Research Paper: Evaluation of Visfatin levels in Gingival Crevicular Fluid in Association between Coronary Heart Disease and Chronic Periodontitis

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ABSTRACT

Introduction: The aim of this study is to evaluate the Visfatin levels in Gingival Crevicular Fluid in Association between Coronary Heart Disease and Chronic Periodontitis.

Materials and Methods: This matched case control study was conducted on 60 CHD patients and 60 controls. These participants were divided to four groups as follow: 1) patients with both CHD and CP (n=30), 2) patients with CHD and without CP (n=30), 3) patients with CP and without CHD (n=30), and 4) healthy subjects (n=30). The amount of visfatin in GCF samples of these participants were evaluated by Enzyme Linked Immunosorbent Assay (ELISA).

Results: The amount of visfatin in GCF of CHD patients with CP and CP patients were significantly higher than healthy subjects (P<0.001).

Conclusion: Visfatin levels in GCF of CHD patients with CP and CP patients can help to better understand the probable inflammatory process of the CHD and CP

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Introduction

The immune components plays important role in create Chronic Periodontitis (CP).(1) The various cytokines including Interleukin-1β and Tumor Necrosis Factor (TNF) are as determinant for periodontal diseases progression.(2) Moreover, increase circulating of IL6 levels is related to periodontal disease severity.(3) The effect of CP on systemic health is expressed in most evidences. Several studies have evaluated the association between CP and some diseases such as diabetes, metabolic syndrome, systematic lupus erythematosus, obesity, CHD and other systemic diseases.(4-8) Various studies expressed that the progression of periodontal diseases in individuals with CHD is more rapid than individuals without CHD.(9) Various inflammation factors such as visfatin, adiponectin, resistin, and leptin are produced and released by adipose tissue.(10) These factors identified in human atherosclerotic plaques.(11) The visfatin is a protein that is secreted by adipocytes, adipose tissue, and macrophages.(12-14) It is guessed that the visfatin secretes IL-1β, TNF, and IL6 during infection and inflammation.(15) The function of these inflammatory factors on CHD reported in different studies. The visfatin levels in GCF was compared between CP patients and healthy subjects in previous studies and the studies suggest that the visfatin increases the progression of CP.(16) However, the amount of visfatin was not measured in GCF of CHD patients with CP. So, the aim of present study is evaluate and compared the amount of visfatin in GCF of CHD patients with and without CP.

Materials and Methods

Study design and sampling

The present case control study was performed from January 2018 to May 2019 in Arak, Iran. The group matching method was performed in this study. This study was approved by Ethical Committee of Arak University of Medical Sciences with (No: IR.ARAKMU.REC.1397.33). All four groups in this study were matched for age, gender, weight, and Body Mass Index (BMI). BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m2).

Sixty CHD patients were selected from cardiovascular clinic and one healthy control was selected for each patient (the final sample size was 120 subjects). All participants were assessed for periodontal diseases by periodontitis. The participants were divided to four groups according to this assessment: 1) patients with both CHD and CP (n=30), 2) patients with CHD and without CP (n=30), 3) patients with CP and without CHD (n=30), and 4) healthy subjects (n=30).

Inclusion and exclusion criteria

After sign the informed consent form by participants some information such as demographic information, interdental and oral hygiene, medical history, smoking, alcohol drinking, and pregnancy were collected. Willing to participate in study was the inclusion criteria. The exclusion criteria were history of antibiotics use, infection in last six month, diabetes, cancers, overweight, obesity, smoking, alcohol drinking, and pregnancy.

Periodontal parameters and CP diagnosis

Three parameters were evaluated in this study.

1)Bleeding of Probing (BOP); for evaluating this parameter, the probe was moved slowly on gingival sulcus length. BOP was expressed as percentage of bleeding sites on probe.

2)Clinical Attachment Level (CAL); this index was assessed by periodontal pocket probing depth to Cemento Enamel Junction (CEJ).

3)Pocket Probing Depth (PPD); this parameter was assessed based on gingival tissue involvement, interval between coronally free gingival with each tooth, and maximum infiltration of probe. By Williams probe, the deepest pocket was considered as scale for each patient. Moreover, the panoramic radiography was received from each participant.

According to American Academy of Periodontology classification, the periodontal

diseases groups had the diagnosis of stage II and grade B.(17) The criteria included age above 35, more than 30% of sites with CAL above of 3mm PPD equal and above of 5mm with bleeding on probe. Periodontal healthy participants had no any evidence of bone loss and attachment loss based on radiography. Also, PPD of these participants was under 3mm.

Gingival Crevicular Fluid collecting

One location was selected for sampling in groups with chronic periodontitis. While, in healthy participants several location with or without inflammation were selected for assure of enough GCF. The sampling location was isolated with cotton rolls and for prevention of saliva contamination, slowly was dried with air. The 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 (PeriotronTM 6000 Proflow Inc., Amityville, NY, USA). The strips were inserted into the crevice until mild resistance was felt, and put to one side in stasis for 30 seconds. The contaminated strips with blood or saliva were discarded. The samples were placed into microcentrifuge

tubes immediately and stored at -20°C to be analyzed. The levels of visfatin in GCF samples were determined using ELISA.

Statistical analysis

The Kolmogorov Smirnoff test was used for evaluating the normality. Comparison of mean was conducted with independent t-test for two group means and with analysis of variance. Also, Post-Hoc Tukey test was used for significant ANOVA results. All data were collected, recorded and analyzed by stata software (v. 11).

Results

This study was performed to compare the Visfatin levels in Gingival Crevicular Fluid in Association between Coronary Heart Disease and Chronic Periodontitis. 120 subjects, were divided to four groups. Three parameters (BOP, CAL, PPD) were evaluated.

The Kolmogorov Smirnov test was used to evaluate the data normality. The results of this test was showed that the distribution of information was normal ($P>0.05$).

The baseline demographic information and periodontal parameters was showed in Table 1.

Table 1. Baseline\Patients demographic data and periodontal parameters

Variables	CHD-CP Mean (SD)	CHD-H Mean (SD)	H-CP Mean (SD)	H-H Mean (SD)	P-value
Age (year)	53.14 (3.21)	52.33 (3.07)	50.27 (3.14)	53.24 (3.26)	0.564
Weight (kg)	67.29 (4.11)	69.87 (3.96)	68.02 (4.29)	67.42 (4.05)	0.657
BMI (kg/m2)	22.73 (1.79)	22.81 (1.58)	23.01 (1.71)	22.91 (1.50)	0.544
PPD (mm)	7.31 (0.91)	0.71 (0.53)	6.73 (0.90)	0.84 (0.37)	<0.001
CAL (mm)	4.01 (0.98)	0.55 (0.57)	4.86 (0.38)	0.64 (0.21)	<0.05
BOP (%)	41.56 (1.79)	2.91 (0.56)	38.5 (1.65)	3.18 (0.33)	<0.05

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

According to this results the significant difference were showed between BOP, PPD, and CAL in various disease status. According to the results of HSD Tukey test, these differences were observed only between CHD-CP and H-CP groups with H-H group in CAL, BOP, and PPD. The mean difference for CAL between the

CHD-CP and H-H was 2.90 ($P<0.05$), for CAL between the H-CP and H-H was 1.75 ($P<0.05$), for PPD between CHD-CP and H-H was 6.87 ($P<0.05$), for PPD between H-CP and H-H was 4.19 ($P<0.05$), for BOP between CHD-CP and H-H was 38.38 ($P<0.05$), and for BOP between H-CP and H-H was 35.32 ($P<0.05$). Also, there

was no significant difference between H-CP and CHD-CP groups.

The mean of the visfatin amounts in different status of diseases were showed in Table 2.

Table 2. Association between visfatin levels and disease status

| Variables | Visfatin levels | | |
	Mean (pictogram/ml)	SD (pictogram/ml)	P value
Disease status	CHD-CP	35.54	4.01	
	CHD-H	14.12	1.82	<0.05
	H-CP	24.71	3.12	
	H-H	12.05	2.79	

The p-values were calculated by ANOVA test and Post-Hoc Tukey test in 0.05 levels of statistical significant

According to these results there were a significant differences in mean of visfatin amount by disease status (P<0.001). The results of HSD Tukey test was showed that these differences were observed between CHD-CP with CHD-H, H-CP, and H-H groups (P<0.001). Also, the mean of visfatin was significantly different between H-CP and H-H groups (P<0.001). The mean differences of disease groups were showed in figure 1.
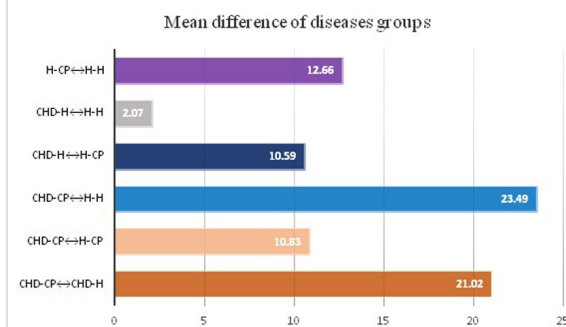

Figure 1. Mean differences of diseases groups from Post-Hoc analysis

Discussion

The aim of this case control study was to evaluate the Visfatin levels in Gingival Crevicular Fluid in Association between Coronary Heart Disease and Chronic Periodontitis.

Visfatin secreted by visceral adipose tissue routinely.(18) and also produced by neutrophils and macrophages.(19) The existence of visfatin amounts in atherosclerotic plaques is identified recently.(20) Visfatin is increased in coronary heart disease, type 2 diabetes mellitus, inflammatory bowel disease, and acute lung injuries.(21-24)present study comprised four groups (CHD with CP, only CHD, only CP, and healthy subject). This groups helped to evaluate the role of visfatin in periodontal diseases and CHD and the relationship between these two diseases. The effect of age, gender, and BMI on visfatin amounts was reduced through matching. The present study was showed that the visfatin amounts in GCF was increased in CHD patients with CP. Also, visfatin amounts was increased with severity of periodontal diseases. This finding was in line with previous studies. The increase of visfatin amounts in periodontal diseases were reported in some studies.(5,16) Also, increase of visfatin in CHD compared with healthy subjects was seen in present study that was similar with previous studies. A study was expressed that there was interaction between periodontal diseases, visfatin amounts, and other systemic diseases.(5) The role of Adipokines in increasing CHD is identified through two ways (direct and indirect), Direct way with effect on paracrine and indirect way through increasing plasma concentration.(25) In a Chen's study, the association between the periodontal parameters and the risk of coronary artery disease in patients with diabetes type 2 was evaluated. In this study visfatin levels, leptin, and the CP reactive protein in peripheral blood were assessed. The results of this study showed that visfatin levels in peripheral blood at the CHD patients and diabetes type 2 were higher than others which were consistent with our results. It should be noted that the this study was conducted in visfatin of serum, but present study was conducted in visfatin of GCF.(26) Another study associated the relationship between the Adiponectins and the serum leptins with metabolic syndrome and the CHD. This study illustrated that the Adiponectins levels in nonsmoker's participants were higher than others, however, the CHD patients had lower Adiponectins levels than healthy participants.(27) In a study conducted by Pardeep et al. the serum and GCF visfatin levels in periodontal disease

were investigated. Some participants had diabetes and others were healthy. This study illustrated that visfatin levels in both GCF and serum groups in patients with diabetes increased.(28)

The finding of visfatin in GCF of the patients may reveal yet another link in the inflammatory cascade involved in pathogenesis of periodontitis as well as systemic effect of CHD. Further studies may include post treatment data to trace the effects of different 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

The amounts of visfatin in GCF of the CHD patients both with and without the CP increased. The finding of visfatin levels in these two diseases can help to better understand the probable inflammatory process of the CHD and periodontitis. However, the further studies with larger sample size to confirm the results of our study are needed.

Authors'contributions

Mandana Ebrahimi Fakhari: Conceptualization, Methodology, Writing - Review & Editing **Amineh Ghazanavi:** Resources, Investigation, Visualization **Mojtaba Bayani:** Data curation, Writing - Original Draft **Mehdi Anvari:** Project administration, Supervision, Funding acquisition

Conflict of Interests

The authors declare no conflict of interest.

Ethical declarations

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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