Original Article

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Plasma fibrinogen and C-reactive protein decreased after nonsurgical periodontal therapy in non-coronary heart disease subjects with periodontitis

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Objective: To evaluate the effect of nonsurgical periodontal therapy (NSPT) on the level of fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (CRP).

Materials and Methods: This study comprised 12 subjects with coronary heart disease (CHD) and 15 subjects without CHD (non-CHD). Each group had 2 subgroups with or without periodontitis. Periodontal parameters and cardiovascular biomarkers were measured at baseline. All subjects received NSPT. All parameters were re-evaluated at 3- and 6-months post-treatment.

Results: At baseline, there were no significant differences in the patients' characteristics. The periodontal inflammation and periodontal disease status results revealed that the non-CHD with periodontitis group had a significantly higher percentage of bleeding on probing + pocket depth > 4 mm + clinical attachment level ≥ 5 mm compared with the CHD with periodontitis group. After NSPT, there was a significant periodontal improvement in all groups. The non-CHD with periodontitis group demonstrated a significant reduction in fibrinogen and CRP level at 6 months after NSPT compared with baseline.

Conclusion: NSPT significantly reduced fibrinogen and CRP in non-CHD subjects with periodontitis.

Keywords: coronary heart disease, C-reactive protein, fibrinogen, periodontal disease, plasminogen activator inhibitor-1

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Introduction

Periodontitis has a bacterial plaque etiology. Plaque stimulates immune cells to release proinflammatory cytokines and inflammatory mediators. Large amounts of local cytokines increase the plasma concentrations of pro-inflammatory biomarkers that then signal the liver or distant organs to produce acute phase proteins, such as C-reactive protein (CRP) and fibrinogen, initiating systemic inflammation [1]. C-reactive protein is produced by hepatocytes and released into the bloodstream in response to acute inflammation [2,3]. Fibrinogen, a soluble protein in blood plasma, is synthesized in hepatocytes and is necessary for blood coagulation. Therefore, fibrinogen plays an important role in hemostasis and thrombosis [4]. Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that inhibits fibrinolysis, leading to increased thrombus formation [5, 6]. It was shown that the level of CRP,

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fibrinogen, and PAI-1 was higher in periodontitis patients compared with the non-periodontitis controls [7, 8].

Once initiated, systemic inflammation is involved in the pathogenesis of atherosclerosis due to the accumulation of inflammatory cells within the arterial wall and thus, increases the local production of chemokines, interleukins, and proteases, resulting in an increased influx of immune cells and atherosclerotic lesion progression [9]. It has been suggested that inflammatory markers, including plasma fibrinogen, CRP and PAI-1, may be associated with coronary heart disease (CHD) incidence [10-12]. A previous study also revealed a higher fibrinogen level in patients with CHD compared with those without CHD [13].

A consensus report evaluating the association of periodontitis and cardiovascular disease (CVD) based on longitudinal studies found that periodontitis increased the risk for CVD independent of other known cardiovascular risk factors [14]. A study also revealed that periodontitis was significantly associated with the prevalence of CHD [15]. Periodontitis causes low-grade systemic inflammation by releasing local inflammatory mediators from the periodontal pockets into the systemic circulation. These inflammatory mediators may enhance the risk of CVD. Elevated systemic inflammation-associated periodontitis might further promote atherosclerosis. Controlling periodontal inflammation might decrease the risk of CVD events in periodontitis patients. A consensus report revealed moderate evidence that nonsurgical periodontal therapy (NSPT) reduced systemic inflammation and improved endothelial function [14]. However, the results from clinical trials were inconclusive and the follow-up period in most studies was 3 months or less [16-20]. The disparate results might be due to subject inclusion criteria [17,19, 21]. In addition, periodontal inclusion criteria or other risk factors for CVD or periodontitis were not clearly specified [17,18]. Thus, the aim of this study was to determine whether NSPT had a beneficial effect on lowering the plasma level of fibrinogen, PAI-1, and CRP in CHD and non-CHD patients with or without periodontitis, and if this beneficial effect, if any, could last longer than 3 months after NSPT.

Materials and methods

In this study, ethical approval was obtained from the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COA.No.MU-DT/PY-IRB 2018/022.0504) and the Ethics Committee, Rajavithi Hospital (043/2562). This study was registered in ClinicalTrials.gov (NCT04305171). The study was conducted from April 2018 to June 2019.

Sample size calculation

The sample size was calculated using 1-month changes in fibrinogen and CRP level, based on Bokhari *et al*. [17]. It was estimated that 8 individuals in the CHD group and 13 individuals in the non-CHD group would be necessary to find a reduction in fibrinogen of 235.6 mg/l (standard deviation, SD = 133.5) and 128.4 mg/ml (SD = 118.1), and a reduction in CRP of 0.2 mg/dl (SD = 0.2) and 0.1 mg/dl (SD = 0.2), in the CHD group and non-CHD group, respectively, with 1% alpha error and 10% beta error.

Subject selection and recruitment

The subjects were outpatients, 35–70-years years, who came to the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University or Rajavithi Hospital, Thailand for a medical checkup. The CHD patients were confirmed using coronary angiography as having

at least 50% diameter stenosis in at least one coronary artery and were clinically stable with the absence of any potentially confounding inflammatory conditions for at least 6 months. Subjects were excluded if they received anticoagulants, or had chronic conditions (e.g. diabetes mellitus, rheumatoid arthritis, malignancy, or autoimmune disease), or acute conditions (e.g. trauma, surgery) or received medications known to affect systemic inflammatory markers (e.g. antibiotics, immunosuppressive drug, or contraceptives) within 3 months, pregnancy or lactation, presence or history of other infections, periodontal treatment within 6 months, or tooth extraction within 2 months prior to the study.

The subjects were referred to the Dental Hospital, Faculty of Dentistry, Mahidol University for periodontal screening. Subjects with at least 14 natural teeth, excluding 3rd molars, were included. Periodontitis was defined as bleeding on probing (BOP), pocket depth (PD) > 4 mm, clinical attachment level (CAL) ≥ 5 mm, and radiographic evidence of bone loss present at the same site of at least 4 teeth. Non-periodontitis was defined as PD ≤ 3 mm. The subjects were informed verbally and in writing about the study and gave written informed consent before participating. The subjects were grouped into CHD and non-CHD groups. Each group divided into subgroups with or without periodontitis.

Socio-demographic information, smoking status (non-smoker, former smoker, current smoker) alcohol consumption (≥10 drinks/week, <10 drinks/week), exercise behavior (no exercise, exercise < 30 minutes, exercise >30 minutes <3 days/week, exercise >30 minutes ≥3 days/week), body mass index (BMI (kg/m^2) [22], and oral health behavior (tooth brushing ≤1 time/day, tooth brushing >1 time/day) of the subjects were recorded.

Periodontal parameters

Periodontal parameters, Plaque score [23], BOP [24], PD, and CAL, were recorded from all teeth, except 3rd molars, by a calibrated investigator. The pocket depth and CAL were recorded to the nearest mm at 6 sites/tooth (disto-buccal, mid-buccal, mesio-buccal, disto-lingual, mid-lingual, and mesio-lingual) using UNC 15 probes (Hu Friedy, IL, USA).

Cardiovascular risk markers

A 10 ml blood sample was obtained using venipuncture at the antecubital fossa and collected in a tube with 3.2% sodium citrate. The samples were centrifuged, and the plasma was collected and frozen at -80 °C. Plasma fibrinogen and PAI-1 were measured with enzyme-linked immunosorbent assay (ELISA) kits (Abcam, Cambridge, UK). C-reactive protein was measured with a highly sensitive C-reactive protein (hs-CRP) ELISA kit.

Study protocol

At baseline, the periodontal parameters and plasma inflammatory markers were recorded. In the CHD patients, medical consultation for discontinuation of antiplatelet drugs was performed before NSPT. The subjects received oral hygiene instruction, supra- and subgingival scaling, and root planing. The procedure was performed using ultrasonic and hand instruments in 2–3 visits and completed within a month. All parameters were recorded again at 3 and 6 months after NSPT (Figure 1).

Intra-examiner agreement

PD and CAL evaluation was repeated within a 1-week interval for 10 individuals. The kappa coefficients for intra-examiner agreement were > 0.80.

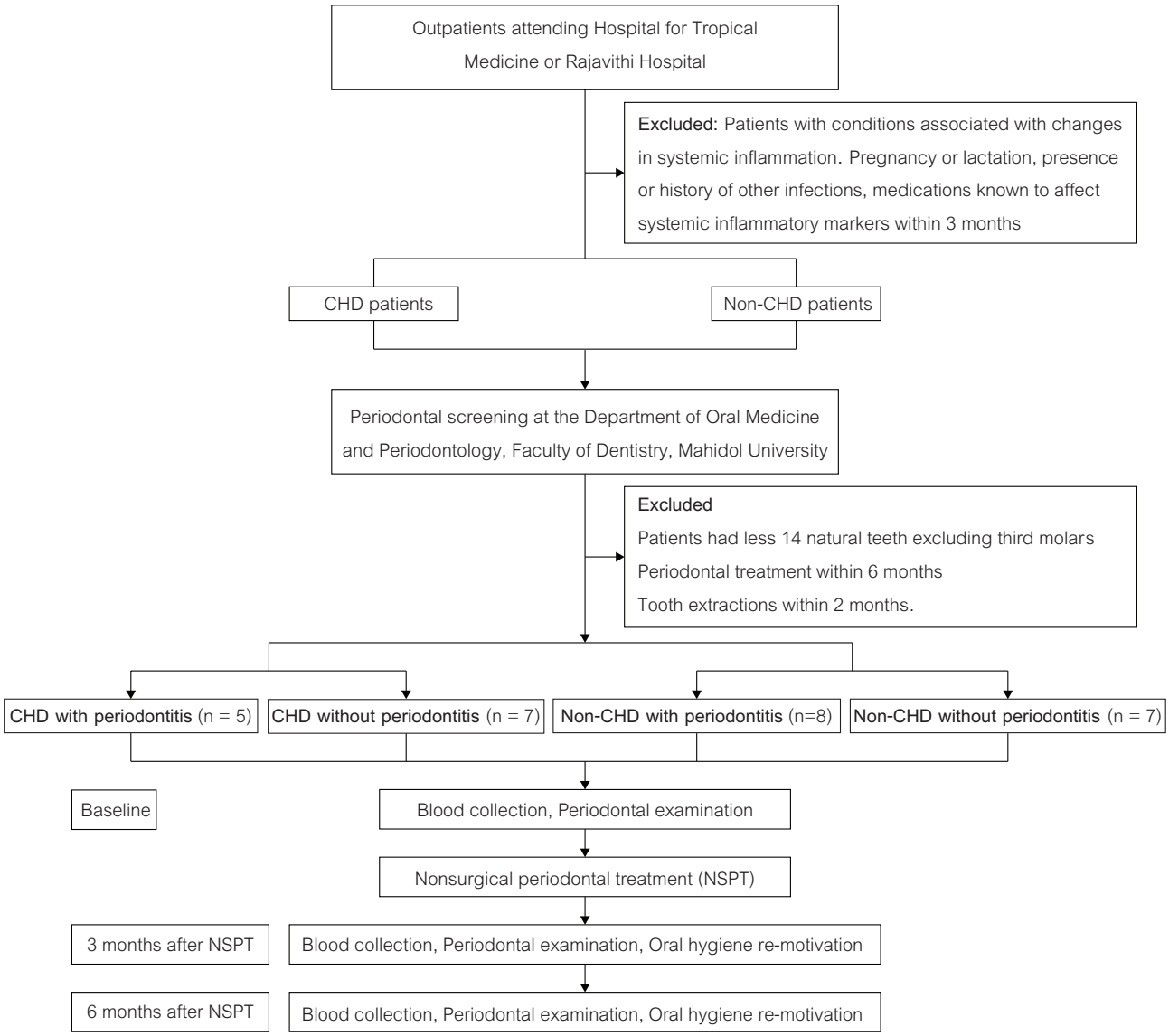

Figure 1 Flow chart of the study

Statistical analysis

The data were analyzed using SPSS for Windows (version 23). The median age, BMI, and number of teeth at baseline were analyzed using the independent-sample Kruskal-Wallis test. The number of males, smokers, alcohol consumption≥10 drinks/week, subjects with education lower than bachelor's degree or no exercise behavior or practiced tooth brushing ≤1 time/day were analyzed using Fisher's Exact Test. Full mouth BOP, plaque score and sites with BOP plus PD>4 mm and CAL≥ 5 mm were calculated as a percentage. Periodontal parameters and level of cardiovascular risk markers were presented as median values. Normal distribution was determined using the Kolmogorov-Smirnov test. Comparisons of the median values of the percentage of sites with BOP plus PD>4 mm and CAL≥ 5 mm were done by the independent-sample Mann-Whitney U Test. Periodontal parameters and cardiovascular risk markers were performed using the Related-samples Friedman's Two-way analysis of variance

by ranks (followed by the Wilcoxon matched-pairs signed-rank) and independent-sample Kruskal-Wallis test (followed by the *Bonferroni* Post-hoc test), for intragroup and intergroup difference, respectively. Significance was set at $P < 0.05$.

Results

Twenty-seven subjects completed the study. Most CHD participants (71.4%) were males (Table 1). None of the participants were current smokers and approximately half of the CHD participants had a history of cigarette smoking. No significant differences in the subjects' characteristics were found between the groups.

Periodontal parameters

At baseline, the participants with periodontitis had a higher percentage of BOP and plaque score compared with those without periodontitis. (Table 2). After NSPT, all periodontal parameters demonstrated a significant improvement in all groups compared with baseline, except for PD in the CHD without periodontitis group and CAL in the CHD and non-CHD without periodontitis groups.

The periodontal inflammation and periodontal disease status results indicated that the non-CHD with periodontitis group a significantly higher %BOP + PD > 4 mm + CAL ≥ 5 mm compared with the CHD with periodontitis group (Table 2). After NSPT, these values were significantly reduced in both groups compared with baseline.

Table 1 Subject characteristics at baseline

Variables	Groups				*P*-value
	CHD		Non-CHD		
	with P (*n* = 5)	without P (*n* = 7)	with P (*n* = 8)	without P (*n* = 7)	
Age; median (Q1, Q3)	68 (54.5, 70)	65 (54, 68)	53 (48.5, 56.5)	65 (62, 66)	0.053
Males; *n* (%)	3 (60)	5 (71.4)	3 (37.5)	2 (28.6)	0.389
Education lower than bachelor; *n* (%)	3 (60)	2 (28.6)	5 (62.5)	2 (28.6)	0.632
Former smokers; *n* (%)	3 (60)	3 (42.9)	3 (37.5)	1 (14.3)	0.467
Alcohol consumption ≥10drinks/wk; *n* (%)	2 (40)	2 (28.6)	3 (37.5)	2 (28.6)	0.306
No exercise behavior; *n* (%)	1 (20)	1 (14.3)	5 (62.5)	1 (14.3)	0.354
BMI; median (Q1, Q3)	24.8 (22.8, 25.7)	25.3 (24.9, 26.2)	26.6 (24.7, 27.2)	20.9 (20.5, 24.9)	0.086
Tooth brushing ≤1time/d; *n* (%)	0	1 (14.3)	2 (25)	0	0.591
No. of teeth; median (Q1, Q3)	19 (16, 25)	26 (18, 28)	27 (20.5, 27.7)	26 (16, 26)	0.196

CHD, coronary heart disease; P, periodontitis; BMI, body mass index

Table 2 Median values of the percentage of sites with bleeding on probing, plaque scores, pocket depth and clinical attachment level

Variables	Time points	Groups				
		CHD		non-CHD		
		with P (*n* = 5)	Without P (*n* = 7)	With P (*n* = 8)	Without P (*n* = 7)	
BOP (% of sites)	baseline	65.5 (62.1, 85.2)	46.7 (34.5, 56.6)	96.9 (86.5, 100)	36.8 (35.2, 48)	†*P* <0.001
	3 months after NSPT	30.1 (26.4, 36.8)	19.6 (12.5, 25)	35.6 (27.9, 44.2)	16.6 (10.9, 21.9)	†*P* <0.001
	6 months after NSPT	28.5 (19.5, 36.7)	16.0 (8.9, 20.3)	29.3 (23.3, 44.1)	14.7 (9.3, 18.7)	†*P* =0.003
	***P*	**0.002**	**0.016**	**0.004**	**0.005**	
Plaque score (% of sites)	Baseline	82.5 (65.5, 90.1)	53.0 (41.6, 65.5)	88.6 (75.2, 92.1)	52.0 (51.2, 61.8)	†*P* =0.002
	3 months after NSPT	41.1 (33.4, 56.2)	25.0 (14.1, 31.1)	35.8 (31.1, 42.2)	40.6 (24.4, 41.6)	†*P* =0.026
	6 months after NSPT	28.2 (24.8, 45.7)	19.0 (12.8, 25.5)	36.9 (25.6, 39.3)	31.2 (23, 36.4)	†*P* =0.081
	***P*	**0.007**	**0.002**	**0.002**	**0.001**	
PD (mm)	Baseline	2.9 (2.7, 3.6)	2.2 (2.1, 2.3)	3.8 (3.5, 4.2)	2.2 (2.1, 2.3)	†*P* <0.001
	3 months after NSPT	2.4 (2.1, 2.7)	2.2 (2.1, 2.2)	2.9 (2.5, 3.2)	1.9 (1.9, 2.1)	†*P* <0.001
	6 months after NSPT	2.4 (2.2, 2.7)	2.2 (2.2, 2.3)	2.8 (2.6, 3.1)	2.2 (2.1, 2.2)	†*P* =0.001
	***P*	**0.015**	0.651	**0.002**	**0.028**	
CAL (mm)	Baseline	4.0 (3.2, 4.3)	2.4 (2.4, 3.0)	4.3 (3.9, 4.4)	2.3 (2.1, 2.7)	†*P* <0.001
	3 months after NSPT	3.5 (2.6, 3.6)	2.3 (2.2, 2.5)	3.6 (3.0, 4.0)	2.1 (2.0, 2.4)	†*P* =0.001
	6 months after NSPT	3.2 (2.6, 3.7)	2.4 (2.3, 2.7)	3.8 (3.1, 4.0)	2.2 (2.1, 3.0)	†*P* =0.001
	***P*	**0.015**	0.565	**0.002**	0.062	

Table 2 Median values of the percentage of sites with bleeding on probing, plaque scores, pocket depth and clinical attachment level (Continued)

Variables	Time points	Groups				
		CHD		non-CHD		
		with P (*n* = 5)	Without P (*n* = 7)	With P (*n* = 8)	Without P (*n* = 7)	
Sites with BOP + PD>4mm + CAL≥5mm (% of site)	baseline	14.4 (12.3, 28.8)	-	28.5 (22.5, 39.2)	-	‡*P* =0.045
	3 months after NSPT	1.9 (0.7, 3.7)	-	9.4 (4.7, 10.8)	-	‡*P* =0.030
	6 months after NSPT	1.1 (0.3, 1.9)	-	6.7 (3.0, 7.6)	-	‡*P* =0.019
	**P*	0.008	-	<0.001	-	

Data are expressed as median (Q1, Q3). *, Friedman test; †, Kruskal-Wallis test; ‡, Mann-Whitney U test; BOP, bleeding on probing; CAL, clinical attachment level; CHD, coronary heart disease; NSPT, nonsurgical periodontal therapy; PD, pocket depth

Levels of plasma fibrinogen, PAI-1 and CRP

No significant difference in the level of all markers was found between the groups at baseline (Table 3, Figure 2). The median values of these markers fluctuated after NSPT in the CHD with or without periodontitis groups. In contrast, the non-CHD with periodontitis group had a significant reduction compared to baseline in fibrinogen and CRP level 6 months after NSPT.

Discussion

Several clinical trials have reported inconclusive results on the effect of NSPT on the levels of cardiovascular risk markers. Most studies found significantly reduced CRP [11,19, 20, 25] and fibrinogen [16, 26, 27] after NSPT in periodontitis patients with or without CHD. In contrast, other studies did not find a significant reduction in these inflammatory markers [18, 28-31] and two studies reported no significant change in PAI-1 in periodontitis patients 3-months post treatment [27, 30]. The discrepancy in the effect of NSPT on the reduction of these biomarkers might be due to subject inclusion criteria, varying from with or without CHD and/or with or without periodontitis [16-21, 26-28, 30]. In some studies, other risk factors that might interfere with the results, such as diabetes mellitus or smoking, were not clearly specified [18,19]. Moreover, the severity of periodontitis can also contribute to the result. A study that included only subjects with limited periodontal breakdown demonstrated a slight reduction in systemic inflammation markers [17]. Thus, in the present study, we examined the effect of NSPT on the levels of these cardiovascular risk markers at 3 and 6 months after NSPT in patients with or without CHD and periodontitis.

In this study, we did not find any significant differences in the inflammatory marker levels between the CHD and non-CHD groups. This might be due to recruiting clinically stable CHD patients and a non-significant difference in the presence of other risk factors, such as smoking and alcohol drinking between the CHD and non-CHD groups at baseline.

Table 3 Median values of cardiovascular risk markers according to periodontal status

Variable	Time points	Groups				
		CHD		Non-CHD		
		with P (*n* = 5)	without P (*n* = 7)	with P (*n* = 8)	without P (*n* = 7)	
Fibrinogen (pg/mL)	Baseline	541.7 (229.0, 1335.6)	429.0 (193.9, 1092.7)	673.8 (485.5, 1062.0)	889.4 (669.3, 1220.0)	†*P* = 0.729
	3 months after NSPT	695.3 (467.9, 951.6)	908.2 (568.0, 1305.7)	608.3 (505.6, 788.1)	752.4 (588.7, 794.0)	†*P* = 0.456
	6 months after NSPT	561.3 (197.6, 802.1)	476.6 (330.1, 1380.3)	355.6 (211.9, 444.3)	446.1 (215.9, 612.1)	†*P* = 0.403
	**P*	0.165	0.565	**0.01**	0.156	
PAI-1 (pg/mL)	Baseline	393.7 (274.8, 580.6)	434.7 (227.0, 537.7)	401.0 (371.5, 563.7)	347.6 (188.3, 542.6)	†*P* = 0.601
	3 months after NSPT	337.6 (184.7, 465.7)	549.4 (435.6, 1013.1)	380.2 (221.3, 603.0)	193.6 (160.0, 547.3)	†*P* = 0.078
	6 months after NSPT	524.6 (252.1, 859.1)	289.3 (274.6, 357,8)	311.0 (242.9, 482.1)	299.2 (145.8, 634.5)	†*P* = 0.568
	**P*	0.819	0.368	0.197	0.867	
CRP (mg/L)	Baseline	0.925 (0.304, 1.585)	0.723 (0.672, 1.080)	1.150 (0.774, 1.877)	0.624 (0.278, 1.270)	†*P* = 0.493
	3 months after NSPT	0.378 (0.220, 1.805)	0.810 (0.615, 1.540)	1.091 (0.478, 2.847)	0.544 (0.292, 1.450)	†*P* = 0.520
	6 months after NSPT	0.542 (0.259, 1.530)	1.040 (0.418, 1.290)	0.862 (0.356, 1.395)	0.964 (0.450, 1.820)	†*P* = 0.869
	**P*	0.819	0.867	**0.008**	0.651	

Data are expressed as median (Q1, Q3). *, Friedman test; †, Kruskal-Wallis test; CHD, coronary heart disease; P, periodontitis; PAI-1, plasminogen activator inhibitor-1; CRP, C-reactive protein; NSPT, nonsurgical periodontal therapy

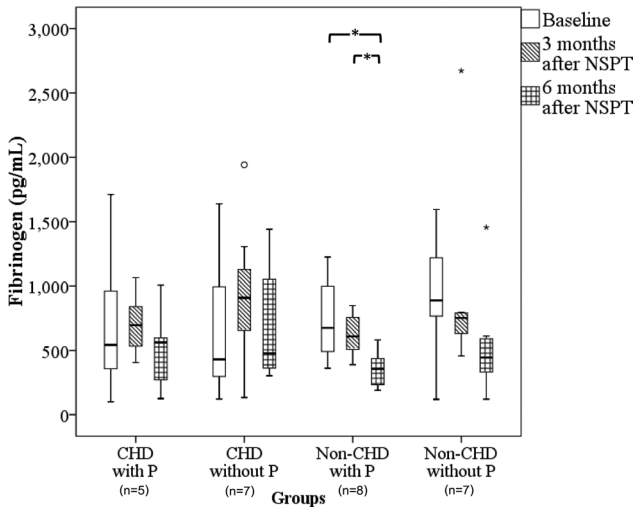

Figure 2A

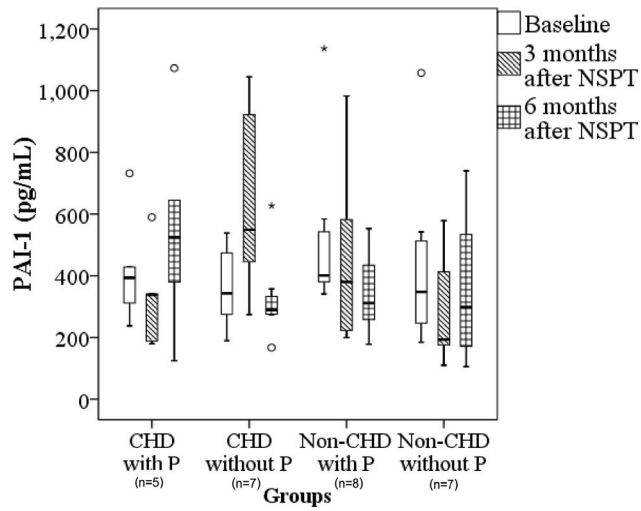

Figure 2B

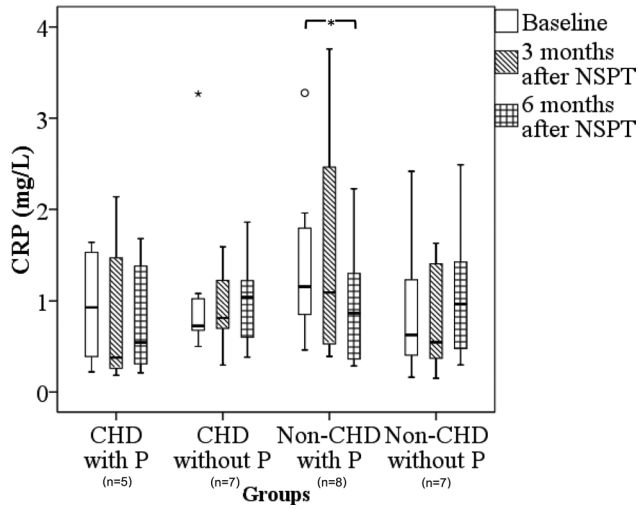

Figure 2C

Figure 2 Levels of fibrinogen (A), PAI-1 (B), and CRP (C) at baseline, 3, and 6 months after NSPT according to CHD and periodontal disease status. (CHD, coronary heart disease; CRP, C-reactive protein; NSPT, nonsurgical periodontal therapy; P, periodontitis; PAI-1, plasminogen activator inhibitor-1; * $P< 0.05$ Friedman test (follow by the Wilcoxon matched-pairs signed-rank))

At 6 months after NSPT, a significant reduction in fibrinogen and CRP was found in the non-CHD with periodontitis group. Several factors have been shown to influence fibrinogen and CRP levels. These factors include inflammation, tissue injury or infection, thrombotic state, or the presence of atherothrombotic disease, etc. [10,32]. In the present study, the reduced fibrinogen and CRP after NSPT in the non-CHD with periodontitis group could be explained by a reduction in periodontal inflammation, leading to a reduction in systemic inflammation. The outcome of our study was consistent with Taylor *et al*. [27] in that fibrinogen was significantly reduced 3 months post treatment in non-CHD with periodontitis patients. There was evidence suggesting that

patients with chronic periodontitis had elevated serum CRP level [8]. This study also showed a higher median value CRP value in the periodontitis subjects at baseline. Systematic review and meta-analysis studies have demonstrated that periodontal therapy reduces CRP levels [11, 25, 33]. Bokhari *et al.* [16] also reported significantly reduced CRP in CHD patients with periodontitis 1- and 2-months post-treatment. D'Aiuto *et al.* [5] reviewed the effect of periodontal treatment on biomarkers and CHD outcomes and they found moderate evidence to support the effect of periodontal therapy in lowering serum levels of CRP. In our study, a significant reduction in CRP level 6 months after NSPT was found in the non-CHD patients with periodontitis. Three months after NSPT, there was no significant difference in fibrinogen and CRP levels. Although the inflammation had subsided, the periodontal pockets that still existed might cause the fibrinogen and CRP levels to stay the same (The percentage of sites with PD>4 mm in the non-CHD with periodontitis patients was 30.79%, 11.4%, and 9.3% at baseline, 3 months, and 6 months post-treatment, respectively, data not shown). Our findings are supported by Slot *et al.* [34] that more residual infection was found with increased residual pocket depth.

In CHD patients, reduced CRP was found 3-months post treatment, however, the difference was not significant. The lack of significance might be due to the small number of subjects. In contrast, the fibrinogen level increased 3-months after NSPT. This result indicated that the CHD factors might cause fluctuating fibrinogen and PAI-1 levels. In addition, Montenegro *et al.* [19] reported that in stable coronary artery disease and periodontitis patients with baseline CRP <3 mg/L, CRP remained unchanged or increased 3 months after NSPT, while a significant reduction in CRP after NSPT was observed only in the group with baseline CRP ≥3 mg/L. In our study, the baseline CRP in the CHD and non-CHD patients with periodontitis ranged from 0.220–1.64 mg/L and 0.46–3.28 mg/L, respectively (data not shown). Thus, our results were consistent with those of Montenegro *et al.* [19].

The significant difference in the percentage of sites with BOP + PD>4mm + CAL≥ 5 mm between the CHD and non-CHD groups at baseline can be explained by the differences in periodontal disease status that might cause reduced fibrinogen and CRP levels. In this study, the CHD with periodontitis group presented with a fewer sites of periodontal inflammation and severe periodontal breakdown compared with that of the non-CHD with periodontitis group, and this could possibly diminish the systemic inflammatory outcome.

Periodontal inflammation was reduced after NSPT in all groups in this study as shown by a significant reduction in BOP. However, CHD patients with or without periodontitis and non-CHD patients with or without periodontitis did not demonstrate a significant difference in PAI-1 level 3- and 6-months after NSPT. Tonetti *et al.* [21] also found no significant difference in PAI-1 3 months post-treatment in severe generalized periodontitis patients. In the non-CHD with periodontitis group, the PAI-1 level decreased after NSPT, however, the difference was not significant. This was consistent with the reduced fibrinogen and CRP in non-CHD without periodontitis, however, the reduction in periodontal inflammation after NSPT might not meaningfully affect the level of PAI-1.

In this study, the risk factors for CHD, such as hypertension or other variables that influence the level of inflammatory markers, *e.g.* diet, hormone levels, total and low-density lipoprotein, cholesterol levels, or medications were not explored. A relatively small sample size in each group is also a limitation. Prospective studies with a larger sample size are required to effectively demonstrate the effect of periodontal treatment on these markers.

In conclusion, NSPT decreased systemic inflammatory marker levels. This decrease led to reduced fibrinogen and CRP in non-CHD with periodontitis patients.

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Conflict of interest

The authors have no conflict of interests.

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