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Original Research

Investigating platelet-activating factor as a potent proinflammatory mediator in coronary atherosclerotic patients

Shler Ghafour Raheem*

Unit of Microbiology, Department of Basic Science, College of Medicine, Hawler Medical University, Erbil, Kurdistan Region-Iraq

*Correspondence to: shler.raheem@hmu.edu.krd

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Abstract: The inflammatory reaction is one of the complications in patients with coronary atherosclerosis. This study aimed to determine the diagnostic value of platelet-activating factor (PAF) compared with high sensitivity C reactive protein (hs-CRP) in coronary atherosclerotic patients. Fifty patients with coronary atherosclerosis and 30 subjects with normal angiography were considered as the control group attending Cardiac Center-Surgical Specialty Hospital - in Erbil city / Iraq. The levels of PAF and hs-CRP were estimated quantitatively using a sandwich enzyme-linked immunosorbent assay and a particle-enhanced immune turbid metric assay, respectively. Lipid profiles and some hematological indexes were also used in this study. The levels of the inflammatory biomarkers of PAF and hs-CRP increased significantly in the patients group compared with controls ($p<0.05$). Although the patients group showed the highest level of low-density lipoprotein (LDL), the difference was not significant ($p>0.05$) compared with the healthy control. However, the incidence of risk factors such as smoking and obesity showed a significant difference ($p<0.05$) in the patients group. Additionally, the PAF level correlated positively and significantly with hs-CRP ($p<0.05$), and negatively with high-density lipoprotein (HDL) ($p>0.05$). Although hs-CRP was a valuable diagnostic marker for coronary atherosclerosis, the PAF level showed to be a better prognostic indicator than hs-CRP in coronary atherosclerosis patients.

Key words: Platelet activating factor; Proinflammatory mediator; hs-CRP; Diagnostic marker; Coronary atherosclerosis.

Introduction

Atherosclerosis is a lipid storage disease and known as a sub-acute inflammatory condition of the vessel wall, characterized by infiltration of macrophages and T cells, that interact with each other and with cells of the arterial wall (1).

Platelet-activating factor (PAF) is a potent pro-inflammatory mediator that detects a defined range of biological and pharmacological effects. It engaged in a wide range of pathophysiological conditions. The primary role of PAF in the cardiovascular system has been shown in platelet and neutrophil aggregation, vascular permeability, microvascular leakage, thrombus formation, leukocyte adhesion to the endothelial cells, and finally initiation and progression of atherosclerosis (2).

Inflammation is a key mechanism of atherosclerosis and white blood cells (WBCs) play a crucial role in the inflammatory process. Although several risk factors are involved in the development of cardiovascular disease (CVD), atherosclerosis has a pivotal role in its pathogenesis (3). Moreover, much evidence supports the role of chronic inflammation in the atherosclerotic process (4). The pathophysiological process of this disease involves the release of many factors such as proteins into the bloodstream, which could later be used as diagnostic biomarkers (5).

CRP levels can be considered a good prognostic indicator and an inflammatory marker in patients with the acute coronary syndrome (6). In addition, the PAF biomarker can be considered as the best-validated marker in prediction of the future cardiac events in patients with suspected cardiovascular diseases such as atherosclerosis. Also, articles summarize the number of possible markers that may play an important role in the pathogenesis of the disease because of its association with some causal mechanisms such as endothelial dysfunction, inflammation, and oxidative and platelet reactivity.

This study aimed to estimate PAF serum level with some inflammatory biomarkers and its potential role as a key contributor to the pathogenesis of atherosclerosis.

Materials and Methods

This study was performed between December 2019 and May 2020. It involved fifty patients with coronary atherosclerosis (26 men and 24 women) with the age ranged between 41-75 years and 30 healthy control (15 men and 15 women) who had normal coronary angiography, with no history of clinical evidence of autoimmune diseases. The age of healthy controls ranged between 40-74 years. Subjects attended Cardiac Center-Surgical Specialty Hospital - in Erbil city. Percutaneous coronary intervention (PCI) underwent for all patients as having >40% stenosis of one major coronary artery disease (CAD). Through the format of a specially designed questionnaire, all participants were subjected to a personal interview. All procedures were in accordance

with the established ethical standards. A total of 5 ml venous blood sample was taken from each participant and transferred into anticoagulant-free tubes, then centrifugationat1000 X G for 15 minutes, serum was isolated and stored at -80 °C. PAF level was measured by enzyme-linked -immunosorbent assay (Thermo Fisher Scientific, USA). Hs-CRP levels were estimated by particle enhanced immunoturbidometric assay using Cobas c111 (Roche Diagnostics GmbH). Lipid profiles were measured on the principle of the enzymatic colorimetric test using Cobasc111 (Roche Diagnostics, GmbH). Body mass index (BMI) was calculated by the formula, BMI = Weight / Height (m) ^{2}.

Table1. Baseline characteristics of coronary atherosclerotic patients.

Characteristics	patients No=50
Age(years)	58.9±2.10
Sex No (%):	
Male (%)	26 (52.0)
Female (%)	24 (48. 0)
Family history of CAD: No%	30 (60.0)
BMI ***	28.3±0.22
Obesity classification: No (%)	
Normal	13 (26)
Overweight	17 (34)
Obese	20 (40)
Inflammatory marker:* **	
PAF (pg/ml)	2.35±0.45
Hs-CRP (mg/l)	10.8±2.49
Lipid profile:	
Total cholesterol(mg/dl)	178.3±5.82
Triglycerides(mg/dl)	125.2±8.7
HDL-Cholesterol (mg/dl)	35.2±1.52
LDL-Cholesterol (mg/dl)	94.5±4.31

Data are presented as mean ± SE and number (%). Body mass index: BMI; Total cholesterol: TC; PAF: Platelet-activating factor; hsCRP: high sensitivity C reactive protein; DHL-C: high-density lipoprotein-cholesterol; LDL-c; LDL: low-density lipoprotein-cholesterol.

Statistical analysis

The statistical package for the social sciences (SPSS) version 23 was used for data analysis. The data were expressed as mean ± SE and the differences were evaluated by Chi-squared test (X^2 test), student's t-test, ANOVA, and Pearson correlation's coefficient test. $P<0.05$ was set as statistically significant.

Results

Table 1 demonstrated the basic characteristics of the coronary atherosclerotic patients group. The average age of the patients was 58.9 with a male predominant 26 (52.0%) and a family history of about 30 (60.0%). The patients group had a body mass index of around 28.3±0.22 and most of them were located within the obese group. Levels of the inflammatory parameters, PAF and hs-CRP were (2.35±0.45), and (10.8±2.49) respectively, the LDL level was high in the patients group.

Table 2 shows inflammatory biomarkers and risk factors distribution between the coronary atherosclerotic patients and control groups. The patients group revealed a significant increase in the inflammatory biomarkers including, PAF, hsCRP and WBC compared with the control group ($P<0.01$). Risk factors as obesity also had significant differences ($P<0.01$), however, the number of smokers as a risk factor in the patient group was significantly more compared to the control ($P<0.05$). Although LDL level increased and HDL decreased in the patients group, the lipid profile results had no significant differences between coronary atherosclerosis patients, and controls ($p>0.05$). The number of individuals with diabetes and hypertension increased in the patients group than the control group with no significant differences ($p>0.05$).

Table 3 explains the relationship between studied parameters in coronary atherosclerotic patients using Pearson correlation. There was a negative significant correlation between PAF, and HDL-C ($r=-0.44$; $P<0.05$), and positive significant correlations between PAF with Hs-CRP ($P<0.05$).

Discussion

Table 2. Distribution of the inflammatory biomarkers and risk factors between the coronary atherosclerosis patients and the control groups.

Variables	Patients No.50	Control No.30	P
Inflammatory biomarkers			
PAF(mg/L)	2.35±0.45	0.38±0.01	0.001
hsCRP(mg/L)	10.8±2.49	2.44±1.31	0.001
WBC(103/µL)	9.61±0.59	8.32±0.34	0.001
LDL(mg/dl)	94.5±4.31	85±0.47	0.295
HDL(mg/dl)	35.2±1.52	46.78±0.87	0.344
Risk factors No (%)			
Smokers	37(48.3)	12(22.5)	0.03
Obese	32(40)	12(15.4)	0.001
DM	28(36.8)	15(19.7)	0.35
Hypertension	30(37.5)	12(25)	0.08

Data are presented mean ± SE and as number (%). Body mass index: BMI; HPA1c: Hemoglobin A1c; PAF: platelet-activating factor; hsCRP: high sensitivity C reactive protein; WBC; White blood cells; DHL-C: high-density lipoprotein-cholesterol; LDL-c; LDL: low-density lipoprotein-cholesterol.

Table 3. Pearson correlation's coefficient to clarify the powerful relationship between inflammatory parameters in coronary atherosclerosis patients.

Parameters		PAF (pg/ml)	Hs-CRP (mg/l)	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
PAF(pg/ml)	r		0.61*	0.13	-0.19	-0.54*	0.18
	P value		0.03	0.72	0.39	0.04	0.39
Hs-CRP(mg/l)	r	0.61*		0.12	-0.18	-0.41	0.32
	P value	0.03		0.59	0.67	0.06	0.24

The incidence of coronary atherosclerosis in the present study was more predominant in males than females, similar to that registered in a previous study that the incidence of the disease develops 7 to 10 years later in women compared with men. The under-recognition of heart disease and change in clinical presentation in women lead to less assertive treatment strategies and a lower engagement of women in clinical trials (7).

Obese individuals in this study were more predominant among the patient group. Such observations were agreeable to the results obtained by another study concerning obesity (8). Inflammation constitutes a mechanistic link between obesity and atherosclerosis, adipokines released by adipose tissue induce insulin resistance, endothelial dysfunction, and general inflammation, all of which can increase atherosclerosis (1).

Smoker habits were significantly increased among the patient group of this study compared with the healthy control, which agrees with the previous result of another study (9). Cigarette smoke promotes inflammation by inducing the production of various pro-inflammatory cytokines (10).

Patients with CAD had a higher WBC compared with healthy controls. This outcome was the same as another study that stated patients with CAD had a higher WBC compared with normal coronary arteriograms and total WBC counts were related to the severity of coronary artery disease, and higher WBC counts enhanced the risk of CVDs (11, 12).

CRP serum level was enhanced significantly in coronary atherosclerosis patients compared to the control group in this study. It was in agreement with a study that showed that the CRP level was significantly enhanced in acute coronary syndrome patients compared to the patients without a history of the disease (13). Additionally, the present result approved that the serum level of CRP as an inflammatory marker had a significant positive correlation with the PAF marker. A correlation was also found between CRP level and common markers of inflammation in the previous studies (9, 14). Proinflammatory cytokine in combination with LDL-C and Hs-CRP played an important role in the obese atherosclerotic patient's pathophysiology that can be used as predictors for the development of disease (15). The serum CRP level is considered the surrogate of chronic inflammation (16). Serum PAF levels in this study were significantly increased in patients with coronary artery atherosclerosis compared with healthy individuals. The plasma PAF activity also increased gradually in stable angina and in acute coronary syndrome in a study by Blankenberg in both gender (in men ($P < 0.0001$) and in women ($P < 0.001$)) as compared with controls (17). Similarly, plasma PAF levels were higher in coronary heart disease patients than in controls in another study (18).

As mentioned, there was a negative significant correlation between PAF, and HDL-C, and a positive significant correlation between PAF with Hs-CRP. several bioactive lipids have been identified in the atherosclerotic plaque, including PAF. The potential relevance of PAF to atherosclerosis remains the subject of debate, and recent results suggest that the potential role of the LDL-associated PAF in atherogenesis may be distinct from that of the HDL-associated enzyme (19). PAF itself is formed in LDL during oxidation and plays an important role in the formation of the atherosclerotic plaque. Some studies showed that PAF activity was associated with HDL particles and plays an anti-inflammatory role. But the relation of LDL and PAF remains controversial (19). In this regard, a genome-wide association study to identify candidate genes (20-23) may be useful.

PAF is an important physiological regulator and factor for initial atherosclerosis. In patients with artery disease, PAF can be better than other biomarkers. In conclusion, the PAF marker could be applied as a novel valuable diagnostic inflammatory biomarker for coronary atherosclerosis. Signaling influences of PAF associated with atherosclerosis.

Conflict of Interest

No conflict of interest.

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