Evaluation of periostin levels in gingival crevicular fluid in association between coronary heart disease and chronic periodontitis

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ABSTRACT

Background: Periostin is a protein, which is essential for periodontal tissue integrity, development and maturity. The aim of this study was to evaluate the role of gingival crevicular fluid (GCF) periostin levels in the association between coronary heart disease (CHD) and chronic periodontitis (CP).

Materials and Methods: This matched case–control study was conducted on 116 participants. The participants were matched for age, gender, and body mass index and divided into four groups as follows: (1) 29 patients with CHD and sever CP (CHD-CP), (2) 29 patients with CHD and without CP (CHD-H), (3) 29 patients without CHD and with sever CP (H-CP), and (4) 29 healthy participants (H-H). The GCF periostin was collected and evaluated using the enzyme-linked immunosorbent assay (ELISA). Finally, the data were analyzed by analysis of variance using the stata software. Significance was assigned at $P<0.05$.

Results: The results showed that there was a significant difference in the GCF periostin levels in the four groups ($P<0.05$). Moreover, according to the results of the Bonferroni's test, differences in the mean periostin levels were significant ($P<0.001$) between CHD-CP and CHD-H, CHD-CP and H-CP, CHD-CP and H-H, and also between H-CP and H-H.

Conclusion: The periostin levels reduced in the CHD patients, especially in the CHD-CP group. The findings reveal a probable role of periostin in the association between CHD and CP.

Key Words: Chronic periodontitis, coronary disease, gingival crevicular fluid, POSTN protein

INTRODUCTION

Coronary heart disease (CHD) is a prevalent disease and also is recognized as a main cause of death worldwide\textsuperscript{[1]}. There are many risk factors for CHD such as smoking\textsuperscript{[2]}, hypertension\textsuperscript{[3]}, obesity\textsuperscript{[4]}, and diabetes mellitus\textsuperscript{[5]}. In addition, the role of inflammation in CHD was determined in some researches\textsuperscript{[6]}.

Chronic periodontitis (CP) is an inflammatory disease that has effects on tooth supportive tissues. The association between CP and some systemic diseases such as multiple sclerosis\textsuperscript{[7]}, lupus erythematos\textsuperscript{[8]}, oral cancer\textsuperscript{[9]}, polycystic ovary syndrome\textsuperscript{[10]}, diabetes\textsuperscript{[11]}, and CHD\textsuperscript{[12]} are also indicated. With respect to evidences, CP progression is faster in patients with CHD in comparison with healthy individuals\textsuperscript{[13]}.

Adipose tissues are producing some inflammatory factors like adiponectin, resistin, leptin, and also cytokines such as tumor necrosis factor (TNF)-$\beta$,
Interleukin (IL) 6. These cytokines play a role in immune response to inflammation.[14]

Periostin is a matricellular protein belonging to the Fasciclin-1 family and is expressed in collagenous tissues and also fiber connective tissues such as heart valves, tendons, cornea, and periodontal ligament.[15] Periostin is an essential protein for tissue integrity, development, and maturity.[16] It is believed that periostin plays a key role as a modulator of periodontal ligament hemostasis.[17] Periostin expression is induced by the use of tumor growth factor (TGF)-β. In addition, periostin expression in wound healing is also indicated.[15]

In earlier studies, the association between CHD and CP was evaluated.[12,18,19] In addition, we compared the gingival crevicular fluid (GCF) periostin in CHD patients with or without CP with healthy participants, in order to evaluate the probable role of periostin in the association between CHD and CP.

**MATERIALS AND METHODS**

**Inclusion and exclusion criteria**

This matched case–control study was conducted on 116 participants. The evaluation of periodontitis was performed for all participants using periodontal parameters such as pocket probing depth (PPD), clinical attachment level (CAL), and bleeding on probing (BOP). Inclusion criteria for CP were inflammatory signs like inflations and red color in gingival margin, PPD ≥5 mm, CAL ≥5 mm and confirmation of periodontal attachment loss using full mouth intraoral periapical radiography and clinical assessment. Inclusion criteria for participants without CP were PPD ≤3 mm and no signs of attachment loss.[20-22] Participants with CHD were entered into the study based on the diagnostic criteria of CHD. All patients with CHD background were examined by a physician, and also by applying following criteria: History of ischemic heart disease, treatment with diltiazem, nifedipine, beta-blockers, and coumarin anticoagulant. In addition, hypertension and diabetes mellitus were determined using the similar method.[23] More evaluations, including total serum cholesterol, triglyceride, high-dose lipoprotein (HDL), and C-reactive protein (CRP) were assessed using registered data in the patients’ medical files. Classification of these biomarkers were accomplished in terms of scientific statement of the American Heart Association (AHA) and American College of Cardiology (ACC).[24] Based on these criteria, participants were categorized to these groups: (1) 29 patients with CHD and sever CP (CHD-CP), (2) 29 patients with CHD and without CP (CHD-H), (3) 29 participants without CHD and with sever CP (H-CP), and (4) 29 healthy participants (H-H). Sever periodontitis is characterized by CAL and PPD higher than 5 mm.[25]

Patients with aggressive periodontitis were excluded from the study. Systemic diseases, including diabetes mellitus, cancers, acquired immunodeficiency syndrome, multiple sclerosis, treatment with a high-dose steroid, radiotherapy, allergy to any drug, pregnancy, alcohol drinking, and cigarette smoking were exclusion criteria in this study. In addition, all participants had no history of periodontal treatment in the past 6 months.

**Study population**

This matched case–control study was approved by the Ethical Committee of Arak University of Medical Sciences with No: IR.ARAKMU.REC.1397.28. In addition, all 116 participants filled out and signed the written informed consent form. Participants in all the study groups were matched for age, gender, and body mass index (BMI).

**Clinical evaluation**

In this study, three periodontal parameters, namely BOP, CAL, and PPD were evaluated. To assess BOP, the probe was slowly moved over the length of gingival sulcus. BOP was expressed as a percentage of sites that bled on probing. The CAL was examined by probing from periodontal packet depth to cement-enamel junction. The PPD was assessed with respect to gingival tissue involvement, interval between coronally free gingival with each tooth, and probe maximum infiltration. The deepest pocket was considered as a scale for each patient using the Williams probe (Hu-Friedy, Chicago, IL, USA). Moreover, digital panoramic radiograph was obtained for each participant.

**Collecting gingival crevicular fluid**

Only one site per individual was selected as a sampling site in periodontitis groups (H-CP and CHD-CP), whereas multiple sites (four sites per individual) with and without inflammation were sampled in healthy groups (H-H, CHD-H) to ensure about collecting adequate amount of GCF. Before sampling, the area was isolated with cotton rolls to prevent saliva contamination; after that, the area was slightly air-dried. The GCF was gathered by the use...
of paper strips (Periopaper, Proflow Inc., Amityville, NY, USA), and also 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 after that they were put to one side in stasis for 30 s. Those strips that were contaminated with blood or saliva were discarded. Samples were immediately placed into microcentrifuge tubes and stored at −20°C until the time of analyzing. The periostin levels in the GCF samples were determined using ELISA.

**Statistical analysis**

Data normality was assessed using the Kolmogorov–Smirnov test. Descriptive statistics, including frequency, percent, mean, and standard deviation (SD) were used to describe and analyze the data. In addition, the analysis of variance (ANOVA) was used for comparison of means between the groups. Therefore, the Bonferroni’s multiple comparison test was utilized to evaluate the mean difference between the groups. All analyses were accomplished using STATA software (v. 11) at a 95% confidence interval (CI).

**RESULTS**

Table 1 shows the baseline information of the study participants. Based on these results, sex ratio (men/women) in CHD-CP, CHD-H, H-CP, and H-H were 14/15, 13/16, 15/14, and 14/15, respectively. The highest age, weight, and BMI means were related to the CHD-H group and equal to 46.56 ± 5.83, 79.25 ± 7.25, and 24.18 ± 4.01, respectively. There was no significant difference between the gender ratio and mean age and also between weight and BMI in the four groups.

Results of the Kolmogorov–Smirnov was not significant \( (P = 0.147) \) and indicated that data had a normal distribution. The mean ± SD of PPD, CAL, and BOP are presented in Figures 1-3, respectively. With respect to these results, the highest mean values for PPD, CAL, and BOP were 6.24 ± 0.91, 4.02 ± 0.68, and 38.24 ± 7.23, respectively and all of them were associated to CHD-CP. The result of ANOVA showed that the mean difference between the groups was significant \( (P < 0.001) \). Results of the Bonferroni’s test demonstrated that mean differences in CAL, PPD, and BOP were significant between CHD-CP and CHD-H and H-H groups \( (P < 0.001) \). In addition, mean differences between H-CP and CHD-H and H-H groups were significant \( (P < 0.001) \). However, the difference between CHD-H and H-H, and also the difference between CHD-CP and H-CP were not significant in any parameters \( (P > 0.05) \).

Table 2 shows the means of periostin levels in the CHD-CP, CHD-H, H-CP and H-H groups. According to these results, mean differences of periostin was significant among the four groups \( (P < 0.05) \). The

**Table 1: Baseline information of the study participants**

| Variables      | CHD-CP | CHD-H | H-CP | H-H | \( P \) |
|----------------|--------|-------|------|-----|--------|
| Total          | 29     | 29    | 29   | 29  |        |
| Gender (n)     |        |       |      |     |        |
| Male/female    | 14/15  | 13/16 | 15/14| 14/15| 0.233  |
| Age (years)    |        |       |      |     |        |
| Mean±SD        | 45.28±6.17 | 46.56±5.83 | 45.11±5.92 | 46.27±6.01 | 0.252  |
| Minimum/maximum| 35/59  | 36/58 | 33/57| 37/59|        |
| Weight (kg)    |        |       |      |     |        |
| Mean±SD        | 74.77±7.93 | 79.25±7.25 | 70.29±6.99 | 73.64±7.07 | 0.117  |
| Minimum/maximum| 61/81  | 63/85 | 58/77| 59/80|        |
| BMI (kg/m²)    |        |       |      |     |        |
| Mean±SD        | 23.89±3.67 | 24.18±4.01 | 22.98±3.11 | 23.54±3.55 | 0.544  |

The \( P \) values were calculated by ANOVA test in 0.05 levels of statistical significant. SD: Standard deviation; BMI: Body mass index; CHD: Coronary heart disease; CP: Chronic periodontitis.

**Figure 1**: Mean of pocket probing depth is shown. Data are presented as means.
mean differences of the four groups of participants are presented in Figure 4. The highest mean difference of periostin was associated with difference between CHD-CP and H-H groups. Furthermore, according to the results of the Bonferroni’s test, differences in the mean periostin levels were significant ($P < 0.001$) between CHD-CP and CHD-H, CHD-CP and H-CP, CHD-CP and H-H, CHD-H and H-H and also between H-CP and H-H.

**DISCUSSION**

The findings of this study showed that there was a difference in the mean values of PPD, CAL, and BOP between the four groups. According to proven role of periodontal parameters in CP\cite{26} and association between CP and CHD,\cite{12,18,19} these values should definitely be higher in CP groups (CHD-CP and H-CP). However, a remarkable point emerged in our study was the increasing of these parameters in the CHD-H groups than healthy participants. However, this result was not significant. In addition, the means of these indices in the CHD-CP were higher than those in the H-CP, although this result was not significant. This result shows the role of CHD in periodontal parameters and progression of CP, which is consistent with the results of the Bokhari’s study.\cite{27} In addition, the results of Mummolo et al. study revealed that development of periodontal diseases in the CHD patients was faster and more aggressive than non-CHD participants.\cite{28}

The main finding of this study was a difference in the mean levels of periostin between the various groups. The finding shows that mean difference was significant between CHD-CP with CHD-H, H-CP, and H-H groups. Furthermore, there was a significant difference in the mean periostin levels between CHD-H and H-CP groups with H-H group that represents the role of both diseases to decrease the amount of periostin. This issue indicates a probable linkage role in association between CHD and CP. With respect to this study findings, the periostin levels had a reduction in the CHD patients, especially in those CHD patients with CP. In addition, the mean differences of periostin between the CHD-H group and healthy group were as same as mean differences of periostin between the CP-H group and healthy group, which indicates an equivalent effect of both diseases on periostin levels. This study is the first

**Table 2: Periostin levels in four disease groups**

| Variables        | Periostin levels Mean±SD (picogram/ml) | $P$  |
|------------------|----------------------------------------|------|
| Disease status   |                                        |      |
| CHD-CP           | 1.34±0.53                              | <0.05|
| CHD-H            | 4.18±1.17                              |      |
| H-CP             | 3.72±1.05                              |      |
| H-H              | 9.83±3.11                              |      |

The $P$ values were calculated by ANOVA test in 0.05 levels of statistical significant. SD: Standard deviation; CHD: Coronary heart disease; CP: Chronic periodontitis
study that evaluated the GCF periostin as a biomarker in the CHD patients with and without CP.

Some studies evaluated decreased levels of periostin in the CP patients. However, these studies evaluated the serum or saliva samples. In Kumaresan’s study, the GCF levels of periostin was assessed in the CP patients who received nonsurgical treatment for their periodontal diseases.\(^\text{[29]}\) They determined that periostin levels were significantly lower in the CP patients in comparison with healthy participants, and this finding is consistent with our findings. In another study, GCF periostin was evaluated in CP and aggressive periodontitis patients, and it was concluded that the GCF periostin was lower in CP patients in comparison with healthy participants.\(^\text{[30]}\)

Periostin reduction in the CHD patients in this study is in agreement with Ling et al. study.\(^\text{[31]}\) However, the serum levels of periostin were evaluated in patients with myocardial infraction.\(^\text{[32]}\) They determined that periostin was positively associated with inflammatory cytokines TNF-\(\alpha\) and IL-6.\(^\text{[33]}\) Their results for diabetic patients were in agreement with our findings. In a study accomplished by Luo et al., Priostin of plasma was elevated in diabetic patients, especially in obese diabetic patients. They declared that, the plasma periostin was positively associated with inflammatory cytokines TNF-\(\alpha\) and IL-6.\(^\text{[34]}\) Their results for diabetes as a systemic disease were not in agreement with our findings. A study was also mentioned that this difference in expression of periostin between systemic diseases and periodontal diseases could be associated with completely different environmental condition in these diseases. Furthermore, the gingival fibroblast is separated phenotypically and maintained the inflammation, and plays a significant role in periostin expression.\(^\text{[32]}\)

This study examined the linkage role of periostin levels in the association between CHD and CP. More researches with larger sample sizes are recommended. In addition, an interventional study that can measure the periostin levels before and after CP treatment is suggested for confirming the role of periostin in the association between CHD and CP.

CONCLUSION

The periostin levels were decreased in patients with CHD, especially in the CHD-CP group. We found a probable linkage role of periostin in the association between CHD and CP. Periostin probably is a potential biomarker for the CP diagnosis in CHD patients, and also is effective for CP prevention. However, more studies are required to confirm this role.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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