Clinical Rheumatology (2019) 38:3139–3143
https://doi.org/10.1007/s10067-019-04708-w

ORIGINAL ARTICLE

The comparison of visfatin levels of gingival crevicular fluid in systemic lupus erythematosus and chronic periodontitis patients with healthy subjects

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Received: 5 May 2019 / Revised: 14 June 2019 / Accepted: 22 July 2019 / Published online: 1 August 2019

Abstract

Background Visfatin is an adipokine and has a crucial role in pro-inflammatory response. The aim of this study was investigating the visfatin levels of gingival crevicular fluid (GCF) in patients with systemic lupus erythematosus (SLE) and chronic periodontitis and healthy subjects.

Materials and methods Sixty non-obese females were selected based on their clinical parameters into four groups: 15 healthy subjects (H-H), 15 systemically healthy individuals with chronic periodontitis (H-CP), 15 SLE patient with CP (SLE-CP), and 15 SLE patients without CP (SLE-H). GCF samples were collected to estimate the levels of visfatin using enzyme-linked immunosorbent assay (ELISA).

Results Investigating the amount of visfatin in the GCF showed that there is a significant difference between visfatin amount of GCF in SLE patients and chronic periodontitis (L-CP) in comparison with other groups ($P < 0.001$).

Conclusion Visfatin levels have correlated positively with all the clinical periodontal parameters and its levels in (L-CP) group are highest in comparative with other groups. This finding suggests visfatin has a possible role in association between these two inflammatory conditions.

Key Point

• *Visfatin in systemic lupus erythematosus*

Keywords Chronic periodontitis · Gingival crevicular fluid · Systemic lupus erythematosus · Visfatin

Introduction

Periodontal disease is one of the two major oral health–related problems that affect a large number of patients all over the world [1]. Periodontitis is a series of infectious and inflammatory diseases, which is the result of interactions between periodontal pathogens with host immune system response, and it can conduce progressive loss of supportive tissue around the tooth and periodontal pocket formation and eventually lead to tooth mobility or tooth loss [2].

SLE is an autoimmune disease which effects on connective tissue in different organs [3]. Therefore, due to the increasing of B cell activity, along with an increase in the production of immunoglobulin G, and immune complexes sediment, and autoantibodies, connective tissues were destroyed [4, 5]. The prevalence of SLE in women is 10 times toward men, and it usually happened between the ages of 15 and 45 [6]. Based on the comprehensive study about controlling rheumatic diseases conducted by the Rheumatology Research Center, the

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prevalence of SLE in Iran is 40 per 100,000 people [7]. However, the SLE global rate is around 20–130 per 100,000 people [8].

Periodontitis and SLE are multifactorial and inflammatory diseases. There are some mechanisms which proposed for explaining the relationship between periodontitis and SLE [9, 10]. Inflammatory cytokines such as IL-1, IL-6, and TNF-α play an important role in the relationship between periodontal disease and SLE [11].

Visfatin is an adipokine that has a crucial role in immune system functions. It is identified as pre–B cell colony-enhancing factor, which is involved in the early development of B cell growth factor and cytokine-like effects, and it also has a role in energy metabolism [12]. Visfatin is a 52-kDa protein, increasing pre–B cell colony release from lymphocytes and improving maturation of B lymphocytes [13]. Furthermore, production of interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), and IL-6 induced by visfatin has also been reported during infection and inflammation [14].

The aim of the present study was investigating the amount of visfatin in GCF of patients with systemic lupus erythematosus and chronic periodontitis beside healthy people.

Materials and method

Study population

The study was performed from August 2017 to September 2018. Sixty non-obese female patients having good cooperation were enrolled in this cross-sectional study. All periodontitis diagnoses in this study are based on generalized severe chronic periodontitis according to criteria defined by the American Academy of Periodontology Disease Classification (greater than 5-mm clinical attachment loss (CAL) in greater than 30% of sites): 15 SLE patients with healthy periodontal condition and 15 patients with SLE periodontitis as well as 15 patients with chronic periodontitis and 15 healthy subjects in terms of systemic and periodontal condition. All of SLE patients with regular follow-up under glucocorticosteroid and monthly intravenous IVCYC therapy for renal disease were selected in outpatient section of Rheumatology Department (Imam Reza clinic, Arak University of Medical Science). SLE patients fulfilled four or more of the revised American College of Rheumatology criteria for the classification of SLE [15]. Patients had their clinical activity measured according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [16], and all SLE patients had SLEDAI scores of ≥ 2. The healthy controls were recruited from volunteers and matched by age, sex, race, and smoking status, which have been considered as risk factors of SLE.

Exclusion criteria were SLE overlapping with other rheumatic diseases (except for secondary Sjögren syndrome), smoking, diabetes, treatment of periodontal disease within the last 6 months, use of orthodontic appliances, use of antibiotics within the last 3 months, need for antibiotics for infective endocarditis prophylaxis during dental procedures, chronic renal insufficiency requiring dialysis or after kidney transplantation, pregnancy or lactation, acute or chronic infectious conditions at the time of the study visit, and diagnosis of neoplastic tumor within the last 5 years. Written informed consent was obtained from those who agreed to participate voluntarily. The protocol of the study was approved by the Ethics Committee of Arak University of Medical Sciences (No. IR.ARAKMU.REC.1397.31).

Clinical periodontal indices (probing pocket depth (PPD), CAL, and bleeding on probing (BOP)) were measured using a calibrated periodontal probe (Hu-Friedy, PCP 15, North Carolina University, Chicago, IL, USA). PPD is the depth of a sulcus or periodontal pocket determined by measuring the distance from the gingival margin to the base of the sulcus or pocket. CAL is the distance between the gingival margin and the cementoenamel junction (CEJ). BOP is the bleeding that is induced by the manipulation of the gingival sulcus. Digital panoramic and full mouth peri-apical radiographs were also used to evaluate the presence and severity of periodontal disease. All clinical examinations, radiographs, and sampling site selections were performed by one examiner. In patients with SLE, medical history recorded which consist of the diagnosis time of SLE, the last active period of disease, kidney involvement, and the time of reference to the hospital, consumable drugs, and the results of their laboratory tests.

GCF sample collection

Only one site per individual was selected as a sampling site in periodontitis groups (H-CP and L-CP), whereas in the healthy groups (H-H and L-H), multiple sites (four sites per individual) with an absence of inflammation were sampled to ensure the collection of an adequate amount of GCF. Before sampling, the area was isolated with cotton rolls to prevent saliva contamination; then, the area was slightly air-dried. GCF was collected using paper strips (Periopaper; Proflow Inc., Amityville, NY, USA) and the volume of fluid in each strip was determined using a calibrated Periotron 6000 (Periotron™ 6000; Proflow Inc., Amityville, NY, USA). Strips were inserted into the crevice until mild resistance was felt, and put to one side in stasis for 30 s. Strips contaminated with blood or saliva was discarded. Samples were immediately placed into microcentrifuge tubes and stored at − 20 °C until analyzed. Levels of visfatin in GCF samples were determined using an enzyme-linked immunosorbent assay (ELISA).

Laboratory test

The samples were assayed for visfatin levels using a highly sensitive ELISA human PBEF visfatin kit (R&D Systems, Minneapolis, MN, USA; catalog number DY4335-05) according to the instructions of the manufacturer, and the assay was duplicated. All reagents were allowed to thaw to room temperature before use. Anti-visfatin antibodies (100 mL) were added to each well and incubated for 1.5 h. The solution was discarded, and the wells were washed five times with wash solution (200 mL each). Then, 100 mL of each standard, positive control, and sample was added into appropriate wells. After incubation, the solution was discarded, and wells were washed four times with wash solution (200 mL each). Prepared horseradish peroxidase-streptavidin solution (100 mL) was added to each well and incubated for 45 min at room temperature. The solution was discarded, and wells were washed five times with wash solution (200 mL each). Tetramethylbenzidine one-step substrate reagent (100 mL) was added to each well and incubated for 30 min at room temperature in the dark. Stop solution (50 mL) was added to each well. Absorbance of the substrate color reaction was read on an ELISA reader using 450 nm as primary wavelength. The total visfatin was determined in nanograms, and the calculation of the concentration in each sample was performed by dividing the amount of visfatin by the volume of sample (nanograms/milliliter).

Statistical analysis

The data were entered and analyzed using the statistical software Stata (V.11). Descriptive analyses were performed, and data are presented as mean, median, standard deviation (SD), and percentiles. The Kolmogorov-Smirnoff test was used for evaluation of the normality. One-way ANOVA was used to compare means and Tukey's post hoc test was performed to show between-group differences.

Results

The results of the Kolmogorov-Smirnoff test showed the normal distribution of all subject ($P > 0.05$). The frequencies of SLE-CP, SLE-H, H-CP, and H-H groups were 23.63% ($n = 13$), 27.27% ($n = 15$), 27.27% ($n = 15$), and 21.83% ($n = 12$) respectively. The mean age of the case and control groups was 44.31 (SD = 9.18) and 43.68 (SD = 8.17).

The baseline patient's demographic data and periodontal parameters are showed in Table 1. According to these results, the means of the CAL, BOP, and the PPD had a significant difference between the disease status ($P < 0.05$). According to the results of Tukey's HSD test, these differences were observed only between the SLE-CP and H-CP groups with the H-H group in CAL, BOP, and PPD. The mean difference for CAL between the SLE-CP and H-H groups was 5.12 ($P < 0.05$), for CAL between the H-CP and H-H groups was 4.97 ($P < 0.05$), for PPD between the SLE-CP and H-H groups was 4.9 ($P < 0.05$), for PPD between the H-CP and H-H groups was 5.06 ($P < 0.05$), for BOP between the SLE-CP and H-H groups was 28.89 ($P < 0.05$), and for BOP between the H-CP and H-H groups was 28.41 ($P < 0.05$). Also, there was no significant difference between the H-CP and SLE-CP groups.

The association between visfatin levels and disease status is showed in Table 2. According to these results, there were significant differences in mean of visfatin levels by disease status ($P < 0.001$). According to the results of Tukey's HSD test, these differences were observed between the SLE-CP group and the SLE-H, H-CP, and H-H groups ($P < 0.001$). Also, the mean of visfatin was significantly different between the H-CP and H-H groups ($P < 0.001$).

Discussion

The findings of this study suggest that visfatin levels are elevated in SLE patients especially in the SLE-CP group. Also, visfatin levels are positively correlated with disease activity. With SLE being the second most common autoinflammatory

Table 1 Baseline/Patients demographic data and periodontal parameters

Variables	SLE-CP mean (SD)	SLE-H mean (SD)	H-CP mean (SD)	H-H mean (SD)	P value
Age (year)	44.17 (3.21)	44.89 (3.07)	45.03 (3.14)	44.99 (3.26)	0.781
Weight (kg)	56.31 (3.89)	55.68 (4.01)	57.89 (3.89)	56.05 (3.88)	0.624
BMI (kg/m^2)	21.89 (1.83)	22.02 (1.17)	23.05 (1.65)	22.65 (2.01)	0.799
PPD (mm)	6.50 (1.35)	2.37 (0.90)	6.66 (1.29)	1.6 (0.73)	< 0.001
CAL (mm)	5.88 (0.84)	1.29 (0.65)	5.73 (1.16)	0.76 (0.49)	< 0.05
BOP (%)	31.23 (2.02)	2.99 (0.62)	30.75 (1.97)	2.34 (0.37)	< 0.05

The *p* values were calculated by ANOVA test in 0.05 levels of statistical significant

Table 2 Association between visfatin levels and disease status

Variables		Visfatin levels		
		Mean (pg/ml)	SD (pg/ml)	*P* value
Disease status	SLE-CP	34.11	3.59	< 0.001
	SLE-H	15.42	2.04	
	H-CP	23.15	3.17	
	H-H	12.59	2.95	
Tukey HSD post hoc		Mean difference (*P* value) (pg/ml)		
SLE-CP	SLE-H	18.69 (< 0.001)		
SLE-CP	H-CP	10.96 (< 0.001)		
SLE-CP	H-H	21.52 (< 0.001)		
SLE-H	H-CP	− 7.73 (0.086)		
SLE-H	H-H	2.83 (< 0.095)		
H-CP	H-H	10.56 (< 0.001)		

The *p* values were calculated by ANOVA test and Tukey's post hoc test in 0.05 levels of statistical significant

disease and the importance of host response to infection in development of periodontal disease, it is very important for SLE patients to have their periodontal health at check. Since periodontal disease is associated with greater systemic inflammation in patients with inflammatory conditions [17], more frequent periodontal inspections and treatments should be considered for these patients. This study also suggested that visfatin levels can be reliably measured from the GCF fluid of patients in order to study inflammatory conditions. This method is less invasive compared with currently used methods including obtaining blood samples or tissue biopsies, while other inflammatory markers have been measured in the GCF fluid of periodontitis patients [18].

Increased baseline visfatin levels compared with healthy individuals are also observed in several other studies as concluded in the meta-analysis by Lee Y et al. [19]. The same pattern can be observed in the results of this study comparing visfatin levels for GCF fluid of SLE patients with those of healthy individuals.

The finding for elevated levels of visfatin in SLE patients is in line with the findings of Mansour H.E.S et al. [20]. While they studied serum visfatin levels in healthy and obese individuals, the same pattern of higher visfatin levels in SLE patients both in healthy and obese conditions was observed there. Also the link between periodontitis and increased visfatin levels was observed in the study by Ghallab N et al. [21]. In their study, visfatin levels present in gingival tissues of patients with type 2 diabetes mellitus (another condition with inflammatory implications) with periodontal disease were compared with those in gingival tissues of healthy individuals with or without periodontitis. The visfatin levels for the diabetic group with periodontitis were greater than those for both the healthy group and the group with periodontitis.

The finding of visfatin in GCF fluid of the patients may reveal yet another link in the inflammatory cascade involved in pathogenesis of periodontitis as well as systemic effect of SLE. Further studies may include posttreatment data to trace the effects of various periodontal treatments on periodontal health and GCF visfatin levels of the patients. Concurrent measurement of systemic and GCF visfatin levels before and after treatment may also be help greater understanding of the interplay between periodontal systemic inflammation.

Conclusion

Visfatin was found to be increased in SLE patients especially in the SLE-CP group. Visfatin levels may be effectively measured from the GCF fluid of the patients which is less invasive compared with preparing blood samples or biopsies. The finding of visfatin as an inflammatory link can help us better understand the inflammatory process of SLE and periodontitis. Therefore, multicenter studies with larger populations are needed to validate the present study findings.

Acknowledgments This study was a part of the dentistry thesis that was funded by Arak University of Medical Sciences.

Compliance with ethical standards

Written informed consent was obtained from those who agreed to participate voluntarily. The protocol of the study was approved by the Ethics Committee of Arak University of Medical Sciences (No. IR.ARAKMU.REC.1397.31).

Disclosures None.

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