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Gingival crevicular fluid levels of visfatin in patients with chronic periodontitis and polycystic ovary syndrome

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Objective
Recently, the association between periodontal diseases and polycystic ovary syndrome (PCOS) has been established, and it has been revealed that visfatin levels increase in chronic periodontitis (CP) and PCOS. However, there was no study comparing the mean visfatin levels between advanced CP and PCOS. Therefore, the probable role of visfatin linking these diseases remains unknown, and this study was conducted to address this gap.

Methods
In this age- and weight-matched case-control study (cases with PCOS disease and controls without it), 110 female participants were divided into 4 groups based on clinical findings. The 1st group participants had both PCOS and advanced CP (n=30), 2nd group participants had only PCOS (n=25), 3rd group participants had only advanced CP (n=23), and 4th group comprised of healthy participants (n=32). Enzyme-linked immunosorbent assay was used to investigate visfatin levels in the gingival crevicular fluid (GCF). Data were collected and analyzed using Stata software (version 11).

Results
The results revealed the significant effect of both PCOS and advanced CP on visfatin levels in the GCF (P<0.05).

Conclusion
According to the results of this study, the visfatin level in the GCF could be the probable link of association between PCOS and advanced CP.

Keywords: Visfatin; Polycystic ovary syndrome; Periodontal disease

Introduction

Visfatin is an adipokine found in several tissues, such as bone marrow, liver, and muscles, and is usually secreted by the visceral fat tissue [1]. Visfatin has an insulin-like effect [2], and studies have shown that the serum visfatin levels in both obese and diabetic women were higher than that of healthy participants [3].

Periodontitis is an infectious and inflammatory disease caused by the interference of periodontal pathogens in the sub-gingival and upper gingival biofilms with the immune system [4]. Advanced chronic periodontitis (CP) can create inflammatory responses of various severities, leading to loss of alveolar bone around the tooth [5].

Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder affecting 412% of women in the reproductive age [6]. The clinical signs of PCOS, such as hirsutism and infertility, are more common in obese women than in non-obese women [7]. Additionally, obesity in PCOS can cause delayed response to various treatments, such as those with gonado-

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tropins and laparoscopy [8,9].

The association between PCOS and advanced CP was evaluated in previous studies [10-12], along with the mechanisms of this association [12]. Both CP and PCOS are related to systematic inflammation [13]. Based on the evidences, oxidative stress was noted as the common link of association between CP and PCOS [14]. Furthermore, PCOS patients are at higher risk of insulin resistance, obesity, dyslipidemia, and cardiovascular diseases [15,16], and the insulin resistance and its increase are related to systemic inflammation. These factors are also related to CP and are considered as the link between the CP and PCOS association [14]. However, the role of some factors, such as gingival levels of visfatin, has not been studied. Some studies reported that the level of visfatin in females with PCOS was more than that of others [17], while other studies reported that the visfatin levels in advanced CP patients was more than that of healthy participants [18]. The association between inflammatory diseases and visfatin levels has been evaluated [19], but no study evaluated the visfatin levels in advanced CP and PCOS patients, or compared them simultaneously with healthy controls.

The major aim of this study was to compare the levels of visfatin between non-obese PCOS females with advanced CP and healthy controls. This comparison would help us evaluate the probable role of visfatin as the link of association between advanced CP and PCOS.

Materials and methods

1. Study population

This matched case-control study included 110 non-obese females. The 4 groups of cases and controls were matched for age and weight. This study was conducted from August to November 2018.

The sample size was calculated with formula given below. Considering the maximum level for *P* as 0.5 and 95% confidence interval, the sample size was calculated as 96 participants, and 120 participants were selected to counteract the possibility of sample loss during the study.

$$n = \frac{Z^2_{1-\alpha/2} \times p(1-p)}{(0.2p)^2}$$

Among the PCOS females, 55 cases were selected, and controls without any systemic diseases were selected against each PCOS case. All clinical assessments were performed for both case and control groups. Sufficient information about the study was provided to all participants. Moreover, the demographic information such as age, sex, job, education levels, weight, and height, along with history of systemic diseases, antibiotics consumption, pregnancy, periodontal diseases treatment, smoking, and oral health were collected. Body mass index was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). All participants were evaluated for periodontal diseases and divided to the following 4 groups: 1) participants with both PCOS and advanced CP (PCOS-CP; n=30), 2) participants with PCOS but without advanced CP (PCOS-H; n=25), 3) participants without PCOS but with advanced CP (H-CP; n=23), and 4) healthy subjects (H-H; n=32).

2. Inclusion and exclusion criteria

Female sex, age of reproduction, and willingness to cooperate were the inclusion criteria for this study. Participants with a history of interfering drugs (antibiotics, oral contraceptives, antihypertensive, and diabetes drugs), infection in the last 6 months, thyroid disorders, hyperprolactinemia, diabetes, hypertension, malignancies, osteoporosis, obesity, overweight, smoking, alcohol consumption, and pregnant women were excluded.

3. Diagnosis of polycystic ovary syndrome

The Rotterdam 2003 criteria were used for PCOS diagnosis. In these criteria, participants presenting any 2 signs from the following were diagnosed with PCOS: change in polycystic ovary (ovaries were more than 10 mm or having 12 or more follicles with diameter of 2–9 mm on one or both sides); oligomenorrhea or chronic anovulation; and hyperandrogenism such as hirsutism. The hirsutism was diagnosed by the Ferimman-Gallway system and was based on the severity and presence of hair on the top lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arms, thigh, and knees. Anovulation was considered as history of 8 or fewer menstrual cycles per year or a menstrual cycle length shorter than 21 days or longer than 40 days.

4. Periodontal parameters and diagnosis of chronic periodontitis

The examination of all periodontal parameters was conducted by one periodontist. All functioning teeth were assessed,

except for partially erupted teeth. The following periodontal parameters were evaluated in this study: bleeding on probing (BOP; this index was measured by moving the probe slowly along the length of the gingival sulcus and expressed as a percentage of the bleeding sites to total probing sites), clinical attachment loss (CAL; this index was evaluated by the depth of the periodontal pocket to the cementoenamel junction), and pocket probing depth (PPD; this index was identified based on the gingival tissue involvement, space between the coronally free gingiva and tooth, and maximum penetration of probe). The William probe was used to evaluate the deepest pocket's depth. Additionally, panoramic radiographs of all patients were recorded.

The groups with periodontal diseases showed generalized moderate-to-severe CP based on the classification of the American Academy of Periodontology and the following criteria [20]: age above 35 years, more than 30% of sites with CAL ≥3 mm, and PPD ≥5 mm with BOP. Periodontal healthy individuals included those with no evidence of radiographic bone loss and attachment loss, with PPD <3 mm.

5. Collecting gingival crevicular fluid

In the advanced CP patients (H-CP and PCOS-CP groups), only one location was selected for sampling. However, in healthy participants, several locations with or without inflammation were selected to ensure the adequate collection of gingival crevicular fluid (GCF). The location of sampling was isolated with cotton rolls and air-dried slowly to prevent saliva contamination. Paper strips (Periopaper; Proflow Inc., Amityville, NY, USA) were used to collect the GCF. The amount of fluid in each strip was diagnosed using calibrated Periotron 6000 (Proflow Inc.). The strips were inserted into the crevice until mild resistance was felt and kept aside in stasis for 30 seconds, while the strips contaminated with blood or saliva were discarded. The samples were placed into microcentrifuge tubes immediately and stored at -20°C until analysis. The levels of visfatin in the GCF samples were determined using enzyme-linked immunosorbent assay (ELISA).

6. Statistical analysis

All data were collected and analyzed using Stata software (version 11; StataCorp, College Station, TX, USA). The descriptive analysis such as mean and standard deviation (SD) were used for description of data. The differences of mean visfatin levels of all groups were evaluated using the *t*-test for 2 means and analysis of variance for more than 2 means. Moreover, the Tukey honest significance difference (HSD) post hoc test was used for calculating differences of mean.

Results

The Kolmogorov-Smirnov test was used to evaluate the normality. The results of this test showed the normal distri-

Table 1. Baseline/patients' demographic data and periodontal parameters

Variables	PCOS-CP	PCOS-H	H-CP	H-H	*P*-value
Age (yr)	45.2±3.2	45.3±3.0	45.3±3.1	45.5±3.3	0.672
Weight (kg)	58.29±4.11	56.87±3.96	58.02±4.29	56.42±4.05	0.593
BMI (kg/m^2)	22.43±1.79	22.13±1.58	22.38±1.71	22.07±1.50	0.879
PPD (mm)	6.86±0.91	0.341±0.53	5.81±0.90	0.69±0.37	<0.001
CAL (mm)	3.92±0.98	1.08±0.57	3.54±0.38	1.21±0.21	<0.05
BOP (%)	33.46±1.79	3.51±0.56	31.53±1.65	2.78±0.33	<0.05
Total hirsutism score	9.91±1.1	9.34±0.9	2.20±1.8	2.91±1.1	<0.05
Regular menses (%)	2.56±0.62	2.91±0.81	89.76±6.8	90.59±7.24	<0.05
Irregular menses (%)	75.13±4.28	82.11±5.73	9.98±1.65	7.78±1.23	<0.05
Amenorrhea (%)	23.24±2.55	16.35±1.98	2.03±0.38	3.64±0.47	<0.05

Values are presented as mean±standard deviation. The *P*-values were calculated by analysis of variance test in 0.05 levels of statistical significance.

PCOS, polycystic ovary syndrome; CP, chronic periodontitis; PCOS-CP, participants with both PCOS and advanced CP; PCOS-H, participants with PCOS but without advanced CP; H-CP, participants without PCOS but with advanced CP; H-H, healthy subjects; BMI, body mass index; PPD, pocket probing depth; CAL, clinical attachment loss; BOP, bleeding on probing.

bution of all participants (P>0.05). The descriptive results revealed that the most frequency of status of disease was related to healthy participants (29.1%, n=32). The frequencies of PCOS-CP, PCOS-H, and H-CP groups were 22.7% (n=25), 27.3% (n=30), and 20.9% (n=23), respectively. The mean age of the case and control groups were 45.28 years (SD=7.98) and 45.54 years (SD=7.35), respectively.

The baseline demographic data and periodontal parameters of all patients are shown in Table 1. According to these results, the means of the CAL, BOP, and PPD values showed a significant difference between the different groups (P<0.05). According to the results of the Tukey HSD test, these differences were observed only between the PCOS-CP and H-H groups and the H-CP and H-H groups for both CAL and PPD indices. The mean difference for CAL between the PCOS-CP and H-H groups was 6.17 (P<0.05), and between the H-CP and H-H groups was 5.12 (P<0.05). The mean difference for PPD between the PCOS-CP and H-H groups was 2.17 (P<0.05), and that between the H-CP and H-H groups was 2.23 (P<0.05).

The associations between the visfatin levels and disease statuses are shown in Table 2. According to these results, there were significant differences in the mean visfatin levels between different disease groups (P<0.05). According to the results of the Tukey HSD test, these differences were observed between the PCOS-CP group and PCOS-H, H-CP, and H-H groups (P<0.05). Additionally, the mean visfatin level was significantly different between the H-CP and H-H groups (P<0.05).

Table 2. Association between visfatin levels and disease status

Variables		Visfatin levels	
		Mean (pictogram/mL)	*P*-value
Disease status	PCOS-CP	37.10±3.41	<0.05
	PCOS-H	16.54±1.96	
	H-CP	21.54±2.90	
	H-H	13.15±3.29	
Tukey HSD post hoc			
PCOS-CP	PCOS-H	20.56	<0.05
PCOS-CP	H-CP	15.56	<0.05
PCOS-CP	H-H	23.95	<0.001
PCOS-H	H-CP	5.24	0.086
PCOS-H	H-H	3.39	0.103
H-CP	H-H	8.39	0.049

Values are presented as mean±standard deviation or mean difference. The *P*-values were calculated by analysis of variance test and post hoc Tukey test in 0.05 levels of statistical significant.
PCOS, polycystic ovary syndrome; CP, chronic periodontitis; PCOS-CP, participants with both PCOS and advanced CP; PCOS-H, participants with PCOS but without advanced CP; H-CP, participants without PCOS but with advanced CP; H-H, healthy subjects; HSD, honest significance difference.

Discussion

The association between the advanced CP and some diseases was analyzed, and one of these diseases was PCOS. In a study conducted by Rahiminejad et al. [21], the prevalence of periodontal diseases in women with PCOS and healthy women was compared. The results of this study showed that the prevalence of periodontal diseases was higher in women with PCOS [21]. Other studies also evaluated the association between the PCOS and advanced CP [10,22]; however, the mechanism of this association was not clear. Some mechanisms were explained in a review study by Tanguturi and Nagarakanti [12], and one of them was the relationship of PCOS with systemic inflammation. Moreover, oxidative stress was another mechanism related to diseases such as diabetes mellitus, metabolic syndrome, and atherosclerosis. The oxidative stress biomarkers were found in the peripheral blood in both advanced CP and PCOS patients [12]. The primary aim of our study was to evaluate the significant association between visfatin levels and presence of both advanced CP and PCOS. Our results showed that the visfatin levels were higher in the PCOS-H group than in healthy participants. Moreover, the participants with both diseases had higher visfatin levels than the others, indicating the probable role of visfatin as a link of association between advanced CP and PCOS. Previous studies, such as the study by Pradeep, showed that both serum and GCF visfatin levels increased in periodontal disease [23]. The association of serum and GCF visfatin levels with periodontal diseases and type 2 diabetes mellitus was determined in another study by Pradeep et al. [24]. They concluded that both serum and GCF visfatin levels were associated with periodontal parameters and severity of type 2 diabetes [24]. Assessing the serum visfatin samples requires invasive methods, but GCF collection is an easy, acceptable, common, and non-invasive method to determine the visfatin levels in the periodontal environment.

The association between visfatin levels and PCOS has been evaluated only in few studies. A case-control study was conducted that showed that the visfatin levels were significantly higher in PCOS hirsute women as compared to others [25]. In a study conducted by Dikmen et al. [26], the serum visfatin levels between PCOS women and healthy controls were compared. The results of this study showed that the serum visfatin in non-obese PCOS women was similar to that of healthy participants. These results were not concurrent with our findings. This study revealed that the serum visfatin levels in overweight and obese PCOS women were higher than that of healthy participants [26]. Another study showed that the visfatin levels in PCOS women was higher and adiponectin levels were lower as compared to others [27]. In a case-control study conducted by Jongwutiwes et al. [28], Asian PCOS women had higher visfatin levels than healthy matched controls. Another study compared the serum visfatin levels of non-obese PCOS women with healthy control group and concluded that the visfatin levels in both PCOS and healthy participants were similar, which was not consistent with our findings [17]. However, a meta-analysis study revealed that the visfatin levels are biomarkers for PCOS [29]. The association between visfatin levels and advanced CP was evaluated in some studies. These studies showed that advanced CP patients have higher visfatin levels than others, which was consistent with our results [30].

This study had certain limitations, including the high cost of the ELISA kit, technique sensitivity of GCF collection and laboratory procedures to evaluate visfatin levels in this fluid, and use of GCF samples only because the aim of this study was to assess the role of visfatin in the link between PCOS and CP (most prevalent oral disease). Thus, it is necessary that similar studies are conducted with a larger sample size, and that the role of both serum and GCF visfatin levels associated with PCOS and CP be measured together.

In conclusion, despite the limitations of the examined sample size, this study is the first to compare visfatin levels in PCOS, CP, and healthy participants, and show the probable role of visfatin in the association between these 2 diseases. Furthermore, GCF visfatin levels could be effectively measured by comparing blood samples or gingival tissue biopsies; however, further multi center studies with larger sample sizes are needed.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study was approved by the Ethical Committee of the Arak University of Medical Sciences (No. IR.ARAKMU.REC.1397.32).

Patient consent

All participants voluntarily participated in the study and provided signed informed consent forms.

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