

Evaluation of cardiac biomarkers in smokers and non-smokers with chronic periodontitis

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ABSTRACT

Objectives: The present study aimed to assess the levels of the cardiac biomarkers in smokers versus non-smokers chronic periodontitis (CP) patients and periodontally healthy subjects, also to correlate cardiac biomarkers level with the severity of CP.

Methods: Eighty selected individuals were divided into three groups: Smokers CP (28), non-smokers CP (32), and healthy control subjects (20). The CP evaluated clinically by plaque index (PI), bleeding index (BI), clinical attachment loss (CAL), and pocket depth (PD). Serum levels of cardiac biomarkers were assayed for the following: Aspartate transaminase (AST), alanine transaminase (ALT), troponin-I (Tr-I), creatinine kinase (CK), and lactate dehydrogenase (LDH).

Results: The statistical analysis of the investigated biomarkers showed that ALT and AST significantly increased in smokers versus non-smokers CP patients ($P \leq 0.05$). In addition, the other biomarkers (Tr-I, CK, and LDH) showed a non-significant increase in smokers compared with non-smokers periodontitis patients ($P \geq 0.05$). Furthermore, the mean values of all biomarkers, Tr-I, ALT, AST, LDH, and CK, expressed highly significant comparisons ($P \leq 0.01$) in smokers and non-smokers CP patients against healthy subjects.

Conclusion: The present study suggested that the cardiac biomarkers affected by CP and worse aggravated by the presence of smoking that could play a bidirectional effect on periodontitis and cardiovascular (CV) conditions. Further researches are needed to elucidate the efficacy of periodontal treatment and serum cardiac biomarkers in systemically healthy and diseased CV patients.

Keywords: Alanine transaminase, aspartate transaminase, creatinine kinase, lactate dehydrogenase, smoking, troponin-I

Introduction

Chronic periodontitis (CP) is a wide-spreading disease caused mainly by anaerobic Gram-negative bacteria in susceptible host. This interaction causes damage to periodontium including loss of attachment and alveolar bone resorption.^[1,2]

Smoking has a negative effect on all human body systems and considered a common risk factor which precipitating many causative diseases of death. The impact of smoking on the cardiovascular (CV) system is mediated by several pathophysiological pathways, including vasomotor effects, irritation, smooth muscle expansion, and platelet dysfunction. Moreover, smoking alters oral microflora in the human being and harms periodontium by several immunological challenges. The complexity of the problem is the slowly increasing severity of periodontal disease and a long-term follow-up is needed to monitor the progression of the disease.^[3]

There is a relationship between periodontal diseases and CV diseases where the treatment of periodontal diseases may be considered now as one of the preventive measures of CV diseases. A few studies have been done using cohort, cross-sectional, or case-control studies with fluctuating results regarding the quality of this relationship.^[4] The pathogenesis of periodontal diseases could explain the relationship between CV disease and CP, where the later arise from bacteria that interact with the host immunity. This challenge releases different cytokines and inflammatory mediators that could lead to systemic inflammation.^[5]

The linking between CP and coronary heart diseases (CHD) has not fully understood. However, this relation complicated by other factors. In light of the fact that not just the hereditary factor assumes a role, but ecological components and other risk factors are also included. In late decades, investigations have gone to concentrate on the relationship between CP and CHD.^[6]

CV biomarkers can assess the heart work by distinguishing the blood elements which related to myocardial infarction. The following biomarkers (ALT, AST, LDH, CK, and Tr-I) and high-sensitive C-reactive protein were significantly increased in CP patients than healthy controls.^[7,8] A study concluded that smoking could be harmful to the CV system, apparently by elevating ventricular wall stress and leading to subclinical myocardial injury. Furthermore, there is a significant relationship between high-sensitive troponins (hs-troponins) and CV disease.^[9]

Although some studies did not affirm such relation between periodontal diseases and CV diseases,^[10,11] a few investigators proposed that the danger of CHD might be improved by periodontal treatment.^[12,13]

Regarding the previous knowledge, this study designed to clarify such a relationship between periodontal diseases and CV diseases by evaluating the cardiac biomarkers in smokers versus non-smokers CP patients and to correlate the level of biomarkers with the severity of CP.

Materials and Methods

Patients

The individuals of this study were recruited from the periodontology clinics at the Teaching Dental Hospital, Faculty of Dentistry, Umm Al-Qura University. The UQUDENT review board was gained (IRB number 111-18), and the consent forms were obtained from all participants before management.

All participants were interviewed to taken the medical and demographic information. A total of 80 patients ranged from 30–55 years old, and they were divided into three groups: Group 1 – smoker CP (28 patients), Group 2 – non-smoker CP (32 patients), and Group 3 – healthy non-smokers control group (20 subjects).

The inclusion criteria of the selected individuals were systemically healthy subjects, either smokers or non-smokers, and Stage II and Stage III (Grade B or C) periodontitis patients (CAL \geq 3 mm).^[14] The exclusion criteria were as follows: Patients have received periodontal treatment in the past 6 months, medically and immunocompromised patients, pregnant and lactating women.

Blood samples

Non-fasting venous blood samples were collected from all participants in teaching dental hospital, then centrifuged at room temperature at 2000×g for 10 min then stored at -80°C until used. The biochemical assay was processed in medical laboratory at Al-Noor Specialized Hospital, Makkah, Saudi Arabia.

Periodontal examination

The periodontal status of all patients was measured using of the following clinical parameters: PI and BI^[15,16] and PD and CAL^[17] for all smokers and non-smokers CP patients.

Laboratory investigations

The selected cardiac biomarkers were investigated according to the required methodology for each one biochemically, except using an enzyme-linked immunosorbent assay technique for measuring the serum levels of Tr-I according to the manufacturer's directions, using Dimension analyzer (Siemens Healthcare, Erlangen, Germany).

The biochemical assaying of ALT, AST, LDH, and CK was determined in serum using of UV/Vis Spectrophotometer (Humastar 200, Automatic Biochemistry Analyzer, Wiesbaden, Germany). The levels of serum (AST and ALT) were determined by the method of Reitman,^[18] LDH by the technique of Amador,^[19] and CK by the method of Tanzer.^[20]

Statistical analysis

The statistical investigation was done using of the SPSS (Statistical Package for the Social Sciences) program version-22. The collected data represented in the following descriptive statistics data mean, standard deviation, minimum, and maximum. The paired and unpaired *t*-test was used to compare the mean values between groups and intergroups; also, Pearson's correlation was used to correlate periodontal parameters against to the evaluated biomarkers. The significance was determined when $P \leq 0.05$. The statistical data were charted and graphed by Microsoft Word version 2016.

Results

Eighty individuals were participated in this study, their mean age was 37.04 years (male 72.5% and female 27.5%). They were categorized into the following groups: Group 1 (smoker CP patients, 35%), Group 2 (non-smoker CP patients, 40%), and Group 3 (healthy subjects, 25%) (Table 1).

Evaluation of clinical parameters

The descriptive statistics of clinical parameters (BI, PI, PD, and CAL) are demonstrated in Table 2. The data were expressed as follows: Mean \pm SD, Group 1 – smoker periodontitis (39.81 \pm 19.08, 45.49 \pm 14.89, 2.76 \pm 0.51, and 3.84 \pm 1.09, respectively) and Group 2 – non-smoker (49.92 \pm 17.46, 52.49 \pm 14.30, 2.81 \pm 0.47, and 4.07 \pm 1.27, respectively). The statistical

Table 1: Demographic data of participants

Age	Mean (37.04 years), SD (9.778)
Gender	Male (72.5%), female (27.5%)
Smoking status	Smoker (35%), non-smoker periodontitis (40%), non-smoker healthy (25%)

analysis showed a significant reduction in BI and PI in smokers compared to non-smokers CP periodontitis patient ($P \leq 0.05$) (Figure 1), while there were no significant differences in PD and CAL ($P \geq 0.05$) (Figures 1 and 2).

Evaluation of cardiac biomarkers

The descriptive statistical data, mean \pm SD, are expressed in Table 3. The statistical comparisons for mean values of ALT and AST showed a significant increase in smokers versus non-smokers CP patients ($P \leq 0.05$). Whereas, non-significant changes ($P \geq 0.05$) when comparing the other cardiac biomarkers (Tr-I, CK, and LDH). Furthermore, the comparisons of smokers and non-smokers CP patients to healthy control subjects, the statistical analysis demonstrated a highly significant increase ($P \leq 0.01$) for all examined cardiac biomarkers: Tr-I, ALT, AST, LDH, and CK (Table 3) (Figures 3 and 4).

Correlation of cardiac biomarkers with clinical parameters

A correlation was significant in Tr-I test in relation to clinical attachment loss in non-smoker CP patients ($P \leq 0.05$, Pearson's correlation; $r = 0.443$), while there was no significant increase in other parameters. The correlation of blood analyses with clinical parameters in smokers and non-smokers CP patients presented in Tables 4 and 5.

Table 2: Statistical comparisons of *t*-test for all clinical parameters

		Comparisons of mean \pm SD		<i>t</i> -test	<i>P</i> value
BI	G1 (39.81 \pm 19.08)	G2 (49.90 \pm 17.44)		-2.139	0.037*
PI	G1 (45.48 \pm 14.88)	G2 (53.60 \pm 16.46)		-1.990	0.05*
PD	G1 (2.76 \pm 0.51)	G2 (2.81 \pm 0.47)		-0.426	0.672
CAL	G1 (3.84 \pm 1.09)	G2 (4.07 \pm 1.27)		-0.729	0.469

BI: Bleeding index, PI: Plaque index, PD: Pocket depth, CAL: Clinical attachment loss, G1: Smokers, G2: Non-smokers. *Significant at *P* value level (≤ 0.05).

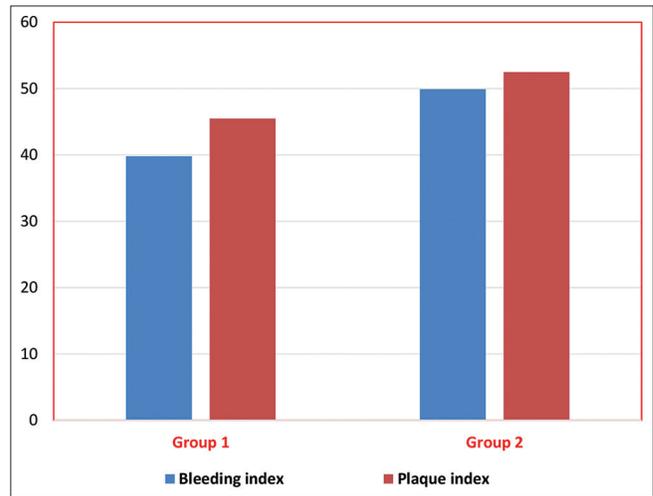


Figure 1: The bleeding and plaque indices of smokers and non-smokers CP patients. G1: Smokers CP, G2: Non-smokers CP

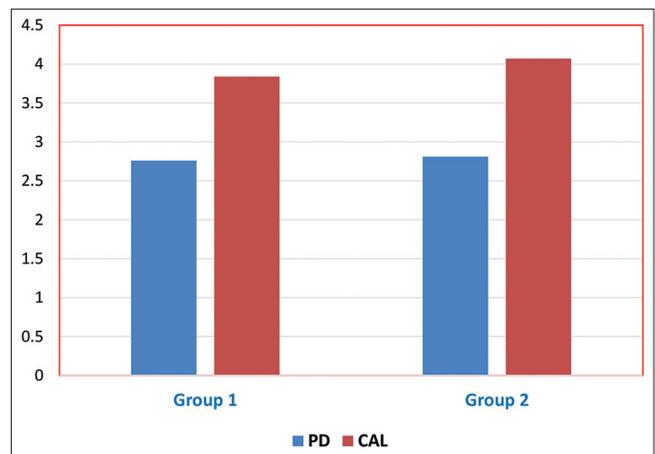


Figure 2: The pocket depth and clinical attachment loss of smokers and non smokers CP patients.

Table 3: Demonstrate statistical comparison of *t*-test for blood analyses

	Normal range	Comparisons of mean \pm SD		<i>t</i> -test	<i>P</i> value
Troponin-I	<0.2	G1 (0.231 \pm 0.022)	G2 (0.224 \pm 0.026)	1.101	0.275
		G1 (0.231 \pm 0.022)	G3 (0.086 \pm 0.035)	17.269	<0.001*
		G2 (0.224 \pm 0.026)	G3 (0.086 \pm 0.035)	15.905	<0.001*
ALT	12.0–78.0	G1 (73.82 \pm 5.47)	G2 (71.40 \pm 3.79)	2.006	0.050*
		G1 (73.82 \pm 5.47)	G3 (41.95 \pm 9.39)	15.860	<0.001*
		G2 (71.40 \pm 3.79)	G3 (41.95 \pm 9.39)	14.805	<0.001*
AST	15–37	G1 (34.46 \pm 2.16)	G2 (33.28 \pm 2.26)	2.061	0.044*
		G1 (34.46 \pm 2.16)	G3 (24.50 \pm 3.59)	11.969	<0.001*
		G2 (33.28 \pm 2.26)	G3 (24.50 \pm 3.59)	10.845	<0.001*
LDH	100–190	G1 (184.92 \pm 4.15)	G2 (182.56 \pm 7.33)	1.507	0.137
		G1 (184.92 \pm 4.15)	G3 (132.75 \pm 16.00)	16.551	<0.001*
		G2 (182.56 \pm 7.33)	G3 (132.75 \pm 16.00)	15.285	<0.001*
CK	24–170	G1 (163.60.92 \pm 6.86)	G2 (160.90 \pm 16.38)	0.812	0.420
		G1 (163.60.92 \pm 6.86)	G3 (75.75 \pm 22.34)	19.623	<0.001*
		G2 (160.90 \pm 16.38)	G3 (75.75 \pm 22.34)	15.832	<0.001*

ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, CK: Creatinine kinase. G1: Smokers CP, G2: Non-smokers CP, G3: Healthy

Table 4: Correlation of cardiac biomarkers levels with clinical parameters in smokers CP

		BI	PI	PD	CAL
Troponin-I	Pearson's correlation	0.316	-0.145	0.025	0.134
	Sig. (two tailed)	0.101	0.460	0.901	0.496
ALT	Pearson's correlation	0.209	0.071	-0.047	-0.271
	Sig. (two tailed)	0.287	0.718	0.812	0.163
AST	Pearson's correlation	0.132	0.0193	-0.075	0.034
	Sig. (two tailed)	0.503	0.325	0.704	0.865
LDH	Pearson's correlation	0.027	-0.038	-0.005	0.149
	Sig. (two tailed)	0.891	0.848	0.978	0.450
CK	Pearson's correlation	0.126	-0.022	-0.175	0.073
	Sig. (two tailed)	0.522	0.911	0.373	0.713

ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, CK: Creatinine kinase

Table 5: Correlation of cardiac biomarkers levels with clinical parameters in non-smokers CP.

		BI	PI	PD	CAL
Troponin-I	Pearson's correlation	-0.151	-0.160	0.318	0.443
	Sig. (two tailed)	0.409	0.381	0.076	0.011 *
ALT	Pearson's correlation	0.062	0.065	-0.124	-0.238
	Sig. (two tailed)	0.738	0.725	0.498	0.190
AST	Pearson's correlation	0.055	0.041	-0.082	-0.235
	Sig. (two tailed)	0.766	0.823	0.654	0.196
LDH	Pearson's correlation	-0.148	0.190	0.036	-0.013
	Sig. (two tailed)	0.418	0.299	0.845	0.944
CK	Pearson's correlation	-0.072	0.015	-0.098	0.034
	Sig. (two tailed)	0.694	0.935	0.595	0.855

Significant at *P* value level (Sig. two tailed ≤ 0.05). ALT: Alanine transaminase, AST: Aspartate transaminase, LDH: Lactate dehydrogenase, CK: Creatinine kinase

Discussion

Periodontal disease shared with systemic or metabolic disorders is highly prevalent among the population. The association between them is the subject of periodontal medicine researches, and a variety of theories about the possible association between the two diseases have been proposed and hypothesized the intervention of an inflammatory responses.^[21] This bidirectional effect between CP and systemic diseases as diabetes, autoimmune, and CV diseases, rheumatoid arthritis, was suggested by the fact that oral infection is a constant potential source for systemic infection and inflammation.^[22]

Systemic infections are recognized to induce a hypercoagulable state and increase blood viscosity. Fibrinogen levels and white blood cell counts are often raised in patients with periodontal disease. Individuals with poor oral health may also have significant elevations in coagulation factor VIII/von Willebrand factor antigen, thereby increasing the risk of thrombus formation. Thus, the periodontal infection may also

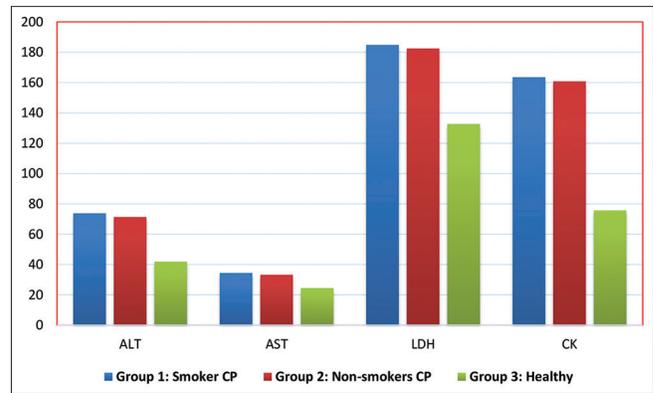


Figure 3: The cardiac biomarker in three studied groups.

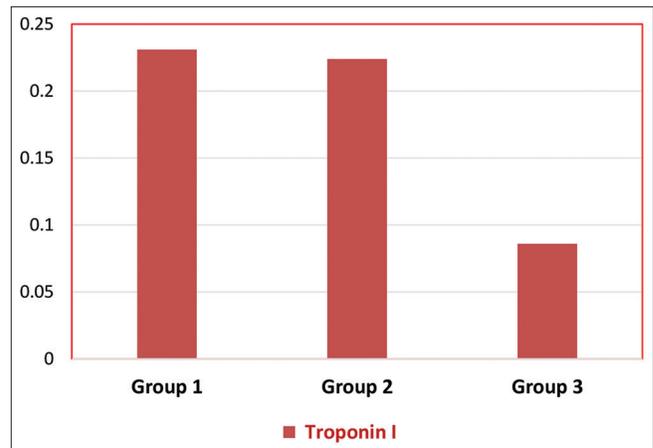


Figure 4: Mean values of troponin-I levels in three examined groups. G1: Smokers CP, G2: Non-smokers CP, G3: Healthy

promote increased blood viscosity and thrombogenesis, which leads to an increased risk for central and peripheral vascular diseases.^[23]

Cigarette smoking is considered a leading preventable cause of CV mortality and morbidity. Epidemiological studies suggested that former smokers, and current smokers, are at higher risk in developing of heart failure even after adjusting for coronary artery disease.^[24] Experimental animal studies suggested that smoking of tobacco may exert direct toxic effects on the myocardium;^[25] additionally, clinical studies showed association between smoking and alteration in cardiac function in subjects without obvious CV disease.^[26] On the other hand, cigarette smoking is an independent risk factor for the initiation, progression, and severity of the periodontal disease. Besides that, smoking can impact the chances of successful periodontal therapy.^[3]

The present study mainly aimed to evaluate the serum cardiac biomarkers level in smokers and non-smokers CP patients in comparison to the healthy control individuals. The periodontal parameters were evaluated clinically, and the cardiac biomarkers were measured biochemically to confirm the possible relationship.

The current study evaluated the clinical parameters of both examined groups through clinical evaluation of PI, BI, PD, and CAL. The PI was significantly increased in non-smokers CP patients, this finding is consistent with Lin *et al.*, 2017,^[27] but disagree with Feres *et al.*, 2015.^[28] The BI has significantly increased in non-smokers CP patients, this outcome inconsistent with Feres *et al.*, 2015 and Lin *et al.*, 2017.^[27,28] For clarification, because the vasoconstriction effect of smoking on gingival blood vessels and increased keratinization of the gingival epithelium, these may reflect less inflammation in gingival tissues of smokers than non-smokers CP patients, this concept is supported by Preber and Bergstrom, 1986.^[29]

Regarding the mean values of PD, the results demonstrated a non-significant comparison between smokers and non-smokers CP patients, this observation in agreement with Feres *et al.*, 2015,^[28] whereas inconsistent with some studies.^[27,30] Concerning to CAL, the results expressed non-significant differences between smokers and non-smokers CP patients. This remark was consistent with some authors,^[28,30] while against the results of Lin *et al.*, 2017.^[27] The insignificant comparisons of PD and CAL in smokers versus non-smokers CP may be referred to as the selected individuals are moderate-to-severe CP, and a large scale of the population may be required.

Troponin is considered a primary cardiac biomarker, notably increased with acute myocardial conditions.^[31] They examined the relationship of acute myocardial infarct size with extension and severity of periodontitis and measured the Tr-I serum levels. They reported that the Tr-I level was highly significant correlated with the severity of periodontitis in acute myocardial infarcted periodontitis patients. According to the investigated levels of Tr-I in the present study, the level of Tr-I was significantly increased in both smokers and non-smokers CP patients in comparison to healthy control subjects. These findings are consistent with the research work of Loo *et al.*, 2012,^[8] they concluded that the increased level of Tr-I may consider a potential risk of CVD development and suggested to use the Tr-I as a monitor to CVD in CP patients. The Tr-I level was slightly increased in smokers than non-smokers CP patients, and this relationship is similar to the results of Loo *et al.*, 2012.^[8] According to the previous outcomes, smoking may increase the possibility of cardiac disorders in systemically healthy CP patients. On the other hand, another study by Vedin *et al.*, 2017,^[32] observed that there is no association between the high-sensitivity troponin-T (Tr-T) and the ratio of tooth loss in several patients with cardiac disease.

AST and ALT enzymes are most commonly associated with tissue damage. Measurement of AST level provides a suggestion of the progression of periodontitis. Therefore, AST could be a potential biomarker for differentiating between active and inactive disease sites.^[33] The statistical comparisons for serum level of ALT and AST were demonstrated highly significant increase in smokers and non-smokers CP patients versus healthy individuals. This observation was supported by

some authors.^[34,7] On other side, Dabra *et al.*, 2012, concluded that the salivary (AST and ALT) considered as biochemical markers for assessing the diagnosis and prognosis of the functional state of periodontal tissues in health and disease and in the evaluation of the periodontal therapy outcomes.^[35]

In this study, the mean values of ALT and AST were increased in smokers than non-smokers CP patients, and a significant difference was obtained. This observation was in accordance with the results of two studies.^[36,37] Based on the previous information, the present study suggested a significant effect of smoking and periodontitis as risks for elevating the serum levels of ALT and AST.

The LDH, CK, and AST biomarkers are intracellular cytoplasmic enzymes that become extracellular when cell death. Hence, the LDH, CK, and AST are used as diagnostic biomarkers of tissue breakdown in chronic liver diseases, acute myocardial infarction, the extent of brain injury following cerebrovascular disease, or infection. The extracellular appearance of LDH and the high level of LDH used to detect cell damages, disease progression, and cell death. This outcome is adapted with the result in the current study that represented a highly significant increase of LDH levels in smokers and non-smokers CP in comparison to healthy control that might support the use of LDH as a potential diagnostic biomarker for periodontal disease progression.^[38,39]

The association between periodontal condition and serum CK levels among young soccer players was studied by Alshail *et al.*, 2016,^[40] they concluded that the increase in bleeding on probing (BOP) and PD was associated with high serum CK level in young soccer players and considered that serum CK level may be revealing compromised oral health. Similarly, we observed a highly significant increase in the levels of CK in smokers and non-smokers in contrast to healthy individuals. Furthermore, the serum levels of LDH and CK were increased in smokers in comparison to non-smokers CP patients and the differences were non-significant.^[41] This notification may reveal the risk of smoking on systemically healthy CP patients.

The correlative analysis of the assayed biomarkers with clinical parameters in both smokers and non-smokers CP patients reflected a non-significant association for all parameters except for CAL and Tr-I in non-smokers CP. For elucidation, the non-significant correlation may be related to the patients who were in the range of moderate-to-severe CP and systemically healthy. For the previous reason, a future study on the association of cardiac biomarkers with periodontal parameters in CVD patients is recommended. Periodontitis and CVD are multifactorial diseases that are precipitated by common risk factors, and the association might be present. Moreover, smoking aggravated the severity of periodontitis and CVD. Hence, the resolution of periodontal infections and tobacco cessation are beneficial, as well as the communication between dentists and cardiologists is strongly recommended.

Conclusion

The present study concluded the following: (1) The serum levels of cardiac biomarkers were more affected in smokers, and non-smokers CP patients versus healthy individuals. (2) Periodontitis considered as one of the potential risk factors that lead to the development and progression of CVD. (3) Smoking played a bidirectional role in aggravating the periodontal and CVD. (4) More researches are needed to clarify the efficacy of periodontal treatment on cardiac biomarkers in the systemically healthy and in diseased CV patients.

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Conflicts of Interest

None declared including specific, related, and/or affiliated financial interest.

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