European Review for Medical and Pharmacological Sciences 2021; 25: 2291-2298

Effect of coexistence of *Chlamydia pneumoniae* and increased epicardial fat thickness on coronary artery disease

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Abstract. – OBJECTIVE: Epicardial fat thickness (EFT) and chlamydia infection are independent cardiovascular risk factors in coronary artery disease (CAD). We aimed to evaluate the effect of coexistence of EFT and chlamydia infection on the presence and severity of CAD in patients with stable angina pectoris (SAP).

PATIENTS AND METHODS: The study included 208 patients with SAP, divided into a CAD group (n=112) and a control group (n=96). The presence of *Chlamydia pneumoniae*-IgG (CP-IgG), EFT, and left ventricular ejection fraction (LVEF) were compared between groups.

RESULTS: CP-IgG, LVEF, and EFT were found to be independent predictors of CAD (CP-IgG, OR=1.559, *p*=0.021; LVEF, OR=0.798, *p*<0.001; EFT, OR=3.175, *p*=0.026). Moreover, a statistically significant interaction was detected between CP-IgG and EFT for predicting the presence of CAD (*p*<0.001). A good positive correlation was found between EFT and Gensini score (r=0.684, *p*<0.001).

CONCLUSIONS: We found that there was an interaction between CP-Ig and EFT for CAD development. This finding suggests that the interaction of CP-IgG and EFT plays a prominent role in the inflammatory process.

Key Words:
Chlamydia pneumoniae, Epicardial fat thickness, Gensini score, Coronary artery disease.

Abbreviations

EFT: Epicardial Fat Thickness, CAD: Coronary Artery Disease, SAP: Stable Angina Pectoris, LVEF: Left Ventricular Ejection Fraction, CP: *Chlamydia Pneumoniae*, HT: Hypertension, ACS: Acute Coronary Syndrome, SAP: Stable Angina Pectoris, CAG: Coronary Angiographic, PAD: Peripheral Artery Disease, ASE: American Society of Echocardiography, MIF: Microimmunofluorescence, Cx: Circumflex Artery, LAD: Left Anterior Descending, D1: first diagonal branch, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, SD: Standard Deviation, DM: Diabetes Mellitus, CI: Confidence İnterval, ICCs: Intraclass Correlation Coefficients, TNF-α: Tumor Necrosis Factor Alpha, HSP: Heat Shock Proteins.

Introduction

Coronary artery disease (CAD) is a leading life-threatening cardiovascular disease that causes high morbidity and mortality[1,2]. Although classic risk factors, such as smoking, hypertension (HT), diabetes, age, gender, and family history are associated with CAD, inflammation is also considered to play a role in the pathogenesis of CAD and to trigger atherosclerotic plaque formation[3]. Notably, this hypothesis is further supported by the identification of macrophage infiltration in atherosclerotic plaques[3,4]. Moreover, the hypothesis has also been supported by numerous studies, and numerous novel theories have been proposed about the inflammatory process[5]. One of these theories posits that some bacterial infections contribute to the atherosclerotic process *via* inflammation, and this contribution has been supported by the strong correlation between *chlamydia pneumoniae* (CP) and atherosclerosis formation[6,7].

Epicardial fat thickness (EFT) is known to be closely associated with CAD formation[8-10]. This association is considered to be caused by the proinflammatory and proatherogenic cytokines secreted by EFT[8,11-13]. The inflammatory

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process induced by cytokines is considered to contribute to atherosclerosis formation[14]. Although the roles of EFT and CP in atherosclerosis formation have been separately examined by numerous studies, most of these studies evaluated patients with acute coronary syndrome (ACS) and, to our knowledge, none of the studies have investigated the role of inflammation and EFT in atherosclerosis formation in individuals without ACS.

In the present study, we aimed to investigate the independent effects of EFT, anti-CP antibody (CP-IgG), and the interaction of these factors on CAD development in patients presenting with stable angina pectoris (SAP).

Patients and Methods

The study included 208 patients with SAP who presented to our outpatient clinic and underwent coronary angiography (CAG) due to a diagnosis or suspicion of CAD between 2017 and 2019. SAP was diagnosed based on the diagnostic criteria of typical angina pectoris in line with the 2013 ESC guidelines on the management of stable CAD[15]. Patients with pregnancy, arrhythmia, chronic kidney disease, history of CAD, coronary stenosis <50%, peripheral artery disease, history of cerebrovascular disease, chronic lung disease, or a family history of hyperlipidemia and malignancy were excluded from the study. Of the 208 patients, the 112 patients found to have CAD were included in the CAD group and the remaining 96 patients who had a normal coronary anatomy were included in the control group. The study was conducted in accordance with the Declaration of Helsinki and the study protocol was approved by the Local Ethics Committee.

Echocardiography examination was conducted in all patients and each patient was queried about the drugs they were receiving. The echocardiography examination was performed at least 15 min after the rest using a Vivid E9 device (Vivid 9 Pro, General Electric Medical Systems, Milwaukee, WI, USA) and an X5-1 transthoracic probe in the left lateral position (two-dimensional, M-mode, color Doppler echocardiography) using parasternal and apical windows. All of the echocardiography examinations were performed in accordance with the American Society of Echocardiography guidelines and the European Standard Echocardiography Guidelines[16].

Measurement of EFT

Parasternal longitudinal and transverse parasternal views were used to measure the EFT on the right ventricular free wall, and the mean value of the two measurements was calculated. An echo-free space between the outer wall of the myocardium and the visceral layer of the pericardium was defined as epicardial fat tissue. Its thickness was measured perpendicularly on the free wall of the right ventricle at end-diastole in three cardiac cycles according to a predefined method[17]. All assessments were performed by two experienced cardiologists.

Blood samples were centrifuged, and serum samples were examined for anti-CP IgG antibody (CP-IgG) using the microimmunofluorescence method with a Chlamydia IgG SeroFIA kit (DADE Behring, Savyon Diagnostics Ltd., Israel) in our microbiology laboratory. The kit had a sensitivity and specificity of 100% and 96.7%, respectively, and subjects were considered seropositive if CP-IgG was ≥1:64.

CAG images were acquired using a biplane angiographic system (Artis zee, Siemens Medical Solutions, Forchheim, Germany). The severity of coronary lesions was evaluated using Gensini score, in which 5 points were assigned for a left main coronary artery lesion; 2.5 points for proximal left anterior descending artery and left circumflex artery (Cx) lesions; 1.5 points for a mid-left anterior descending (LAD) lesion; 1 point each for the first diagonal branch (D1), obtuse marginal branch, and the right coronary artery lesions; 0.5 points for second diagonal (D2) or posterolateral branch of CX lesions; 1 point for narrowing between 0% and 25%; 2 points for narrowing between 25% and 50%; 4 points for narrowing between 50% and 75%; 8 points for narrowing between 75-90%; 16 points for narrowing between 90% and 99%; and 32 points for a 100% occluded lesion. Subsequently, a defined coefficient for each segment of coronary stenosis was multiplied by a score corresponding to the degree of points[18,19]. All assessments were performed by two experienced cardiologists.

Patients with a systolic blood pressure >140 mmHg and a diastolic blood pressure >90 mmHg were considered hypertensive. Moreover, patients with a fasting blood glucose >126 mg/dl and patients receiving treatment due to a previous diagnosis of diabetes mellitus (DM) were considered diabetic. Patients were divided into two groups based on their smoking history: current smokers and never smokers.

Statistical Analysis

All statistical tests were conducted using the Statistical Package for the Social Sciences 19.0 for Windows (SPSS Inc., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess normality of the data. Continuous data were expressed as mean ± standard deviation (SD) and categorical data were expressed as percentages. The Chi-square test was used to assess differences in categorical variables between groups. The relationships among parameters were assessed using Pearson's or Spearman's correlation coefficient according to the normality of the data. Student's *t*-test or the Mann-Whitney U test was used to compare unpaired samples as needed. Univariate and multivariate logistic regression analyses were used to identify independent variables of ACS. Independent variables used in univariate analysis included age, gender, chlamydia, left ventricular ejection fraction (LVEF), creatinine, EFT, HT, DM, and smoking. After performing univariate analysis, variables that were found to be statistically significant were entered into the multivariate logistic regression analysis using the stepwise method. The results of univariate and multivariate regression analyses were presented as odds ratios (ORs) with 95% Confidence Intervals (CIs). Furthermore, the interaction analysis was performed with a generalized linear model for CP-IgG and EFT. Finally, 15 patients were randomly selected to assess the respective intra- and interobserver variabilities for EFT and the Gensini score, expressed as intraclass correlation coefficients (ICCs). Results were considered to be statistically significant at a 2-sided *p*-value of <0.05.

Results

The study included 112 patients with CAD and 96 controls without CAD. Table I presents the clinical and demographic characteristics of participants in both groups. No significant differences were found between the groups with regard to age and gender. The CAD group included 94 (84%) men and 18 (16%) women, and the control group comprised 74 (77%) men and 22 (23%) women. The mean age was 60.83 ± 12.09 years in the CAD group and 58.60 ± 10.61 years in the control group. The CP-IgG level was significantly higher in the CAD group compared to the control group (77 [68%] *vs.* 31 [32%]; $p<0.001$). Similarly, LVEF was significantly lower in the CAD group compared to the control group (46.66 ± 13.04 *vs.* 62.60 ± 4.15; $p<0.001$). No statistically significant differences were found between the two groups with regard to biochemical and blood parameters. However, EFT was significantly higher in the CAD group compared to the control group (3.16 ± 0.81 *vs.* 2.51 ± 0.67; $p<0.001$). Moreover, the incidence of HT and DM was significantly higher in the CAD group compared to the control group (HT, 50 patients [44%] *vs.* 30 patients [31%], $p=0.048$; DM, 40 patients [35%] *vs.* 20 patients [20%], $p=0.018$; Table I).

Table I. Clinical and demographic characteristics.

Parameters	CAD group (n = 112)	Control group (n = 96)	p
Age (years)	60.83 ± 12.09	58.60 ± 10.61	0.163
Gender (male) (%)	94 (84%)	74 (78%)	0.216
Chlamydia, n (%)	77 (68%)	31 (32%)	< 0.001
LVEF (%)	46.66 ± 13.04	62.60 ± 4.15	< 0.001
Hemoglobin (g/dl)	14.38 ± 3.93	14.40 ± 1.57	0.953
Hematocrit (%)	44.35 ± 8.15	44.39 ± 5.06	0.960
Platelet count ($10^3/\mu L$)	232.14 ± 72.24	242.95 ± 60.02	0.240
Sodium (mmol/l)	137.94 ± 2.93	138.38 ± 2.97	0.286
Potassium (mmol/l)	4.21 ± 0.53	4.18 ± 0.59	0.715
AST (IU/dl)	25 (18-39)	22 (18-30)	0.102
ALT (IU/dl)	22 (14-31)	19 (14-25)	0.172
Creatinine (mg/dl)	0.85 ± 0.24	0.82 ± 0.16	0.134
EFT (mm)	3.16 ± 0.81	2.51 ± 0.67	< 0.001
Gensini score	38 (22-74)	–	–
Hypertension, n (%)	50 (44%)	30 (31%)	0.048
Diabetes mellitus, n (%)	40 (35%)	20 (20%)	0.018
Smoking, n (%)	72 (65%)	66 (69%)	0.688

CAD: Coronary artery disease, LVEF: Left ventricular ejection fraction, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, EFT: Epicardial fat thickness.

The patients were divided into two groups according to the presence of CP-IgG. LVEF was lower in the IgG (+) group compared to the IgG (-) group (47.6 ± 14.0 *vs.* 60.9 ± 5.8; $p<0.001$; Figure 1). IgG (+) CAD patients had a higher mean Gensini score compared to IgG (-) CAD patients (63.0 ± 31.4 *vs.* 19.7 ± 10.9; $p<0.001$; Figure 2). Moreover, a good positive correlation was found between EFT and Gensini score (r=0.684, $p<0.001$; Figure 3).

Factors associated with CAD, including age, gender, CP-IgG, LVEF, creatinine, EFT, HT, DM, and smoking, were initially analyzed by univariate analysis. Of these, CP-IgG, LVEF, and EFT were found to be statistically significant on univariate analysis and were then analyzed by multivariate analysis, which indicated that CP-IgG, LVEF, and EFT are independent predictors of CAD (CP-IgG, OR=1.559, $p=0.021$; LVEF, OR=0.798, $p<0.001$; EFT, OR=3.175, $p=0.026$; Table II). Moreover, a statistically significant interaction was detected between CP-IgG and EFT for predicting the presence of CAD ($p<0.001$).

Reproducibility

Intra- and interobserver reliability of the EFT and the Gensini score were assessed in 15 randomly selected subjects and expressed as ICCs. ICCs for intra- and interobserver reliability for EFT were 0.92 (95% CI, 0.86-0.95) and 0.90 (95% CI, 0.82-0.94), respectively, and 0.92 (95% CI, 0.85-0.94) and 0.91 (95% CI, 0.84-0.96), respectively, for Gensini score.

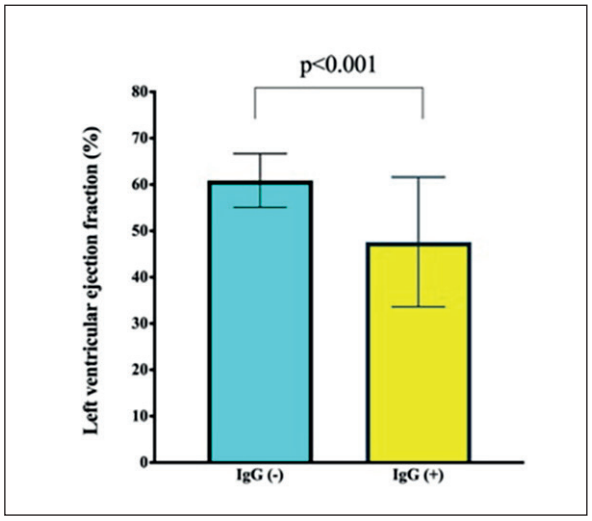

Figure 1. Comparison of left ventricular ejection fraction between chlamydia pneumoniae IgG (+) and IgG (-) patients (47.6 ± 14.0 *vs.* 60.9 ± 5.8; $p<0.001$).

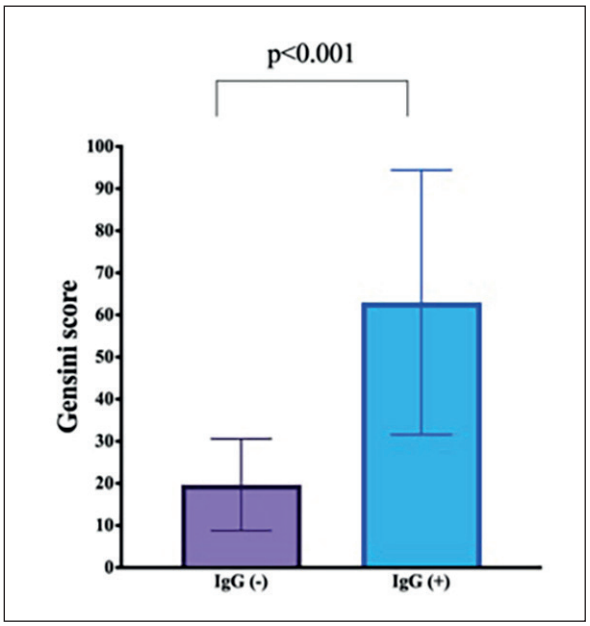

Figure 2. Comparison of gensini scores between chlamydia pneumoniae IgG (+) and IgG (-) patients (63.0±31.4 *vs.* 19.7±10.9; $p<0.001$).

Discussion

The present study investigated the interaction between CP-IgG and EFT in patients with SAP and obtained the following results:

1. A significant difference in Gensini score was observed between IgG (+) and IgG (-) CAD patients.
2. EFT was significantly and positively correlated with Gensini score in CAD patients.
3. CP-IgG, LVEF, and EFT were found to be independent predictors of CAD.

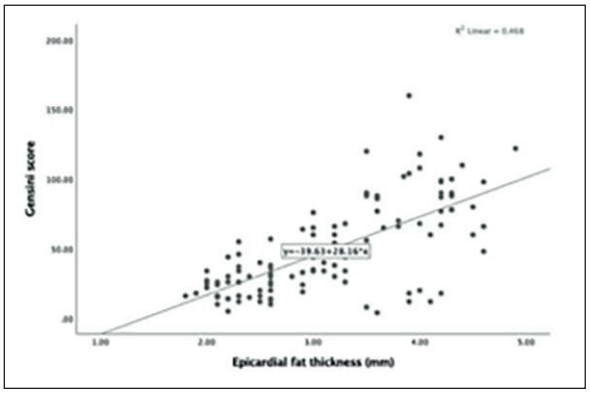

Figure 3. Correlation between EFT and Gensini Score in coronary artery disease patients.

Table II. Multivariate logistic regression analysis to determine the independent predictors of coronary artery disease.

Variable	Univariate			Multivariate		
	OR	95% CI	*p*	OR	95% CI	*p*
Age (years)	1.017	0.993-1.042	0.163			
Gender	1.111	0.937-1.280	0.468			
CP-IgG	5.020	2.783-9.055	< 0.001	1.559	1.121-1.994	0.021
LVEF	0.779	0.723-0.839	< 0.001	0.798	0.737-0.864	< 0.001
Creatinine	2.425	0.388-15.163	0.334			
EFT	3.144	2.072-4.771	< 0.001	3.175	2.103-4.411	0.026
Hypertension	2.544	0.483-12.770	0.279			
Diabetes mellitus	1.257	0.628-1.892	0.262			
Smoking	1.138	0.865-1.418	0.412			

CP: Chlamydia pneumonia, LVEF: Left ventricular ejection fraction, EFT: Epicardial fat thickness, CI: Confidence interval.

4. There was a statistically significant interaction between CP-IgG and EFT for predicting the presence of CAD.

To date, numerous risk factors and pathophysiological mechanisms have been implicated in the development of CAD. However, the contribution of infectious agents to these risk factors and pathophysiological mechanisms is not a recent subject. The earliest studies[20,21] reporting on this contribution demonstrated a relationship between herpes simplex virus and cytomegalovirus and the atherosclerotic process. Following these studies, other studies showed that the levels of CP-IgG antibodies were significantly elevated in patients with ACS and stable CAD compared to control subjects[22]. This elevation has been confirmed by numerous studies investigating the relationship between CP and CAD, and a plethora of theories have been proposed by the authors of these studies regarding the pathophysiology of this elevation.

Inflammation induced by proinflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukins, is considered to trigger the atherosclerotic process[23], which in turn leads to insulin resistance, ultimately resulting in atherosclerosis[24]. This relationship between CP and atherosclerosis is further supported by the identification of lipoprotein-containing immune complexes in atherosclerotic plaques[25] and by the identification of these complexes in almost 70% of patients with acute myocardial infarction[26,27]. Additionally, this relationship could also be explained by the remarkable resemblance between the heat shock proteins (HSPs) expressed by many bacteria and human HSPs: the antibodies produced against bacterial HSPs are thought to contribute to this process by cross-reacting with human HSPs[28]. Taken together, these findings indicate that there are numerous pathophysiological mechanisms involved in the relationship between CP and atherosclerosis development. Considering these mechanisms, it is not surprising that a relationship was detected between Gensini score and CP-IgG in our study.

To our knowledge, there are very few studies in the literature investigating the relationship between CP and Gensini score, and these studies have mostly been conducted in patients with ACS[29,30]. It is commonly known that sudden changes in Gensini score may occur in ACS patients due to the unstable plaque burden and the presence of thrombus in these patients. These changes, in turn, may produce different outcomes within several hours. In SAP patients, however, Gensini scores tend to be more consistent due to the presence of stable lesions. In contrast to studies focused on ACS patients, we evaluated SAP patients and found a strong positive correlation between Gensini score and CP-IgG, which suggests that CP may not only cause CAD, but also lead to common vascular diseases.

In our study, a strong negative correlation was found between LVEF and CP-IgG, which was consistent with the findings reported in the literature[29,31-33]. However, since previous studies have evaluated LVEF only in ACS patients, it is not clear whether the findings presented in those studies were associated with ACS or CP infection. In our study, unlike in previous studies, we evaluated LVEF in SAP patients and found that LVEF levels were significantly lower in CAD patients compared to control subjects, which suggests that

the CP agent is likely to have a negative impact on LVEF independent of ACS. Nevertheless, further studies with long-term follow-up periods are needed to substantiate this hypothesis.

As previously mentioned, the relationship between CP and atherosclerosis is associated with numerous mechanisms, particularly inflammation. Epicardial fat is a tissue in humans that exerts proinflammatory effects by secreting proinflammatory and proatherogenic cytokines and has been shown to contribute to atherosclerosis development[34-37]. This contribution has been further supported by the studies reporting a relationship between EFT and CAD[38,39]. Accordingly, it is not surprising that both CP infection and EFT were found to be independent predictors of CAD in the present study, which also implies that the toxic and proatherogenic cytokines secreted by epicardial fat may contribute to the atherosclerotic process independent of CP infection. However, although these two factors could be considered to exert their effects *via* inflammatory processes, our findings indicated that these effects are exerted through different mechanisms. Moreover, although the findings obtained in the present study are not sufficient to thoroughly explain the relationship between EFT and CP-IgG, they indicated that the coexistence of increased EFT and CP-IgG is likely to precipitate CAD.

Limitations

Our study had some limitations. First, it was a single-center study and had a small patient population. Second, it did not evaluate mortality (due to insufficient follow-up data on the patients) or how quickly the investigated parameters precipitated CAD. Finally, the study presented no information regarding antibiotic usage among patients, mainly because it is almost impossible to obtain information on the dosing of antibiotic drugs and whether these drugs prevent CAD development in CP patients.

Conclusions

The present study demonstrated that CP plays a role in the etiology of CAD, as it has been shown in numerous studies in the literature. Although the relationship between EFT and CAD has been documented previously, the present study concurrently evaluated two different factors that are known to increase the risk of CAD (EFT and CP-IgG). Both of these factors, despite exerting their effect via inflammation, were found to be independent predictors of CAD, which suggests that CAD may have multiple pathophysiological mechanisms. Patients with these two conditions should be promptly diagnosed and evaluated for CAD to prevent the formation of cardiovascular diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

No funding is declared for this article.

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