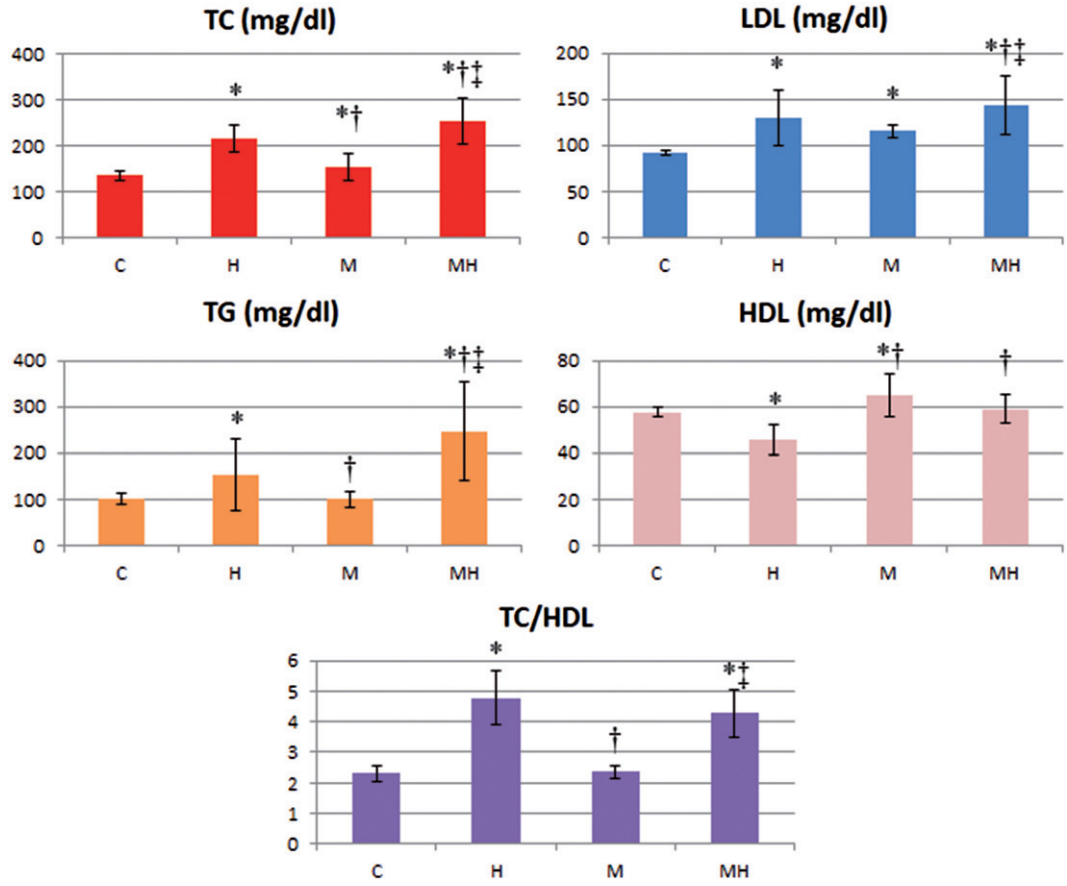


**Figure 1.** Serum lipid parameters of the groups. C, control; H, hyperlipidemia; M, menopause; MH, menopause and hyperlipidemia. *Significant difference from Group C ($p < .05$, Mann–Whitney *U* test). †Significant difference from Group H ($p < .05$, Mann–Whitney *U* test). ‡Significant difference from Group M ($p < .05$, Mann–Whitney *U* test).

Data among groups were analysed by a chi-squared test for the categorical parameters and both a Mann–Whitney *U* test and a Kruskal–Wallis H test for continuous variables. Though covariates affecting the results were carefully distributed among groups, the effect of age, which was different among study groups after Bonferroni correction, was adjusted in multivariate linear regression analyses to identify the predictive indicators of periodontal and oxidative parameters. To evaluate the relationships between parameters independently of the study groups, age adjusted partial correlation analyses were also made in the whole population; $p < .05$ was considered statistically significant.

## Results

Age was higher in postmenopausal groups (M and MH) than in premenopausal groups (C and H), and no significant difference was shown regarding sociodemographics (education level, monthly income, oral hygiene habits) other than age (data not shown). Distribution of the lipid parameters according to the study groups is indicated in Figure 1. The increase of the lipids in Group H compared to Group C was clear. Group M had higher TC, LDL, and HDL levels than Group C. Though TG and TC/HDL were lower in Group M than in Group H, TC, LDL, and HDL were similar between Group H and M. Group MH had the highest TC, TG, and LDL levels.

**Table 1.** Comparisons of the periodontal and oxidative parameters between groups (Mean ± Standard Deviation).

| Variables | C ($n = 18$) | H ($n = 16$) | M ($n = 17$) | MH ($n = 16$) | $p$* |
|---|---|---|---|---|---|
| PI | 1.10 ± .65 | 1.50 ± .63$^{\dagger}$ | 1.20 ± .44 | 1.50 ± .43$^{\dagger\S}$ | .037 |
| GI | 1.01 ± .42 | 1.28 ± .39 | 1.12 ± .37 | 1.51 ± .29$^{\dagger\S}$ | .002 |
| PD | 2.59 ± .46 | 3.03 ± .43$^{\dagger}$ | 3.31 ± .37$^{\dagger\ddagger}$ | 3.38 ± .26$^{\dagger\ddagger}$ | .000 |
| CAL | 2.68 ± .61 | 3.21 ± .67$^{\dagger}$ | 3.59 ± .35$^{\dagger\ddagger}$ | 3.77 ± .62$^{\dagger\ddagger}$ | .000 |
| MT | 2.61 ± 1.79 | 4.81 ± 3.53$^{\dagger}$ | 11.71 ± 7.30$^{\dagger\ddagger}$ | 10.63 ± 6.054$^{\dagger\ddagger}$ | .000 |
| 8-OHdG | 21.52 ± 3.91 | 27.38 ± 11.34$^{\dagger}$ | 26.79 ± 6.67$^{\dagger}$ | 40.46 ± 17.25$^{\dagger\ddagger\S}$ | .000 |
| MPO | 7.07 ± .70 | 7.82 ± 1.41 | 8.47 ± 2.81 | 10.81 ± 1.88$^{\dagger\ddagger\S}$ | .003 |

C: control; H: hyperlipidemia; M: menopause; MH: menopause and hyperlipidemia; PI: plaque index; GI: gingival index; PD: probing pocket depth; CAL: clinical attachment level; MT: missed teeth; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; MPO: myeloperoxidase.
*Intergroup differences were analysed by Kruskal–Wallis H test.
$^{\dagger}$Significant difference from Group C ($p < .05$, Mann–Whitney *U* test).
$^{\ddagger}$Significant difference from Group H ($p < .05$, Mann–Whitney *U* test).
$^{\S}$Significant difference from Group M ($p < .05$, Mann–Whitney *U* test).

Periodontal parameters were increased by menopause and/or hyperlipidemia, and menopause groups (Group M and Group MH) had the highest PD and CAL levels (Table 1). Positive correlations were shown between periodontal and lipid parameters in age adjusted partial correlation analyses (Table 3). The age adjusted multivariate linear regression analyses revealed that compared to Group C, Group H was related to an increase in PD levels, but no significant effect of Group M was observed regarding periodontal parameters. All periodontal parameters except PI were increased in Group MH in comparison to Group C. PD and MT were also higher in Group MH when compared to Group H (Table 2).

**Table 2.** Age adjusted multivariate linear regression analysis (β [95% CI]) of periodontal and oxidative parameters between groups.

| Dependent variables | C-H | C-M | C-MH | H-MH |
|---|---|---|---|---|
| PI | 0.21 (−0.17, .72) | 0.07 (−0.30, 0.37) | 0.30 (−0.05, 0.28) | −0.17 (−0.30, 0.12) |
| GI | 0.21 (−0.10, .44) | −0.10 (−0.27, 0.19) | **0.38 (0.01, 0.21)** | 0.16 (−0.08, 0.19) |
| PD | **0.36 (0.05, .65)** | 0.30 (−.07, 0.39) | **0.62 (0.12, 0.33)** | **0.39 (0.01, 0.30)** |
| CAL | 0.26 (−0.05, .76) | 0.25 (−0.10, 0.44) | **0.50 (0.10, 0.44)** | 0.30 (−0.05, 0.46) |
| MT | 0.31 (−0.15, 3.77) | 0.39 (−0.30, 5.68) | **0.53 (0.84, 3.31)** | **0.39 (0.29, 4.14)** |
| 8-OHdG | **0.39 (0.58, 12.65)** | **0.57 (0.18, 6.57)** | **0.91 (6.09, 12.35)** | **0.60 (3.97, 14.79)** |
| MPO | 0.33 (−0.24, 1.67) | 0.03 (−1.16, 1.28) | **0.71 (0.42, 1.70)** | **0.85 (0.70, 2.96)** |

C: control; H: hyperlipidemia; M: menopause; MH: menopause and hyperlipidemia; PI: plaque index; GI: gingival index; PD: probing pocket depth; CAL: clinical attachment level; MT: missed teeth; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; MPO: myeloperoxidase.
Bold denotes statistical significance ($p < .05$).

Salivary 8-OHdG and MPO levels were higher in menopausal groups than in premenopausal ones (Table 1). Group MH was related to an increase in salivary MPO levels compared to both Group C and H. Saliva 8-OHdG levels were increased in Group H and M when compared to Group C, but the increase was highest in Group MH (Table 2). Positive correlations between MPO and periodontal parameters were indicated. Saliva 8-OHdG levels were also positively correlated to MT (Table 3).

**Table 3.** Significant age adjusted partial correlations among periodontal, oxidative and lipid parameters.

| Variables | | 8-OhDG | MPO | TC | TG | LDL | HDL | TK/HDL |
|---|---|---|---|---|---|---|---|---|
| 8-OhDG | r | | .679† | .408† | .299* | | | .334† |
| | *P* | | .000 | .001 | .014 | | | .006 |
| MPO | r | .679† | | .369† | .443† | | | .286* |
| | *P* | .000 | | .008 | .001 | | | .044 |
| PI | r | | | .353† | .359† | | | .306* |
| | *P* | | | .003 | .003 | | | .012 |
| GI | r | | .315* | .390† | .401† | | | .331† |
| | *P* | | .026 | .001 | .001 | | | .006 |
| PD | r | | .444† | .414† | .356† | .360* | | .302* |
| | *P* | | .001 | .001 | .003 | .021 | | .013 |
| CAL | r | | .498† | .380† | .285* | .349* | | .269* |
| | *P* | | .000 | .001 | .020 | .025 | | .028 |
| MT | r | .480† | .453† | .364† | | | .281* | |
| | *P* | .000 | .001 | .002 | | | .021 | |

8-OHdG: 8-hydroxy-2′-deoxyguanosine; MPO: myeloperoxidase; TC: total cholesterol; TG: triglyceride; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; PI: plaque index; GI: gingival index; PD: probing pocket depth; CAL: clinical attachment level; MT: missed teeth.
*$p < .05$.
†$p < .01$.

## Discussion

Many studies have been reported regarding the relationship between periodontal disease and systemic conditions, such as hyperlipidemia [17], diabetes [36], osteoporosis [37], and menopause [31]. Though the role of proinflammatory cytokines, such as tumor necrosis factor-α [4,36,38], interleukin (IL)-1β [4,36,38], IL-6 [4], serum lipoprotein associated inflammatory mediators [39], and oxidative stress [11,17], has been indicated in many previous studies that have investigated both the effects of hyperlipidemia on periodontal disease and the mechanisms linking these two pathologic conditions, none of the reports investigated the role of menopause in this relationship. In menopause, various metabolic and physiologic alterations occur depending on the decreased ovarian hormone production and prevalence of CVD risk factors, such as hypertension, diabetes mellitus, hyperlipidemia, and metabolic syndrome increases [40]. Reports indicate that periodontal status also gets worse after menopause [30,31]. To the best of the author's knowledge, this is the first study evaluating the effect of menopause on the relationship between hyperlipidemia and periodontal disease in terms of oxidative status.

An increase in periodontal parameters in hyperlipidemic groups was shown, and a significant increase in PD by hyperlipidemia was indicated in age adjusted linear regression analyses. Additionally, positive correlations between periodontal and lipid parameters were indicated. The effect of hyperlipidemia on the worsening of the periodontal status was reported in various studies [4,5,11,17,39,41]. Our results are similar to the literature in that hyperlipidemia is a risk factor for periodontitis. Periodontal parameters were shown to have increased in menopausal groups and were highest in Group MH, though no significant difference was found between Group M and C in age adjusted analysis. Estrogen inhibits the expression of inflammatory cytokines, which are important in bone destruction. Therefore, an estrogen deficiency can contribute to bone loss and an increase in gingival inflammation during periodontitis [30]. Though decreasing bone mineral density by menopause is considered to worsen the periodontal disease severity, the studies are controversial [37,42]. The study population included women without osteoporosis. However, estrogen levels were not evaluated, and menopausal duration was limited to 1-5 years to prevent time-related effects. When compared to that of Group H, the increase in PD and MT in Group MH corroborates the hypothesis that menopause aggravates an already worsened periodontal status by hyperlipidemia.

Oxidative stress has a crucial factor in the pathogenesis of systemic conditions [43]. Furthermore 8-OHdG is an important indicator of oxidative DNA damage [44]. In diet-induced hyperlipidemia, 8-OHdG levels were reported to have increased [45]. Saliva 8-OHdG levels were also shown to be associated with periodontal destruction in a number of studies [10,12,15,16]. Fentoglu et al. [17] reported elevated 8-OHdG levels in patients with hyperlipidemia and periodontitis. MPO, another oxidative enzyme, is stored in neutrophils and has a key role in the cleaning of phagocytized bacteria and therefore has the potential to cause tissue damage [46]. MPO release is stimulated by active neutrophils under inflammatory conditions like hyperlipidemia [47]. MPO, having also been considered a promising marker of periodontal disease

activity, may play a critical role in the pathogenesis of periodontal destruction [24]. Increased saliva MPO levels were demonstrated in patients with periodontitis [13,22,23]. In the present study, the MPO and 8-OHdG levels increased by hyperlipidemia and the positive correlations among oxidative stress markers, lipid parameters, and periodontal parameters all support the previous studies [15,17,23,24,45,47].

After menopause, TC, TG, and LDL levels increase, while HDL levels slowly decrease in relation to the decreasing estrogen levels [48]. Decreased estrogen levels increase oxidative stress related to the concentration and chemical structure of the hormone [29]. It has been reported that estrogen deficiency may increase MPO activity [49] and 8-OHdG levels [50]. In the present study, the levels of TC, LDL (Figure 1), salivary MPO, and 8-OHdG (Table 2) were shown to be elevated in menopause groups independently of hyperlipidemia. Furthermore, the combination of menopause and hyperlipidemia can cause a synergistic effect on salivary oxidative stress, as shown in the findings, which place the highest oxidative parameter levels in group MH.

Though very rigid inclusion criteria, such as no history of antilipemic therapy, hormone replacement therapy, obesity, and osteoporosis, were chosen in our study, some limitations complicated the interpreting of the results. The cause–effect relationship could not be indicated because of the cross-sectional study design. The population had mild to moderate periodontitis. The relationship between hyperlipidemia and/or menopause and various degrees of periodontal disease could not be revealed.

In conclusion, saliva 8-OHdG and MPO levels may be used as an indicator of the relationship between periodontal disease and hyperlipidemia that is aggravated by menopause. Menopausal status should be considered a conflicting factor in studies evaluating the relationship between periodontal and systemic disease; larger population-based studies are needed to confirm the results.

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## Disclosure statement

The authors declare no conflict of interest related to this study.

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