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Similarity of Adipocytokines Level in Radial and Coronary Artery Associated with Epicardial Adipose Tissue Thickness

[1]Abdul Hakim Alkatiri, [2]Syakib Bakri, [3]Ilhamjaya Patellongi, [1]Peter Kabo, [4]Gatot Susilo Lawrence, [1]William Horas and [1]Mirnawati Mappiare

One of the most important anti-inflammatory protein is adiponectin. It acts to prevent the deleterious of TNF-α on endothelial cell by reducing level of adhesion molecules. To investigate the correlation between serum tumor necrosis factor (TNF)-α, high sensitivity C-reactive protein (hs-CRP) and adiponectin level in coronary artery (CA) and those in the radial artery (RA). This study also sought to investigate the association of those results with epicardial adipose tissue (EAT) in coronary artery disease subjects. A cross-sectional study of 110 Indonesian subjects who underwent transthoracic echocardiography (TTE) and diagnostic coronary angiography were studied. Blood samples from RA and CA were collected before coronary angiography. Pearson correlation analysis was used to determine the relationship between mean values and all statistical analysis was done by using SPSS. This study found forty three subjects with severe coronary artery disease (CAD) and thicker EAT (8.5+6.9 vs 6.1+2.6, $p<0.001$). Analysis of the correlation between serum level of TNF-α in RA versus CA showed for linear correlation in non-severe CAD ($r = 0.57$, $p<001$), severe CAD ($r = 0.67$, $p<001$) and all subjects ($r = 0.61$, $p<0.001$). Serum level of hs-CRP showed strong correlation for non-severe CAD ($r = 0.99$, $p<0.001$), severe CAD ($r = 0.98$, $p<0.001$) and all subjects ($r = 0.99$, $p<0.001$). Furthermore, analysis of serum level of adiponectin for non-severe CAD ($r = 0.87$, $p<001$), severe CAD ($r = 0.86$, $p<0.001$) and all subjects ($r = 0.86$, $p<0.001$) also showed linear correlation in both sample site. A linear correlation between TNF-α, hs-CRP and adiponectin in RA and CA associated with EAT in CAD subjects.

Key words: Inflammatory markers, adipocytokines, epicardial adipose tissue, coronary artery disease, adiponectin

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Abdul Hakim Alkatiri
Department of Cardiology,
Faculty of Medicine, Hasanuddin,
University, Makassar, Indonesia

Tel: +62 411 590939
Fax: +62 411 590939

[1]
Department of Cardiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[3]Department of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[4]Department of Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

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INTRODUCTION

Inflammation plays a key role in the pathogenesis of atherosclerosis[1,2]. EAT is located inclose proximity to coronary artery and has been reported to be a source of inflammatory mediators[3].

TNF-α is the one of the most important mediators of inflammation where secreted not by adipocytes but instead by infiltrating macrophages in adipose tissues. This marker activates the transcription factor-kβ, which organizes inflammatory changes in vascular tissue[4,5].

The CRP has been shown to be an important marker of vascular inflammation and a predictor of atherosclerosis[6]. Synthesis of CRP is mainly hepatic and some reported the extra-hepatic such as inflamed coronary artery. The CRP synthesis and secretion by cell in atherosclerostic lesion by paracrine/autocrine loops could result in local concentration of CRP far in excess of plasma concentration[7]. The CRP secretion is augmented 100-fold in presence of macrophage conditioned media (MCM)[8].

One of the most important anti-inflammatory protein is adiponectin. It acts to prevent the deleterious of TNF-α on endothelial cell by reducing level of adhesion molecules. Secretion of adiponectin inversely related to the expression of pro-inflammatory adipokine[4]. The mechanism for the anti-atherosclerotic effect of adiponectin is not fully understood. Some studies have shown direct effect of adiponectin on smooth muscle cell and endothelial[9].

The aim of this study was to investigate the correlation between serum TNF-α, hs-CRP and adiponectin level in CA and those in the RA. This study also investigate the association of those results with EAT in CAD subjects.

MATERIALS AND METHODS

Recruitment of subjects: Those patients who recruited were 110 patients with clinical CAD, age 38-71 years (83 males and 27 females), who underwent coronary angiography from September, 2015-February, 2016. All subjects gave written informed consent and this study was approved by Hasanuddin University and Wahidin Sudirohusodo Hospital ethic committee.

Excluded from this study were individuals with acute coronary syndrome (<7 days), severe inflammation or infection disease, subjects with evidence of severe hepatic, pulmonary or renal disease and subjects using steroids or non-steroid anti-inflammatory drugs (NSAIDs) medication.

Blood sample collection: Subjects were put in supine position during procedure. The collection of blood sample from radial artery as the systemic site and coronary blood was taken from coronary osteal through catheter before contrast were given.

Measurement of EAT thickness: The EAT thickness was measured by TTE using a vivid 7 (GE, Horten, Norway) while the patients were lying on the left lateral decubitus position. EAT thickness was measured at the end of the diastole perpendicular to right ventricular free wall[10-12].

Coronary angiography: Coronary angiography was performed through radial access, using Tiger catheter with standardized projection and the results of this examination indicated the degree severity of stenosis. Angiograms were recorded in multiple projections with a biplanar digital cardiac imaging system (Philips Integris DCI, Eindhoven, the Netherlands). The severity of coronary artery lesions was scored using a modified Gensini score[13-15]. In brief, the coronary circulation was divided into eight proximal segments, the percentage by which each lesion in the proximal coronary circulation narrowed the artery was assessed according to the maximal narrowing of the diameter of the artery in all projections. The extent and severity of proximal coronary disease was assessed by assigning points to each lesion as follows: $\leq$50% of the luminal diameter, 1 point, 50-74% stenosis, 2 points, 75-99% stenosis, 3 points and total obstruction, 4 points. The points of each lesion in the proximal coronary circulation were summed and a score for severity of coronary atherosclerosis was obtained. According to the modified Gensini score for classification of the degree of coronary stenosis, the results of total scores can be separated into mild lesions (1-6 points), moderate lesions (7-13 points) and severe lesions (>13 points). Accordingly, the study population was classified into two angiographic groups: Patients with non-severe CAD (Gensini score $\leq$13) and patients with severe CAD (Gensini score >13)[15,16].

Measurement of immunoassay for tissue inflammatory mediators and adipocytokines: Serum TNF-α level were measured by enzyme immunoassay kit Quantikine HS Human TNF-α (R and D Systems, Inc., Minneapolis, USA). Briefly 200 µL serum transferred to a 96-well plate coated with a monoclonal antibody specific for human TNF-α followed by incubation for 3 h at room temperature. The optical density of the streptavidin-POD enzyme conjugate was determined using a model 680 microplate reader (Bio-Rad, CA, USA) at 450 nm.

Serum hs-CRP concentration was measured using particle-enhanced immunoturbidi-metric method with Cobas® c501 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Anti-CRP antibodies coupled to latex microparticles reacted with CRP in the sample to form an antigen/antibody complex. The lower detection limit of the hs-CRP assay was 0.15 mg L^{-1}.

Serum total adiponectin were determined using a sensitive enzyme-linked immunosorbent assay kit (SEKISUI MEDICAL Co., Tokyo, Japan). The tests were performed according to the recommendation of the manufacture. Optical density was read using a microplate reader 270 (Biomeriux, France).

Statistical analysis: Continuous variables were expressed as mean±standard deviation. Categorical variables were

compared with chi-square test. The Pearson correlation analysis was used to determine the relationship between mean values. A p-value of less than 0.05 was considered statistically significant. All statistical analysis were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA)[17].

RESULTS AND DISCUSSION

Demographic, echocardiographic and biochemical findings of subjects: There were 83 male and 27 female in the study population. The mean age was 56.2±7.8 years. The demographic, echocardiographic and biochemical findings of this study groups are presented in Table 1. The age, body mass index (BMI), waist circumference, hypertension, dyslipidemia and family history were similar between patients with non-severe and severe CAD. Male gender, smoking and subject with type 2 diabetes mellitus (T2DM) were significantly more in the severe CAD group. Subject with non-severe CAD had significantly higher ejection fraction than were patients with severe CAD. According to the laboratory inflammatory marker, there were no significant differences between the groups.

Figure 1 demonstrates the relationship between TNF-α, adiponectin and hs-CRP concentration in RA and CA. It was found that the inflammatory markers level in both sites were not significantly different in all subjects.

In Fig. 2, it was found that TNF-α level in radial and coronary artery in subjects with non-severe CAD, severe CAD and in all subjects did not show significantly different result.

Figure 3 demonstrates the correlation of hs-CRP levels in RA and CA in patients with non-severe and severe CAD. It was found that there was also no significantly different level of hs-CRP in RA and CA in non-severe CAD, severe CAD and all subjects.

Figure 4 demonstrates correlation of adiponectin levels in RA and CA also in subjects groups. There was no significant difference of adiponectin level between RA and CA in the groups.

It also found that the level of TNF-α and hs-CRP were higher while the adiponectin level was lower in subjects with severe CAD. But these results were not statistically significant as seen in Table 2.

To the best of authors knowledge, this is the first study to analyze the correlation between RA and CA inflammatory markers level in CAD patients in Indonesia. This study showed a linear correlation between TNF-α, hs-CRP and adiponectin level in both sites. Several studies evaluated inflammatory markers level from different sites[2,18-21]. Fontana *et al*.[18] evaluated the correlation between adipokine level in portal vein and radial artery in 25 obese subject. They found that plasma TNF-α, resist in, macrophage chemoattractant protein-1 (MCP-1) and adiponectin concentration were similar in both site. In contrast with level of interleukin-6 (IL-6) and leptin in both site. IL-6 level was higher in portal vein compared with radial artery (42.1±41.8 vs 28.5±27.6 pg mL^{-1}, p =0.007). Leite *et al*.[19] compared hs-CRP level in peripheral vein in left forearm and coronary sinus in CAD patients. Subjects were divided into two groups (stable angina, 20 patients and unstable angina, 20 patients). The result suggested a strong linear correlation between hs-CRP levels in peripheral vein and coronary sinus for both stable angina ($r = 0.993$, $p<0.001$) and unstable angina ($r = 0.976$, $p<0.001$) and for the entire

Table 1: Differences in clinical, echocardiographic and biochemical of the subjects by severity of coronary stenosis

Variable (n = 110)	Non-severe CAD (n = 67)	Severe CAD (n = 43)	p-value
Demographic characteristics			
Age (years)	55.3±7.9	57.8±7.3	0.102
Gender (male/female)	44/23	39/4	0.002
BMI (kg m^{-2})	25.7±4.4	24.9±2.9	0.305
Waist circumference (cm)	92.6±13.6	92.1±11.6	0.848
Hypertension, n (%)	28 (41.8)	21 (48.8)	0.298
Smoker, n (%)	25 (37.3)	27 (62.8)	0.008
Diabetes mellitus, *n* (%)	11 (16.4)	14 (32.6)	0.042
Dyslipidemia, n (%)	63 (94.0)	38 (88.4)	0.239
Family history, n (%)	6 (9.0)	5 (11.6)	0.441
Echocardiographic characteristics			
Ejection fraction (%)	61.7±7.4	57.7±6.9	0.006
EAT thickness (mm)	6.1±2.6	8.5±6.1	<0.001
Inflammatory Markers			
TNF-α (pg mL^{-1})			
• Radialis artery	6.3±8.8	7.1±10.0	0.668
• Coronary artery	7.6±15.1	8.6±14.7	0.750
hs-CRP (mg L^{-1})			
• Radialis artery	4.2±8.1	6.0±8.4	0.283
• Coronary artery	4.3±8.3	6.0±8.7	0.294
Adiponectin (µg mL^{-1})			
• Radialis artery	4.2±2.5	3.8±2.6	0.549
• Coronary artery	4.1±2.7	4.0±2.7	0.907

Values are presented as mean±standard deviation or number (%), EAT: Epicardial adipose tissue, BMI: Body mass index, TNF-α: Tumor necrosis factor alpha, hs-CRP: high sensitivity C-reactive protein

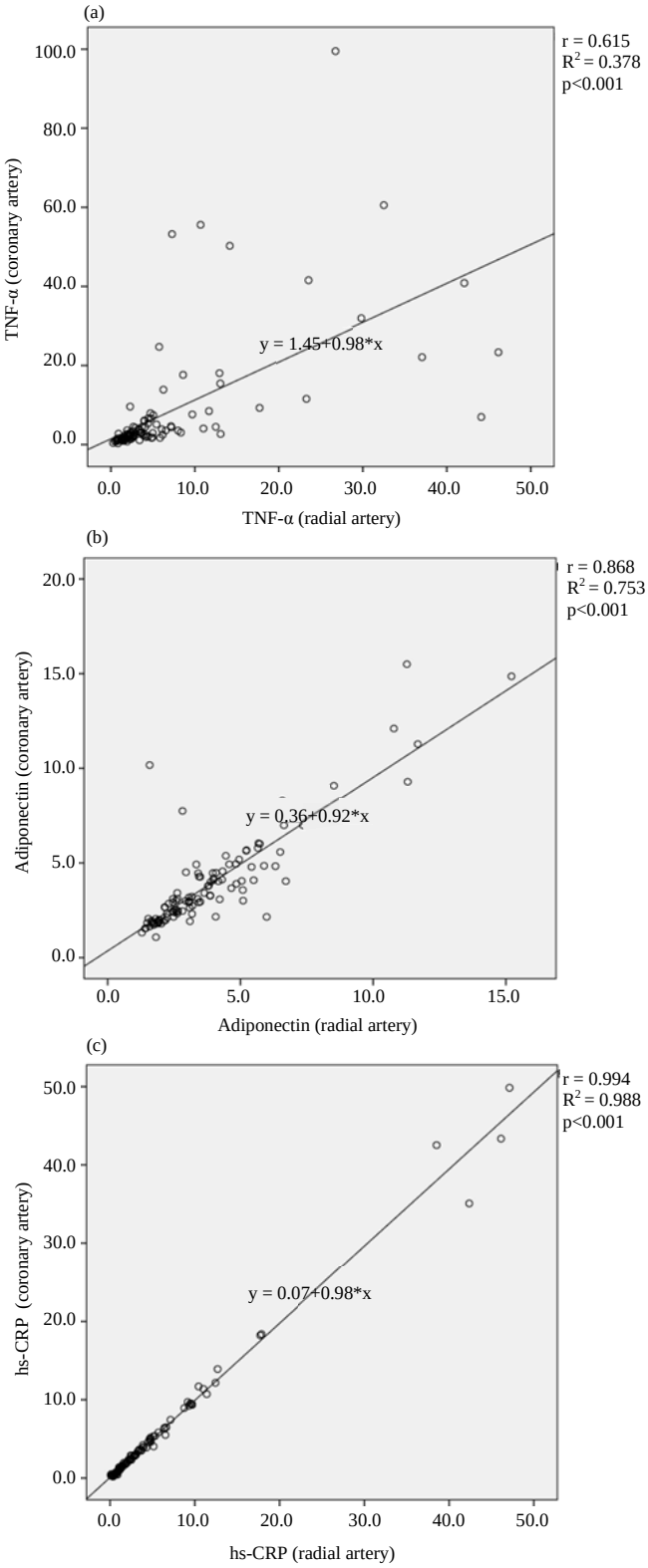

Fig. 1(a-c): Relationship between (a) TNF-α, (b) adiponectin and (c) hs-CRP level in radial and coronary artery in RA and CA

sample (r = 0.985, p<0.001). Sacks *et al.*[20] evaluated the adipokine level in femoral artery and coronary sinus in 11 patients who underwent radioablation. They found that there were no significant differences between concentrations

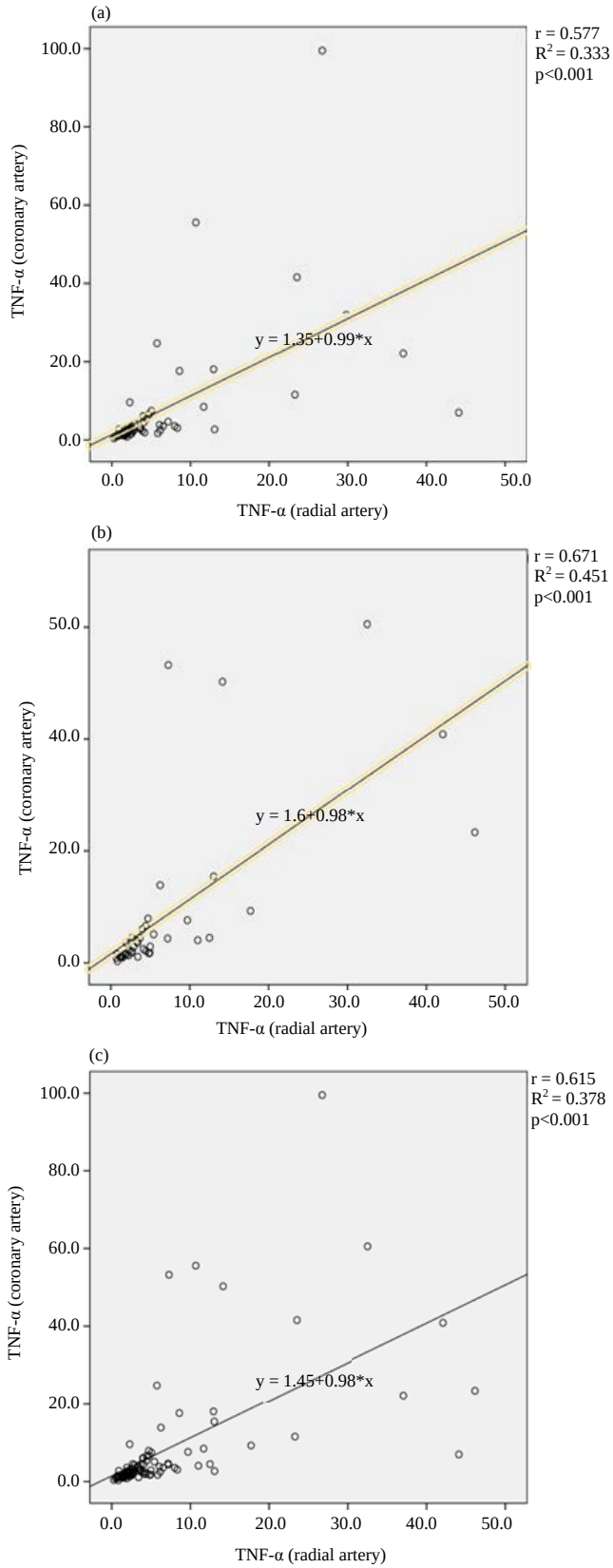

Fig. 2(a-c): Correlation between radial and coronary artery level of TNF-α in patient with, (a) Non-severe CAD, (b) Severe CAD and (c) All subjects

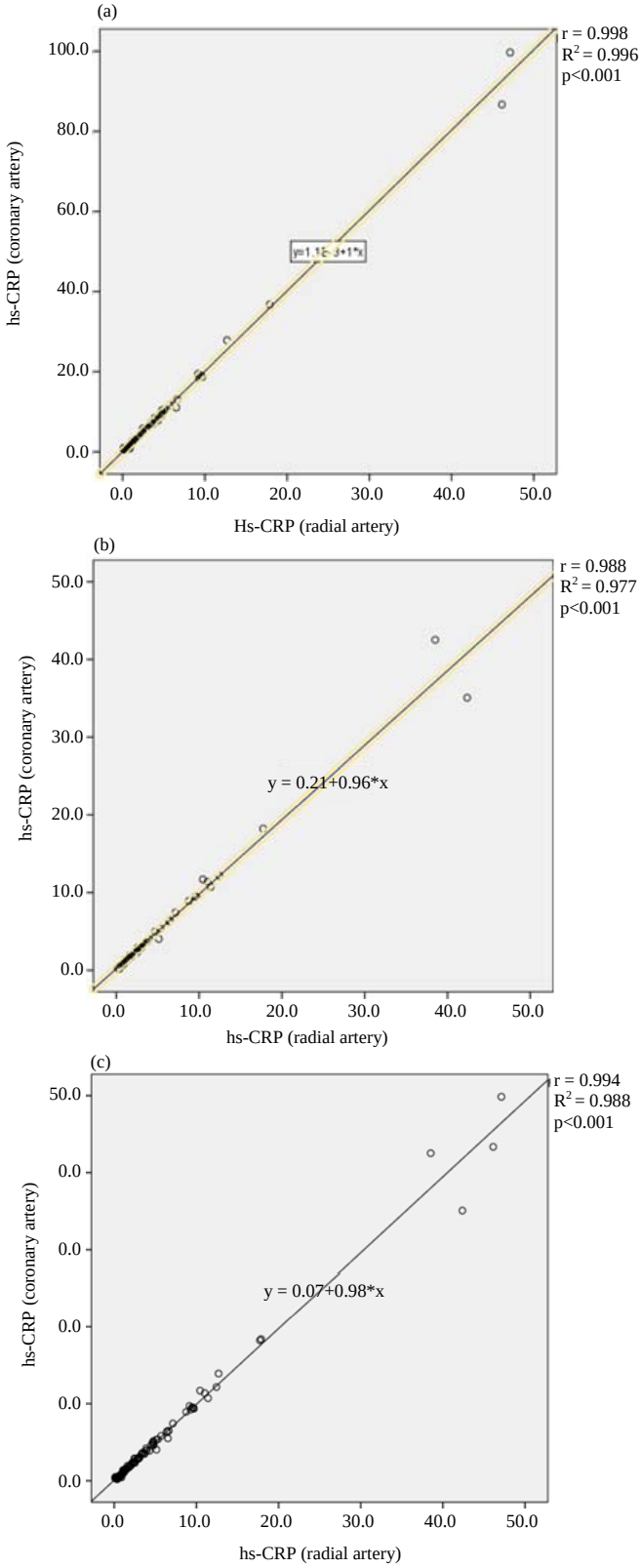

Fig. 3(a-c): Correlation between radial and coronary artery level of hs-CRP in patient with, (a) Non-severe CAD, (b) Severe CAD and (c) All subjects

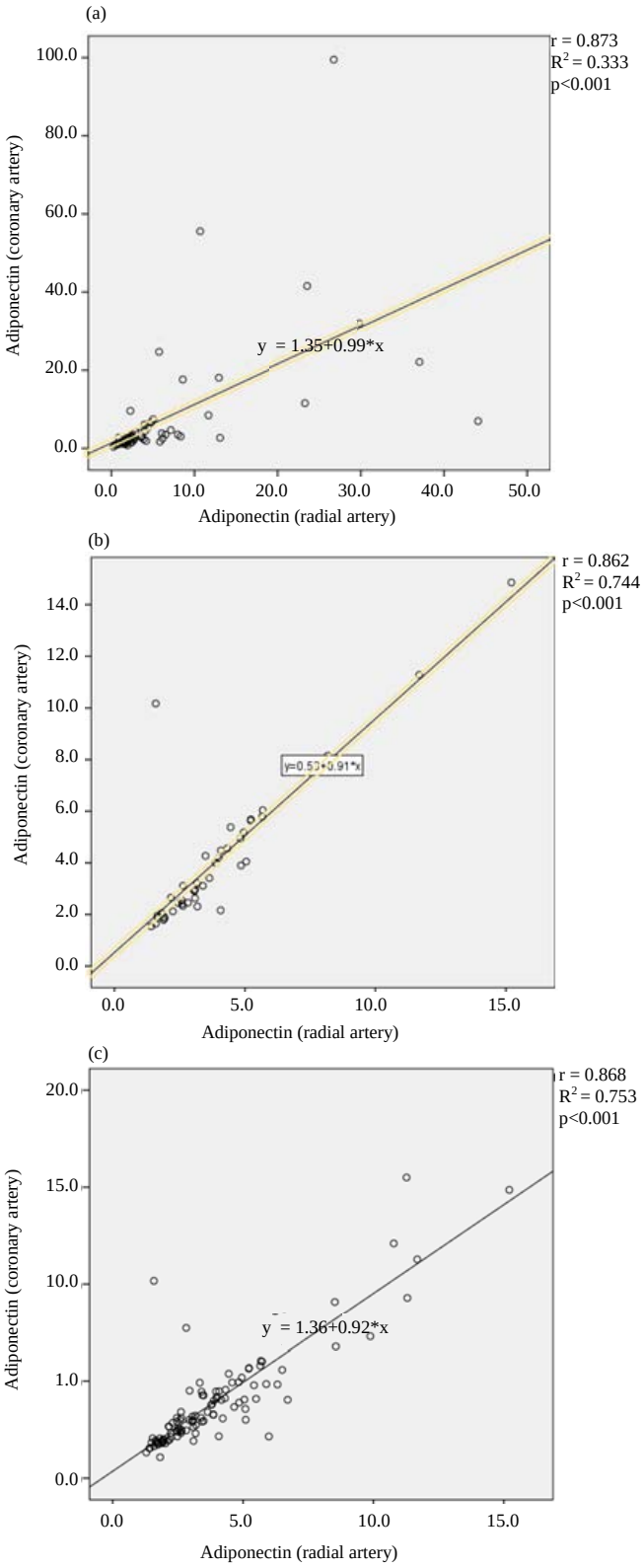

Fig. 4(a-c): Correlation between radial and coronary artery level of adiponectin in patient with, (a) Non-severe CAD, (b) Severe CAD and (c) All subjects

Table 2: Radial and coronary artery level of all markers in non-severe and severe CAD subjects

Inflammatory markers	Sample site		p-value
	Radial artery	Coronary artery	
TNF-α (pg mL^{-1})			
Non-severe CAD	6.3±8.8	7.6±15.1	0.668
Severe CAD	7.1±10.1	8.6±14.7	0.750
All subject	6.6±9.3	7.9±14.9	0.730
hs-CRP (mg L^{-1})			
Non-severe CAD	4.2±8.2	4.3±8.2	0.283
Severe CAD	6.0±8.7	6.0±8.4	0.294
All subject	4.9±8.4	4.9±8.3	0.272
Adiponectin (μg mL^{-1})			
Non-severe CAD	4.2±2.5	4.1±2.6	0.549
Severe CAD	3.9±2.6	4.0±2.7	0.907
All subject	4.0±2.5	4.1±2.7	0.712

Values are presented as mean±standard deviation or number (%), TNF-α: Tumor necrosis factor alpha, hs-CRP: High sensitivity C-Reactive Protein

of adiponectin, leptin, resistin, aPAI-1, MCP-1, TNF-α, IL-1β, IL-6, IL-8 and VEGF in both sites. Zhou *et al*.[21] evaluated adiponectin concentration in peripheral and coronary sinus vein plasma that measured with enzyme-linked immunosorbent assay in CAD and non-CAD patients. They showed that peripheral and coronary sinus plasma adiponectin level were significantly lower in CAD patients than non-CAD patients (5.9±1.4 vs 10.3±2.2 μg mL^{-1}, p<0.01 and 4.5±1.9 vs 12.7±3.1 μg mL^{-1}, p<0.01, respectively). Cheng *et al*.[2] demonstrated that the tissue level of adiponectin in abdominal fat was significantly lower than epicardial fat in CAD patients. In addition according to Marseglia *et al*.[22], obesity is a major cause of morbidity and mortality and leads to several diseases, including metabolic syndrome, diabetes mellitus, cardiovascular, fatty liver diseases and cancer. In the different research, findings from Rajkovic, N., *et al*.[23] the basal levels of adipocytokines and inflammatory markers was done in 18 obese (BMI $\geq$30 kg m^{-2}) (group A), 21 overweight (25 kg m$^{-2}\leq$BMI and it, 30 kg m^{-2}) (group B), 25 non-obese T2D patients (group C) and 15 non-obese controls (group D).

The CRP may contribute to development of the atherosclerotic lesion through its role in complement activation, cell adhesion and recruitment, thrombosis, the expression of regulatory cytokines, apoptosis and lipid[19,24]. It supports this findings that hs-CRP level was higher in severe CAD than non-severe CAD group.

Adiponectin is abundantly present in the circulation and decreases the expression of multiple adhesion molecules through the modulation of nuclear factor kβ signaling in different cell types, including endothelial cells. Adiponectin also dramatically suppresses the secretion of TNF-α from human monocyte/macrophages and thus protects against atheroma formation[9,25]. It is compatible with this findings that TNF-α level in severe CAD group that obtained from coronary artery was higher than from radial artery. In contrast, adiponectin level in severe CAD group that obtained from coronary artery was lower than from radial artery.

Recent study[25] found that inflammatory markers level in subject with severe CAD were higher than non-severe CAD. This findings might be correlated with EAT thickness that significantly greater in severe CAD compared with non-severe CAD (6.1±2.5 mm vs 8.5±2.4, p<0.001). Two potential mechanisms for this association have been proposed. First, EAT is a component of visceral adiposity and is related to metabolic syndrome and cardiovascular risk factors, secondly, EAT has paracrine and endocrine functions. It can secrete numerous bioactive molecules (adipokines) such as adiponectin, resist in and inflammatory cytokines (IL-1b, IL-6 and TNF-α). Sacks and Fain[26] pointed out the paracrine and vasocrine signaling effects of epicardial adipokines for the development of atherogenesis. In patients with severe CAD, inflammatory adipokines are expressed and secreted in greater amount from EAT and therefore the potential exists that one or more adipokines might be released from EAT into coronary blood under pathophysiological circumstances[26]. Study By Hu *et al*.[27] found the effects of a low-carbohydrate diet (40 g/day, n = 75) versus a low-fat diet (30% kcal/day from total fat, 7% saturated fat, n = 73) on biomarkers representing inflammation, adipocyte dysfunction and endothelial dysfunction in a 12 month clinical trial among 148 obese adults free of diabetes and CVD.

A significant decrease in the analysed regulatory molecules, i.e., leptin receptor and adiponectin, was found in blood plasma of the patients with untreated type 2 diabetes[28]. The pathogenic profiles of adipokines expressed in fat tissues can alter the vascular inflammation burden and have remote influences on the cardiovascular system. The impact of adipocytokines has been recently extended from endocrine to paracrine roles. EAT lack fascial structures to separate themselves from underlying myocardium and the phenomenon with increased infiltration of inflammatory cells in EAT had been observed in CAD patients. The EAT thickness was found to be correlated with the severity of CAD in patients with CAD. These reports suggested that adipocytokines derived from EAT might have direct pro-inflammatory responses in CAD pathogenesis[2,29]. This study is based on a cross sectional analysis of a single center cardiac catheterization. In addition, we cannot confirm whether inflammatory markers result from EAT, atherosclerotic process itself, or both.

CONCLUSION

This research finds evidence that the adipocytokines concentration level in the coronary artery and radial artery in

coronary artery disease subjects are not significantly different. This research also shows no significant correlation between the concentration level and EAT thickness.

SIGNIFICANCE STATEMENTS

This research showed that there is no significant difference between the adipocytokines level in central and peripheral circulation in patients with coronary artery disease. This study will help the researcher to find out deeply the function and the regulation of the tissue inflammation expression of those adipocytokines. Further studies are needed to investigate the correlation between tissue inflammation expression and atherosclerosis process.

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