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Original Article

Endocan and Interleukin-6 Levels in Individuals with polycystic ovary syndrome and periodontal inflammation: A prospective cross-sectional stud

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Abstract

Aim: Periodontitis and polycystic ovary syndrome (PCOS) are relationship to both chronic low-grade inflammation and systemic inflammation. In spite of common risk factors, the link between periodontitis and PCOS has not yet been clear. The aims of the current cross-sectional study are to determine serum and saliva endocan and interleukin-6(IL-6) levels, and to evaluate the correlation between these two biomarkers in women with periodontitis and PCOS.

Materials and Methods: 87 individuals divided into four groups; PCOS with periodontitis (PCOSP), PCOS but periodontally healthy (PCOSPH), systemically healthy with periodontitis (SHP) and control group. Demographics, periodontal parameters, serum and saliva endocan and IL-6 values were evaluated.

Results: Serum endocan values in all the study groups were significantly greater than those in the control group. Saliva endocan values in PCOSP group were greater than those in the control group. Serum IL-6 values in both the PCOSP and SHP groups were significantly greater than those in the control group. Saliva IL-6 values in both periodontitis groups were significantly greater than those in the control groups. There were significant positive correlations between the serum and saliva endocan and saliva IL-6 values ($p<0.01$). Additionally, there was significant correlation between the saliva endocan and serum IL-6 levels ($p<0.05$).

Conclusion: Although both periodontitis and PCOS itself is increased endocan and IL-6 levels compared to those seen in healthy individuals, this increase was generally more pronounced in individuals with both PCOS and periodontitis. It can be thought that this increase may be modified by the inflammatory process in periodontal disease.

Keywords: Cytokines; gynecology; periodontitis; saliva; Women's health

INTRODUCTION

Periodontitis is an inflammatory disease characterized by tissue destruction, microorganisms and the host defense, and causes local and systemic increases of pro-inflammatory common systemic mediators for example tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) (1). Vascular dilatation, increased capillary permeability, blood flow, and leukocytes, monocytes/macrophages, epithelial cells, and endothelial cells are occurred in the inflammatory process. In order to increase the number of cells provided for microbial challenge, the inflammatory response is increased by the production of cytokines and chemokines (2). Various studies suggest a relationship between systemic diseases, including metabolic syndrome (3), diabetes , cardiovascular disease (CVD) (4) and periodontal disease.

Polycystic ovary syndrome (PCOS) is a multi-systemic disease characterized by hyperandrogenism, polycystic ovaries and chronic anovulation (5). It is a reproductive and metabolic disease related to raise risk of cardiovascular events (6). Additionally, PCOS is related to several risk factors such as dyslipidemia, obesity, insulin resistance, glucose metabolism disturbance and hypertension and chronic low grade inflammation (5-7).

Endothelial cell-specific molecule 1, a soluble proteoglycan, is also known as endocan. It is stated that

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this is secreted predominantly by endothelial cells under the command of inflammatory cytokines (8). Endocan has also been reported to play a role in angiogenesis, vascular permeability and tumor progression (9-11). It is supported that there may be a relationship between endocan and inflammation, by a growing body of evidence. Elevated endocan values have been determined in several diseases for example diabetes (12), coronary artery disease (13) and systemic diseases (e.g. Behcet's disease (14), systemic lupus erythematosus (15) and inflammatory bowel disease (16)).

Interleukin-6 family cytokines are described as cytokines that use the common signaling receptor subunit 130kDa. While the production of IL-6, a hormonally regulated cytokine is stimulated by catecholamines, it is suppressed by glucocorticoids and estrogens (17). At the same time, during inflammatory stress, IL-6 -as a one of the major cytokines- stimulates the hypothalamic-pituitary-adrenal axis (18). It has been shown that circulating IL-6 levels correlate with insulin resistance and obesity, both of which commonly co-present with PCOS (19).

Both periodontitis and PCOS are relationship to systemic inflammation and CVD's (1, 3, 4, 6). These two conditions are thought to be associated with common pathophysiological events. A growing body of evidence suggests a possible association between these two diseases (1, 20-23). In spite of common risk factors, the link between periodontitis and PCOS has not yet been clear. To the best of the authors' knowledge, there is no study evaluating the relation between periodontitis and PCOS with regards to endocan and IL-6 parameters.

The goals of this study were to assess whether serum and saliva endocan and IL-6 values are altered in individuals with periodontitis and PCOS, and to detect whether there is a relationship between endocan and IL-6 levels.

MATERIALS and METHODS

Study Groups

This prospective cross-sectional study was approved by the Ethics Committee of Ataturk University's Faculty of Dentistry, Erzurum, Turkey (2017/14) in accordance with principles embodied in the Declaration of Helsinki of 1975 (as revised in 2000). All participants were recruited from the Department of Periodontology, Faculty of Dentistry and the Department of Obstetrics and Gynaecology, Faculty of Medicine, both of Ataturk University. The participants were fully informed of this study's aims and methodologies, and informed consent was gotten from each participant.

The sample size was detected using serum endocan levels variations among the PCOS but periodontally healthy (PCOSPH) group and systemically and periodontally healthy (SHPH) group based on obtained in previous study (24). To achieve 80% power and determine a minimum clinically significant difference of 2.33 ng/ml with a 95% confidence level, at least 15 patients were required per group.

Between April and November 2017, age- and body mass index (BMI)-matched, non-obese individuals participated in this cross-sectional study. The four groups comprising a total of 87 individuals were formed, as follows; 1) PCOS with P [PCOSP, (n:23)], 2) PCOSPH, (n:23), 3) systemically healthy with periodontitis [SHP, (n:21)] and 4) a control group comprising SHPH individuals, (n:20).

Body mass index was calculated by dividing the body weight (in kg) by the square of height (in mm). Additionally, waist circumference (WC) was measured and the waist-hip ratio (WHR) was calculated for each participant (25).

The inclusion criteria were as follows: 1) newly diagnosed with PCOS, 2) had never smoked, 3) had never use alcohol, 4) BMI ≥18 and <25 kg/m2, and 5) the presence of ≥18 teeth.

The exclusion criteria were as follows: 1) history of any systemic disease other than PCOS, 2) pregnant or lactating, 3) had received any periodontal treatment in the previous six months, 4) had taken any medicine such as oral contraceptives, anti-inflammatories, antibiotics, steroid hormones, hypertensives, or insulin-sensitizers in the previous three months (as these medications can affect the metabolic criteria (5)), 5) hemoglobin A1c (HbA1c) ≥ 6.5% and 6) possible confounding factors, including history of Cushing syndrome, non-classic congenital adrenal hyperplasia, hyperprolactinemia, thyroid dysfunction or androgen-secreting tumours.

PCOS was diagnosed according to the presence of any two of the three Rotterdam criterias (1) chronic anovulation, 2) hyperandrogenism and 3) polycystic ovaries appearance in ultrasound) (5), systemically healthy subjects had regular menstrual cycles, were matched for BMI, had no signs of hyperandrogenism and had undergone ultrasound exclusion for PCOS.

This present study was recorded at ClinicalTrials.gov in August 2017; its clinical trial registration number is NCT 03264846.

Dental Examinations

All participants were clinically assessed at their first visit to the Periodontology Department of Ataturk University, Faculty of Dentistry by a single calibrated clinician (AT). Ten randomly selected patients were used for calibration. The examiner evaluated the patients twice at an interval of 48 h for probing depth (PD) measurement. Calibration was accepted if measurements at baseline and at 48 h were similar, to the millimeter, at the ≥ 90 % level.

Clinical measurements were taken of periodontal parameters, including plaque index (PI) (26) , gingival index (GI) (27) , PD, clinical attachment loss (CAL) and the percentage of bleeding on probing (BOP). Periodontal clinical measurements were made at six different areas of each tooth excluding abutment teeth, third molars and dental implants using a Williams probe with Michigan markings (Hu-Friedy, Chicago, IL). PD is described as the length (in mm) from the gingival margin to the base of the

pocket; CAL is described as the length (in mm) between the cemento- enamel junction and the apical part of the pocket.

Periodontally healthy individuals were those with BOP < % 20, no attachment loss, no history of periodontal disease, and PD ≤ 3 mm (28). Periodontitis was diagnosed as two or more interproximal sites with CAL ≥ 4 mm, not on the same tooth, or two or more interproximal sites with PD ≥ 5 mm, not on the same tooth (29).

Sample Collection and Preparation

All serum and saliva samples were collected from the individuals on the second to fifth days of the spontaneous or progesterone- induced menstrual cycle, in the morning following overnight fasting.

Serum Samples. Venous blood was collected in vacutainer tubes (BD Vacutainer SSTTM II Advance Tube, Becton Dickinson, Franklin Lakes, NJ.) from the antecubital vein, via the conventional venepuncture method. The centrifuge of venous blood samples was made at 3,000 rpm for 8 min and at +40 C.

Saliva Samples. Subjects were requested not to drink fluids (except water) or chew gum for the same period, and abstention was controlled prior to biological sample collection. All saliva samples of about 4 ml were obtained by expectoration into polypropylene tubes (Vacuette, Grenier Bio-One) before taking clinical periodontal measurements. To remove cell debris, the centrifuge of saliva samples were performed immediately upon collection, at 3,000 rpm for 8 min and at 40 C).

All centrifuged serum and saliva samples were transferred into small Eppendorf tubes and stored at -800 C.

Endocan and IL-6 Levels

Serum and saliva endocan values were measured in the direction of the manufacturer's company by ELISA (Human Endothelial Cell Specific Molecule 1 ELISA Kit, Elabscience, China). Additionally, measurements of serum and saliva IL-6 levels were undertaken through the use of ELISA (Human Interleukin 6 ELISA Kit, Elabscience, China). All measurements are presented in pictogram per millilitre (pg/ml).

Statistical Analyses

Statistical analyses were undertaken using software (SPSS for Windows, Version 20 SPSS Inc., Chicago, IL, USA and Excel, Microsoft, Redmond, WA). The normality of the data distribution was analyzed via a Kolmogorov-Smirnov test and statistical analysis determined that demographic, clinical, and laboratory parameters were non-normally distributed. Differences among the groups for variables were analyzed with Kruskal-Wallis and Mann-Whitney U tests. A Spearman correlation test was used to detect any possible relationship in the clinical data and the endocan, IL-6 values. Statistical significance was considered as any p value lower than 0.05.

RESULTS

Clinical and Demographic Findings

Tables 1 and 2 presents are presented the mean values of demographical features and clinical periodontal measurements of the patients.

No statistically significant differences were observed among the groups in terms of age, BMI, WHR or HbA1c scores (p>0.05).

Plaque index values in both the PCOSP and SHP groups were statistically significantly greater than control group (SHPH). GI values in both the PCOSP and SHP groups were statistically greater than in both the PCOSPH and SHPH groups. GI values in the PCOSPH group were statistically greater than in the SHPH group. BOP (%) values in the PCOSP group were greater than those in the SHP group. The PD, CAL and BOP (%) values in both the periodontitis groups were statistically significantly greater than in both the PCOSPH and SHPH groups (p<0.05).

Table 1. Demographic Parameters of All Groups

	PCOSP (n:23)	PCOSPH (n:23)	SHP (n:21)	Control (SHPH) (n:20)
Age	26.86 (6.73)	25.65 (5.55)	26.42 (5.56)	25.10 (4.07)
BMI (kg/m^2)	22.40 (2.71)	22.38 (2.07)	22.70 (1.90)	22.12 (2.21)
WHR	0.73 (0.04)	0.73 (0.06)	0.73 (0.05)	0.72 (0.04)
WC (mm)	73.86 (6.95)	73.21 (7.39)	72.71 (8.25)	71.55 (8.25)
HbA1c (%)	4.8 (0.365)	4.8 (0.122)	4.7 (0.539)	4.7 (0.48)

PCOSP: Polycystic ovary syndrome with periodontitis, PCOSPH: Polycystic ovary syndrome but periodontally healthy, SHP: Systemically healthy with periodontitis, BMI: Body mass index, WHR: Waist hip ratio, WC: Waist circumferences, kg: kilogram, mm: millimeter. Values are presented mean±standart deviation

Laboratory Findings

While serum endocan values in the PCOSP, PCOSPH and SHP groups were statistically significantly greater than those in the SHPH group, there was no statistically significantly difference among those three groups (PCOSP, PCOSPH, SHP). The saliva endocan values in the PCOSP group were statistically greater than those in the SHPH group.

Serum IL-6 values in both the PCOSP and SHP groups were statistically significantly greater than those in the SHPH group ($p<0.05$). Saliva IL-6 values in both the periodontitis groups were statistically significantly greater than those in the SHPH group ($p<0.05$). Additionally, saliva IL-6 values in the PCOSP group were significantly greater than those in the PCOSPH group ($p<0.05$). Endocan and IL-6 values are summarized in Table 3 and Fig.1.

Correlations

Serum endocan levels had statistically positive correlations with whole clinical periodontal parameters, except for PI; serum IL-6 values, meanwhile, had significant positive correlations with PI, GI, and PD. Saliva endocan values had statistically significant positive correlations with PI and with serum and saliva IL-6 levels. Saliva IL-6 values also had statistically significant positive correlations with all clinical periodontal parameters, except for BOP, serum IL-6 and serum endocan values. Correlations are summarized in Table 4.

Table 2. Clinical Periodontal Parameters of All Groups

	PCOSP (n:23)	PCOSPH (n:23)	SHP (n:21)	Control (SHPH) (n:20)
PI	1.09 (0.45)ǂ	0.37 (0.30)	1.02 (0.40)ǂ	0.28 (0.37)
GI	0.91 (0.70)ǂ†	0.12 (0.17)ǂ	0.86 (0.56)ǂ†	0.05 (0.11)
PD (mm)	3.31 (0.56)ǂ†	1.30 (0.31)	3.15 (0.51)ǂ†	1.26 (0.38)
CAL (mm)	3.63 (0.89)ǂ†	1.31 (0.29)	3.35 (1.09)ǂ†	1.28 (0.63)
BOP(%)	68.27 (14.07)ǂ†	5.18 (4.29)	61.93 (18.17)ǂ†	2.00 (5.19)

PCOSP: Polycystic ovary syndrome with periodontitis, PCOSPH: Polycystic ovary syndrome but periodontally healthy, SHP: Systemically healthy with periodontitis, PI: Plaque index, GI: Gingival index, PD: Probing depth, CAL: Clinical attachment level, BOP: Bleeding on probing, mm: millimeter.
Values are presented mean±standart deviation
ǂ Significantly different from control group, p< 0.05
†Significantly different from PCOSPH group, p< 0.05

Table 3. Comparision of epidemiological, clinical, biochemical and radiological findings between thr groups

	PCOSP (n:23)	PCOSPH (n:23)	SHP (n:21)	Control (SHPH) (n:20)	P1	P2	P3
Serum Endocan (pg/mL)	673.8 (411.2-820.8)ǂ	684.5 (475.8-779.2)ǂ	595.5 (129.5-788.5)ǂ	406.5 (186.6-560.5)	<0.001	0.009	<0.001
Saliva Endocan (pg/mL)	27.1 (16.8- 136.9)ǂ	24.3 (16.2-167.5)	27.3 (15.9-177.3)	17.8 (15.8-173.4)	0.035		
Serum IL-6 (pg/mL)	22.6 (8.5-186.4)ǂ	14.2 (8.7-156.2)	14.8 (8.5-312.3)ǂ	11.9 (8.5-52.8)	0.007	0.0049	
Saliva IL-6 (pg/mL)	26.6 (8.5-180.5)†	12.5 (8.1-184.4)	15.6 (8.7-199.9)ǂ	11.0 (7.8-54.7)	0.001	0.026	

PCOSP: Polycystic ovary syndrome with periodontitis, PCOSPH: Polycystic ovary syndrome but periodontally healthy, SHP: Systemically healthy with periodontitis, IL-6: Interlelukin 6, pg: picogram, mm: millimeter. P1: Compared with PCOSP and Control, P2: Compared with SHP and Control, P3: PCOSPH and Control.
Values are presented median (min-max)
ǂ Significantly different from control group, p< 0.05
† Significantly different from PCOSPH group, p< 0.05

Table 4. The Correlations Between Periodontal Clinical Measurements and Laboratory Parameters

	PI		GI		PD		CAL		BOP%		Saliva IL-6		Saliva Endocan		Serum IL-6	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Serum Endocan	0.169	0.117	0.243*	0.023	0.296**	0.005	0.256*	0.017	0.269*	0.012	0.280**	0.009	-0.095	0.382	0.013	0.908
Serum IL-6	0.320**	0.003	0.276**	0.010	0.257*	0.016	0.148	0.172	0.014	0.168	0.722***	<0.001	0.235*	0.028		
Saliva Endocan	0.263*	0.014	0.144	0.183	0.130	0.229	0.050	0.645	0.036	0.739	0.280**	0.009				
Saliva IL-6	0.444***	<0.001	0.348***	0.001	0.305**	0.004	0.303**	0.004	0.204	0.058						

PCOSP: Polycystic ovary syndrome with periodontitis, PCOSPH: Polycystic ovary syndrome but periodontally healthy, SHP: Systemically healthy with periodontitis, IL-6: Interleukin 6, PI: Plaque index, GI: Gingival index, PD: Probing depth, CAL: Clinical attachment level, BOP: Bleeding on probing, mm: millimeter.
*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)
*** Correlation is significant at 0.001 level (2- tailed)
r; Spearman correlation coefficient

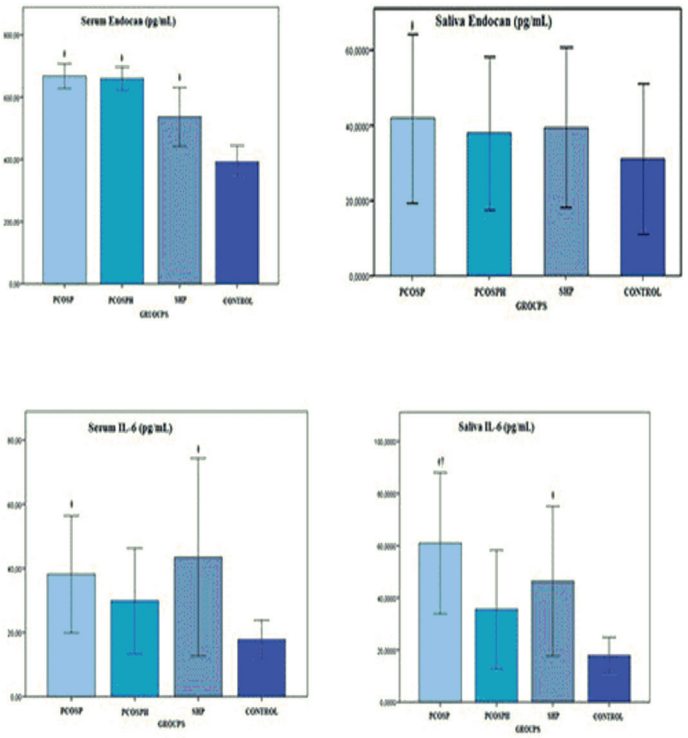

Figure 1. Endocan and IL-6 Levels in Serum and Saliva

PCOSP: Polycystic ovary syndrome with periodontitis, PCOSPH: Polycystic ovary syndrome but periodontally healthy, SHP: Systemically healthy with periodontitis, pg: Picogram, mL: Milliliter

ǂ Significantly different from control group, p< 0.05
† Significantly different from PCOSPH group, p< 0.05

DISCUSSION

The current study, comparing clinical periodontal parameters to saliva and serum levels, hypothesized that IL-6 and endocan levels, both of which are known to be released under the control of inflammatory cytokines may be linked in patients presenting with periodontitis and PCOS. Endocan and IL-6 values were observed to be greater in the PCOSP group than in healthy control individuals, this result was consistent with the current study's hypothesis.

Although there has not been clear results about the biological role of endocan in the control of inflammation, it was stated that endocan could be implicated in the regulation of the lymphocyte function related to antigen-1 (LFA-1)/ intercellular adhesion molecule-1 (ICAM-1) pathway. Therefore, it was also reported that endocan may affect both the recruitment of circulating lymphocytes to inflammatory areas and LFA-1-dependent leukocyte adhesion and activation (30). Turer et al. (31) report that serum endocan values in patients with periodontitis are statistically greater than those in their control group. In another, serum endocan values in individuals with PCOS were determined to be statistically significantly greater than those in the control group (24). Consistent with these studies' findings, the current study found serum endocan values to be significantly greater in all study groups (PCOSP, PCOSPH and SHP) than in the SHPH group. This increase in serum endocan levels may have been attributed to the fact that inflammatory events are encouraged. While the highest serum endocan level was found in the PCOSP group, there were no statistically significant differences among the study groups. Although periodontitis and PCOS work to increase serum endocan values alone, no statistically significant additional effect of increasing serum endocan levels was found in the presence both of these two diseases (in the PCOSP group).

A study reporting elevated gingival crevicular fluid endocan levels in individuals with periodontitis states that endocan may be used as a diagnostic and prognostic biomarker for periodontal disease (31). Upon evaluating

the saliva endocan results in the present study, saliva endocan values in the presence of only PCOS or only periodontitis were found to be greater than those in control group; nonetheless, this difference was not statistically significant. Saliva endocan values in the PCOSP group were statistically significantly greater than those in the control group, and the presence of both PCOS and periodontitis appeared to have a contributory effect on saliva endocan values. This finding may be expressed by the synergistic effect of these two diseases, or by the tendency of these patients to occur inflammation. Comparing periodontal clinical parameters of this present study with the study of Turer et al.,(31) it seems relatively low. The reason why there was no statistically significant difference between study groups could be the fact that periodontitis was not separated as active-passive periods in this study.

In addition to studies that have found there to be no significant relationship between periodontitis and serum IL-6 levels (32, 33), other studies report that serum IL-6 level increases with periodontitis (34, 35). In the current study, serum IL-6 values were observed to be statistically greater in the periodontitis groups, relative to the control group. Various studies report increased (1, 34), decreased (35) or no relationship (36) between serum IL-6 values in PCOS patients, compared to systemically healthy controls. In the current study, PCOS itself did not cause any statistically significant increase in serum IL-6 values, but these values were statistically significantly greater in both periodontitis groups (PCOSP, SHP). There were no statistically significant differences among the study groups. According to these results, the presence of periodontitis itself affected the inflammatory process more so than the presence of PCOS.

While some studies report saliva IL-6 values being greater in individuals with periodontitis than in healthy controls (37, 38), others report no difference between the groups (39, 40). In the current study, saliva IL-6 values were observed to be statistically significantly greater in both periodontitis groups than in the control group. Ozcaka et al.(1) state that individuals with PCOS and gingivitis had greater saliva and serum IL-6 concentrations than healthy individuals or periodontally healthy individuals with PCOS. Similarly, in the current study, saliva IL-6 values were observed to be statistically significantly greater in the PCOSP group than in the PCOSPH group. According to these findings, periodontitis had an additional effect on the increase in saliva IL-6 values in PCOS individuals. This can be explained by the contributory effect created by the combination of these diseases, both which bear an inflammatory character. Furthermore, in the current study, chronic periodontitis by itself seems to be more effective in enhancing saliva IL-6 values than PCOS by itself.

In individuals with PCOS, correlations have been reported between serum, saliva, and gingival crevicular fluid IL-6 values and TNF-α values and clinical periodontal parameters of gingivitis (1). Similarly, the current study, correlations were detected between serum and saliva IL-6 values and clinical periodontal parameters. Despite there being significant correlations between serum endocan levels and whole clinical periodontal parameters (except for PI), there was no correlation between saliva endocan and clinical periodontal parameters. This discrepancy may be related with that the individuals pay attention at their oral hygiene only at the dental appointment. Additionally, a correlation was seen between serum endocan and saliva IL-6 values. The current study also found that saliva endocan levels correlate with both saliva and serum IL-6 levels. This may be due to the regulation of endocan by inflammatory cytokines (8).

The current study's limitations contain its cross- sectional design, the absence of active/ passive periodontal pocket distinction, and the fact that no evaluations were undertaken of other inflammatory cytokines (e.g. TNF-α and IL-β) or pro-angiogenetic biomarkers such as vascular endothelial growth factor.

CONCLUSION

Within the limitations of this study, individuals presenting with both periodontitis or PCOS tended to have increased endocan and IL-6 levels compared to healthy individuals. These results indicate that increased endocan and IL-6 levels in both PCOS and periodontitis patients may promote the inflammatory events. To better explained the role of IL-6 and endocan at the pathophysiology of both PCOS and periodontitis, further studies subclassifying patients with PCOS and periodontal disease severity and/ or randomized controlled trials evaluating the effects of periodontal treatment are needed.

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Competing interests: The authors declare that they have no competing interest.

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Ethical approval: This study was approved by the Ethics Committee of Ataturk University's Faculty of Dentistry, Erzurum, Turkey (2017/14) in accordance with principles embodied in the Declaration of Helsinki of 1975 (as revised in 2000).

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