Plasma Concentrations of Adiponectin in Patients with Coronary Artery Disease and Coronary Slow Flow

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ABSTRAK

Latar belakang: *Adiponektin, hormon yang disekresikan oleh adiposit yang berperan pada homeostasis energi dan memiliki efek antiinflamasi, antioksidan, endothelium serta efek protektif terhadap endotelium dan miokard dengan fungsi regulasi yang positif terhadap mikrosirkulasi koroner. Meskipun secara fisiologis peran adiponektin masih belum diketahui secara pasti,namun adiponektin berperan pada proses inflamasi atau metabolisme lipid, yang berkontribusi terhadapa proses aterosklerosis. Pada studi ini, kami melakukan evaluasi kadar konsentrasi adiponektin pada pasien CAD, aliran darah lambat dan subjek sehat.* ***Metode:*** *penelitian ini dilakukan dengan design cross-sectional yang melibatkan 30 pasien CAD, 30 pasien SCF dan 30 pasien subjek sehat dari Desember 2017-Februari 2018 di RSUD dr. Zainoel Abidin, Banda Aceh, Indonesia. Kadar plasma adiponektin di ukur dengan menggunakan alat immunosorbent enzyme-linked (ELISA) sesuai dengan spesifikasi alat.* ***Hasil:*** *terdapat hasil yang signifikan bermakna secara statistik di antara subjek CAD, SCF dan subjek sehat dalam hal usia, jenis kelamin, tekanan darah sistolik, kolesterol total, trigliserida dan kreatinin dengan p<0,001. Rerata kadar konsentrasi adiponektin pada pasien CAD secara signifikan menunjukkan nilainy yang lebih rendah dibandingkan pasien dengan SCF dan subjek sehat (CAD 3,40 (0,87) µg/ml; SCF 4,58 (2,32) µg/ml; subyek sehat 5,65 (4,87) µg/ml; P<0,001).* ***Kesimpulan:*** *penelitian ini menunjukkan kadar plasma adiponektin yang rendah merupakan molekul penting yang berhubungan dengan aterosklerosis. Kadar plasma adiponektin mungkin berhubungan dengan peran terjadinya patofisiologi dari penyakit kardiovaskular baik pasien CAD dan CSF.*

Kata kunci: *Adiponektin, penyakit arteri koroner, Aliran darah lambat.*

ABSTRACT

Background: *Adiponectin, an adipocyte-secreted hormone involved in energy homeostasis, has broad anti-inflammatory, antioxidant, and endothelium- and myocardial-protective effects, together with a potentially positive regulatory function in coronary microcirculation. Although the physiological role of adiponectin has not yet been fully elucidated, it may well be involved in the regulation of many of the inflammatory processes or lipid metabolisms that contribute to atherosclerosis. In this study we investigate the plasma concentration of adiponectin in patients with coronary artery disease (CAD), those with coronary slow flow (CSF) and in healthy subjects.* ***Methods:*** *this study was conducted according to a cross-sectional design involving 30 CAD, 30 CSF, and 30 healthy subjects. These subjects were sourced from the Dr. Zainoel Abidin Center Hospital, Banda Aceh, Indonesia, between December 2017 and February 2018. The plasma concentration of adiponectin was measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications.*

Results: *there were statistically significant differences at p<0.001 between the CAD, CSF, and healthy-subject groups in terms of age, sex, systolic blood pressure, total cholesterol, triglycerides, and creatinine. Mean plasma concentrations of adiponectin in patients with CAD were significantly lower than in patients with CSF and in healthy subjects (CAD: 3.40 (0.87) μg/ml; CSF: 4.58 (2.32) μg/ml; healthy subjects: 5.65 (4.87) μg/ml; P<0.001).* ***Conclusion:*** *the findings suggest that low plasma adiponectin concentration is associated with atherosclerosis. Plasma concentrations of adiponectin may be related to the pathophysiology role of cardiovascular disease in both CAD and CSF patients.*

Keywords: *Adiponectin, coronary artery disease, coronary slow flow.*

INTRODUCTION

Adiponectin is one of the most intensively discussed secretion products of white fat cells, having been increasingly implicated in the pathogenesis of atherosclerosis and insulin resistance.[1] A previous study detected adiponectin in catheter-injured vascular walls of rats but not in intact vascular walls. Separate studies have shown that plasma adiponectin accumulates in the subendothelial space of the vascular wall at an early phase of catheter injury and that it can be detected around macrophages in the injured human aorta at the site of a thrombus.[2,3]

Data on the prospective impact of adiponectin plasma-concentration determination in cardiovascular disease in humans are evolving. Several clinical studies have demonstrated a strong association between low plasma adiponectin levels and coronary artery disease (CAD). In addition, decreased adiponectin concentrations have been shown to be implicated in the pathogenesis of cardiovascular risk as being related to endothelial dysfunction. Despite these advances in understanding, the exact relationship between plasma concentrations of adiponectin and CAD remains unclear in clinical practice.[4] In this study, we focus particularly on the plasma concentration of adiponectin in patients with CAD, CSF, and in healthy subjects.

METHODS

This cross-sectional study was conducted between December 2017 and February 2018 in the Division of Cardiology, Internal Medicine Department, Faculty of Medicine, University of Syiah Kuala, Banda Aceh and the Cardiac Catheterization Laboratory, Dr. Zainoel Abidin Hospital, Banda Aceh. Patients aged >26 years were consecutively recruited into one of three groups: CAD, CSF, and healthy subjects. All study participants had been referred for coronary angiography because of exertional chest pain suggestive of stable angina pectoris. Exclusion criteria were the presence of total stenosis of coronary arteries, patients who had undergone coronary artery bypass graft, and healthy subjects with significant comorbidity, including hypertension, diabetes mellitus, the use of anti-inflammatory drugs other than aspirin, renal and/or hepatic dysfunction, BMI >22.9 kg/m^2 or <18.5 kg/m^2, smoking, and alcohol consumption.

The study has been approved by the Ethical Review Committee of the Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia; reference number 476/TGL/KEPK FK USU-RSUP HAM/2017 on September 14, 2017 and all subjects provided informed consent for the study.

Laboratory Measurement

Venous blood was drawn from all subjects after an overnight fast. Routine hematology, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, urea, and creatinine levels were measured using conventional methods. The concentration of adiponectin (μg/ml) was measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's specifications (R&D Systems).

Coronary Angiography

Coronary angiography was performed using the standard Judkins technique. A contrast agent was injected manually during coronary angiography (6–10 ml of contrast agent at each position using right and left, and cranial and caudal angulations). Coronary angiography recordings were taken at the left anterior oblique, cranial and

right anterior oblique, and caudal and horizontal positions. All angiographic examinations were conducted by two cardiologists who were blind to the clinical characteristics of the patients. They assessed the flow in coronary arteries using the thrombolysis in myocardial infarction (TIMI) frame-count method described by Gibson et al.[5]

TIMI frame count was calculated from the difference between the first and last frames. Since the distance between proximal and distal bifurcation in the left anterior descending (LAD) coronary artery is longer than in other coronary arteries, LAD TIMI frame count is significantly higher than those of the right coronary artery (RCA) and the circumflex (Cx) artery. The cutoff values in respect of length for normal visualization of coronary arteries were 36.2 (SD 2.6) frames for the LAD, 22.2 (SD 4.1) frames for the left Cx (LCx), and 20.4 (SD 3) frames for the RCA. The corrected cutoff value for the LAD coronary artery was 21.1 (SD 1.5) frames. All participants with a corrected TIMI frame count greater than the two standard deviations of the published range for the particular vessel were considered as having CSF. The mean TIMI frame count for each patient and control subject was calculated by dividing the sum of the TIMI frame count for LAD, LCx, and RCA by 3.

Statistical Analysis

Continuous variables are presented as mean (standard deviation) and categorical variables are presented as n (%) prevalence. Conformity to normal distribution of the continuous variables was examined using the Shapiro–Wilk test. According to data distribution and number of groups, a parametric (analysis of variance [ANOVA], t test) or nonparametric (Kruskal–Wallis, Mann–Whitney) test was then performed. Post hoc comparisons were performed with the appropriate test for data distribution. The Pearson's or Spearman correlation test was used for evaluation of the correlation between variables. A value of $P<0.05$ was considered statistically significant.

Table 1. Demographic and clinical characteristics of the subjects in the three groups

Variables	CAD (n = 30)	CSF (n = 30)	Healthy subjects (n = 30)
Age (years), median (range)	55 (37–65)	52 (37–65)	35 (27–51)
Sex-male, n (%)	29 (96.0)	10 (33.0)	17 (56.0)
BMI (kg/m^2), median (range)	23.7 (21.6–29.0)	22.7 (18.1–31.2)	23.2 (18.5–25.1)
SBP (mmHg), median (range)	130 (112–152)	126.50 (109–147)	116.50 (107–128)
DBP (mmHg), mean (SD)	84.30 (7.13)	84.93 (9.63)	78.10 (8.84)
Hemoglobin (g/dl), median (range)	14.2 (10.1–17.1)	13.2 (8.7–16.6)	13.2 (11.6–17.5)
Hematocrit (%), mean (SD)	42.17 (4.93)	39.90 (4.85)	39.93 (3.44)
WBC (ul), median (range)	8350 (5900–14,900)	8700 (4600–16,600)	7700 (4500–10,100)
Platelets (103/ul), mean (SD)	263.4 (59.4)	270.7 (74.2)	278.1 (65.2)
Neutrophil-lymphocyte count ratio, median (range)	1.6 (0.8–19)	1.3 (0.8–5.1)	1.3 (0.8–1.7)
Total cholesterol (mg/dl), median (range)	198 (112–461)	194 (127–336)	149 (90–198)
LDL cholesterol (mg/dl), mean (SD)	50.4 (18.31)	49 (17.7)	64.8 (14.5)
HDL cholesterol (mg/dl), median (range)	121.5 (30–225)	129 (50–252)	111 (62–156)
Triglyceride (mg/dl), median (range)	143.5 (88–231)	106 (55–373)	94 (21–129)
Urea (mg/dl), mean (SD)	30.9 (11.03)	23.8 (6.62)	23.3 (5.20)
Creatinin (mg/dl), median (range)	1.0 (0.6–1.6)	0.8 (0.6–1.3)	0.7 (0.5–1.0)
Fasting glucose (mg/dl), median (range)	101 (71–218)	109 (89–154)	98 (82–117)
2 hours post-prandial glucose (mg/dl), median (range)	129 (100–226)	133 (103–226)	126 (97–176)

Data presented as mean (SD) or n (%) prevalence. Nonparametric tests were used and the medians of these data are presented: body mass index (BMI); systolic blood pressure (SBP); diastolic blood pressure (DBP); white blood count (WBC); low-density lipoprotein (LDL); high-density lipoprotein (HDL).

RESULTS

The study included 90 subjects: 30 patients with CAD (the CAD group); 30 patients with only coronary slow flow (the CSF group) and 30 healthy subjects. Demographic and clinical characteristics of the patients are summarized in **Table 1**.

Based on the clinical data, there was a statistically significant difference (at p<0.001) between the CAD, CSF, and healthy-subject groups in terms of age, sex, and SBP. Using another correlation analysis, total cholesterol, serum triglycerides, and creatinine were also significantly higher in subjects with CAD compared to both subjects with CSF and healthy subjects (p<0.001).

The baseline characteristics of the TIMI frame counts for CAD, CSF, and healthy subjects are shown in **Table 2**. The corrected TIMI frame counts for RCA and mean corrected thrombolysis in myocardial infarction (cTFC) were significantly higher in patients with CSF than in those with CAD (123.10 (SD 27.89) vs. 105.85 (SD 20.27), P = 0.034; 91.85 (SD 12.56) vs. 83.75 (SD 13.64), P=0.006, respectively) and the most common target vessel in the CSF group was RCA (93.3%, P = 0.038) for all three coronary arteries.

Mean plasma adiponectin concentrations were found to be significantly lower in the CAD and CSF subjects than in the healthy subjects (3.40 (0.87) μg/ml; 4.58 (2.32) μg/ml; and 5.65 (4.87) μg/ml, respectively; P<0.001)

DISCUSSION

The objective of the study was to investigate whether the plasma levels of adiponectin differed between subjects. The study demonstrated that the plasma levels of adiponectin differed significantly between groups of patients with CAD, CSF, and healthy subjects.

CAD, also known as ischemic heart disease or coronary heart disease, has become one of the major causes of death worldwide. Atherosclerosis, which is the main cause of CAD, is an inflammatory process involving the vascular wall cells and activation of markers of inflammation such as monocytes, T lymphocytes, pro-inflammatory cytokines, chemoattractant cytokines (chemokines), and growth factors.[6] Vessel-wall inflammation is a central feature in the initiation, progression, and terminal stages of atherosclerosis, leading to plaque, rupture, and thrombosis, and many systemic markers of

Table 2. Baseline characteristics of TIMI frame counts for angiographic data of patients with CAD and CSF

TIMI frame	CAD (n = 30)	CSF (n = 30)	P value
RCA (frame), mean (SD)	28.07 (8.82)	26.83 (3.87)	0.486
LCX (frame), mean (SD)	34.4 (10.34)	28.37 (2.76)	0.003
LAD (frame), mean (SD)	47.27 (10.11)	42.37 (7.77)	0.04
cTFC LAD (frame), mean (SD)	27.8 (5.9)	24.67 (4.8)	0.029
cTFC (frame), mean (SD)	30.13 (7.94)	26.83 (3.553)	0.042
Target vessel, n (%)			
- RCA	22 (73.3)	28 (93.3)	0.038
- LCx	4 (66.6)	25 (83.3)	0.136
- LAD	26 (86.6)	25 (83.3)	0.718

Right coronary artery (RCA); left circumflex artery (LCx); left anterior descending artery (LAD); corrected thrombolysis in myocardial infarction (cTFC).

Table 3. Between-group comparison of adiponectin level in CAD, CSF, and healthy subjects

	Mean (SD)	Median (min-max)	95% confidence interval	P value
CAD (n = 30)	3.40 (0.87)	3.35 (1.48–4.95)	2.99–3.81	< 0.001
CSF (n = 30)	4.58 (2.32)	4.15 (1.63–9.99)	3.50–5.67	
Healthy subjects (30)	5.65 (4.87)	4.87 (3.27–9.45)	4.80–6.50	

Kruskal–Wallis analysis, A post hoc Mann–Whitney test showed healthy vs. CSF p = 0.045; healthy vs. CAD p<0.001; and CAD vs. CSF p = 0.157

inflammation have been investigated and linked to the prediction of future cardiovascular events and to identification of patients at risk.[7,8]

During the last decade, much interest has been focused on adiponectin as an important modulator of inflammatory response during the initiation and progression of atherosclerosis in the vascular wall.[9] Adiponectin is a collagen-like protein produced specifically by adipose tissue that is abundantly present in the circulation. A recent report concluded that plasma adiponectin concentrations were related to the severity of coronary atherosclerosis, although the difference fell short of significance, in that adiponectin concentrations decreased as the number of significantly narrowed coronary arteries increased.[10] In vitro and in vivo studies have reported that circulating adiponectin shows multiple protective effects on the vascular endothelium by inhibiting various crucial steps in the atherosclerotic process. Adiponectin has been shown to attenuate monocyte attachment to endothelial cells by reducing adhesion-molecule expression, suppressing macrophage-to-foam cell transformation, and decreasing proliferation and migration of vascular smooth-muscle cells.[11-13] In our study, we found significantly differences in the levels of plasma serum adiponectin between the three groups, with the level being lowest in the CAD group. However, plasma concentration of adiponectin has been differently reported by Lim et al.[14], whose study showed that adiponectin concentrations did not correlate with coronary atheroma or coronary stenosis scores.

In the literature, there are a number of clinical reports identifying the crucial role of endothelial dysfunction in the pathogenesis of CSF. Selcuk et al.[9] showed that plasma adiponectin concentrations were significantly decreased in patients with CSF compared to those with normal coronary flow. It has been suggested that loss of the anti-inflammatory effects of adiponectin and increased endothelial expression of proinflammatory factors directly contribute to endothelial dysfunction by allowing vascular proinflammatory reactions to occur more readily.[15] In this mechanism, decreased concentrations of circulating adiponectin may be attributed to impaired endothelial function resulting from slow-flow-induced vascular damage that gives rise to the accumulation of adipocytokine in the vessel wall.[16] Our study showed that plasma concentration of adiponectin was lower in patients with CSF than in healthy subjects.

Our study had some limitations. First, the number of participants was relatively low. Second, the patients did not undergo intravascular ultrasonography (IVUS) to detect atherosclerotic

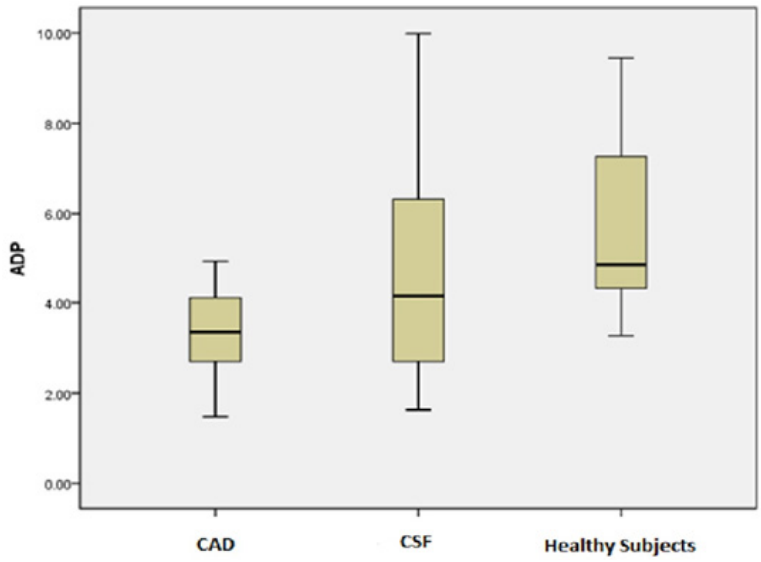

Figure 1. Comparison of plasma concentrations of adiponectin in patients with CAD, CSF and healthy subjects

changes in the coronary arteries. Finally, we did not measure the inflammatory indicators as supporting evidence for the mechanisms of pathophysiology in cardiovascular disease.

CONCLUSION

The results of this study show that plasma adiponectin is an important molecule associated with atherosclerosis, and, for the data obtained, the level of adiponectin is lowest in the CAD group. These findings may have important implications for understanding the physiopathologic role of cardiovascular disease in both CAD and CSF patients and therefore for the development of therapeutic strategies.

CONFLICT OF INTEREST

The authors have declared no financial conflicts of interest.

REFERENCES

1. Schnabel R, Messow CM, Lubos E, et al. Association of adiponectin with adverse outcome in coronary artery disease patients: results from the Athero Gene study. Eur Heart J. 2008;29:649–57.
2. Okamoto Y, Arita Y, Nishida M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res. 2000;32:47–50.
3. Ouchi N, Kihara S, Arita Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation. 2001;103:1057–63.
4. Hashimoto N, Kanda J, Nakamura T, et al. Association of hypoadiponectinemia in men with early onset of coronary heart disease and multiple coronary artery stenoses. Metabolism. 2006;55:1653–7.
5. Gibson CM, Cannon CP, Daley WL, et al. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation. 1996:93;879–88.
6. Hua XP, Zhang XD, Kwong JSW, Zeng XT, Zhang ZJ, Wei WL. Tumor necrosis factor-alpha G-238Apolymorphism and coronary artery disease risk: a meta-analysis of 4,222 patients and 4,832 controls. Ther Clin Risk Manag. 2015;11:1429–36.
7. Razi MM, Abdali N, Asif SM, Azharuddin M. Association of inflammatory cytokines/biomarkers with acute coronary syndrome and its correlation with severity and hospital outcome. J Clin Prev Cardiol. 2017;6:44–9.
8. Rosenson RS, Koenig W. Utility of inflammatory markers in the management of coronary artery disease. Am J Cardiol. 2003;92(suppl):10i–18i.
9. Selcuk H, Selcuk MT, Temizhan A, et al. Decreased plasma concentrations of adiponectin in patients with slow coronary flow. Heart Vessels. 2009;24:1–7.
10. Nakamura Y, Shimada K, Fukuda D, et al. Implications of plasma concentrations of adiponectin in patients with coronary artery disease. Heart. 2004;90:528–33.
11. Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation. 2000;102:1296–301.
12. Ouchi N, Kihara S, Arita Y, et al. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation. 2001;103:1057–63.
13. Matsuda M, Shimomura I, Sata M, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem. 2002;277:37487–91.
14. Lim HS, Tayebjee MH, Tan KT, Patel JV, Macfadyen RJ, Lip GYH. Serum adiponectin in coronary heart disease: ethnic differences and relation to coronary artery disease severity. Heart. 2005;91:1605–06.
15. Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. Curr Opin Lipidol. 2003;14:561–6.
16. Okamoto Y, Arita Y, Nishida M, et al. An adipocyte derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res. 2000;32:47–50.