

RESEARCH ARTICLE

Comparison of Tumor Necrosis Factor- α Level in Coronary Artery Disease and Coronary Slow Flow of Thrombolysis in Myocardial InfarctionMuhammad Diah^{1,*}, Rahmawati², Aznan Lelo³, Zulfikri Muhktar⁴, Dharma Lindarto⁵, Ferry Sandra⁶¹Division of Cardiology, Department of Internal Medicine, Faculty of Medicine, Universitas Syiah Kuala/Dr. Zainoel Abidin Centre Hospital, Jl. Teuku Tanoh Abee, Banda Aceh, Indonesia²Department of Internal Medicine, Faculty of Medicine, Universitas Syiah Kuala, Jl. Teuku Tanoh Abee, Banda Aceh, Indonesia³Department of Pharmacology, Fakultas Kedokteran, Universitas Sumatera Utara, Jl. Dr. Mansyur No.5, Medan, Indonesia⁴Department of Cardiology, Fakultas Kedokteran, Universitas Sumatera Utara, Jl. Dr. Mansyur No.5, Medan, Indonesia⁵Division of Endocrine Metabolic, Department of Internal Medicine, Fakultas Kedokteran, Universitas Sumatera Utara, Jl. Dr. Mansyur No.5, Medan, Indonesia⁶Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No.260, Jakarta, Indonesia

*Corresponding author. E-mail: diah6262@gmail.com

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Abstract

BACKGROUND: Tumor necrosis factor (TNF)- α , an important primary pro-inflammatory cytokine, has a crucial role in the pathogenesis of atherosclerosis. Since the pathophysiological mechanism of coronary slow flow (CSF) is not fully understood, we investigated the level of TNF- α in coronary artery disease (CAD), CSF and healthy subjects.

METHODS: This study was conducted in cross-sectional design involving 16 CAD, 18 CSF and 18 healthy subjects. Coronary angiography was recorded at the left anterior oblique, cranial, right anterior oblique, caudal, and horizontal positions. The flow in coronary arteries of the subjects were assessed using Thrombolysis in the Myocardial Infarction (TIMI) frame count method. Peripheral blood-derived serum was collected and level of

TNF- α was determined by using highly sensitive enzyme-linked immunosorbent assay (ELISA).

RESULTS: No significant difference in level of TNF- α in CAD, CSF and healthy subjects (2.72 ± 2.64 pg/mL, 1.88 ± 0.8 pg/mL, 1.64 ± 0.35 pg/mL, respectively) ($p=0.087$). In addition, there was no correlation between the concentration of TNF- α and TIMI frame count ($r < 0.2$, $p > 0.05$).

CONCLUSION: There was no significant difference of TNF- α level in CAD, CSF and healthy subjects. In addition, there was no correlation between the TNF- α level with TIMI frame count as well. Nevertheless, further clinical studies with more subjects are needed.

KEYWORDS: TNF-alpha, coronary artery disease, coronary slow flow

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Introduction

WHO has reported the cardiovascular disease (CVD) is progressively becoming the principle causes of death all over the world since 2008. The WHO health report has also estimated the mortality caused by CVD will rise from 17.1 million in 2004 to 23.4 million in 2030.(1) Atherosclerosis occurs due to the outcome of inflammatory and fibro-

proliferative events, by which some factors happen in response to the localization of atherogenic lipoproteins on the intima layer of the blood vessel wall. A number of studies have found that signs of systemic inflammation can become powerful future predictors of cardiovascular events in healthy, low risk individuals without overt cardiovascular disease and those with coronary artery disease (CAD). Increase in concentration of inflammatory mediators can foresee the adverse cardiovascular events.(2,3)

An important primary pro-inflammatory cytokine that has a crucial role in the pathogenesis of atherosclerosis is the tumor necrosis factor (TNF)- α . TNF- α is produced by several types of cells especially macrophages and is involved in systemic inflammatory conditions. This cytokine affects the myocardium in some diseases such as myocarditis, ischemic heart disease and cardiac dysfunction.(4) Previous studies suggested that TNF- α levels was correlated with coronary atherosclerosis.(5) Although elevated level of TNF- α in CAD is known, only a few data is available for the level of TNF- α in coronary slow flow (CSF). Hence, the present study was conducted to investigate whether the difference of TNF- α level in CAD, CSF and healthy subjects.

Methods

Study Subject

The protocol of this cross-sectional study was approved by the Ethical Review Committee, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (No. 205/KE/FK/2013). Prior to the study, all subjects have signed the written informed consent. The study was conducted from November 2013 to February 2014 in the Division of Cardiology, Department of Internal Medicine, Faculty of Medicine, Universitas Syiah Kuala and Cardiac Catheterization Laboratory at dr. Zainoel Abidin Regional General Hospital, Banda Aceh. Subjects aged >26 years were consecutively recruited. Subjects who had clinical symptoms of chest pain typical of angina and undertook coronary angiography, were then divided into groups of CAD and CSF subjects based on the results of angiography. Meanwhile, subjects who didn't have chest discomfort confirmed by electrocardiography and didn't undertake coronary angiography, were grouped as healthy subjects. Subjects with total stenosis coronary artery and coronary artery bypass graft were excluded. Subjects with significant comorbidities, including hypertension, diabetes mellitus, the use of anti-inflammatory drugs other than aspirin, renal or hepatic dysfunction, body mass index (BMI) >22.9 kg/m² or <18.5 kg/m², smoker, and alcohol consumption, were excluded as healthy subjects.

Coronary Angiography and Thrombolysis in the Myocardial Infarction (TIMI)

The standard Judkins technique was employed to perform the coronary angiography. The coronary angiography

was recorded at the left anterior oblique, cranial, right anterior oblique, caudal, and horizontal positions. These examinations were done by two cardiologists who did not have any information on the clinical characteristics of the subjects.

Furthermore, the flow in coronary arteries of the subjects were assessed using TIMI frame count method.(6) The differences between the first and last frames in the TIMI frame count were calculated. The cut-off values from the length for normal visualization of coronary arteries were 36.2 \pm 2.6 frames for Left Anterior Descending Artery (LAD), 22.2 \pm 4.1 frames for Left Circumflex Artery (LCx), and 20.4 \pm 3 frames for Right Coronary Artery (RCA). The corrected cut-off value for LAD coronary artery was 21.1 \pm 1.5 frames. The mean TIMI frame count for each subject was calculated by dividing the sum of the TIMI frame count of LAD, LCx and RCA by 3.

Sample Collection, Preparation and Measurement

Five mL peripheral blood sample of each subject was obtained from the antecubital vein and collected in non-heparinized glass tube. The tube was put on ice immediately after collection for 30-45 min. After that, the tube was centrifuged at 3000 rpm for 15 min. Serum was collected and stored at -20°C freezer.

For the concentration of TNF- α measurement, serum was thawed and immediately processed with Human TNF- α Quantikine HS enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA). Other blood tests for hemoglobin, hematocrit, white blood cell, platelet, erythrocyte sedimentation rate (ESR), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, urea, creatinine, uric acid, fasting glucose and 2-hour post prandial glucose, were performed using standard methods in dr. Zainoel Abidin Hospital.

Statistical Analysis

Conformity to normal distribution of the continuous variables was examined with the Shapiro-Wilk test. Parametric or non-parametric tests were performed based on the data distribution and number of groups. Conferring to data distribution, post hoc comparisons were conducted with the suitable test. The paired-sample t-test was used to compare the dependent groups and the Pearson's or Spearman correlation test was used to evaluate the correlation between variables. The $p < 0.05$ was considered as statistically significant.

Results

Fifty-two subjects were included in the study, 16 with CAD, 18 only CSF and 18 healthy subjects. Demographic and clinical characteristics of the subjects are summarized in Table 1. There were significant differences in terms of age, BMI, and SBP of CAD, CSF and healthy subjects. CAD subjects had the highest BMI and SBP levels. Based on laboratory results, platelet, triglyceride, urea, creatinine, uric acid and 2-hour post prandial glucose were also significantly different. CAD subjects had the highest creatinine, uric acid and 2-hour post prandial glucose levels. CSF subjects had the highest triglyceride and urea levels. Healthy subjects had the highest platelet count.

TIMI Frame Counts of CAD and CSF subjects are shown in Table 2. There was not any significant difference

of TIMI frame count for RCA, LCx, LAD, cTFC LAD and cTFC in both groups. The most common of target vessel in CSF subjects was RCA (94.44%) of all three coronary arteries. The number of RCA in CSF subjects was significantly different compared to the number of CSF in CAD subjects ($p<0.001$).

ELISA results showed that TNF- α levels of CAD was the highest. However, the TNF- α levels of CAD, CSF and healthy subjects were not significantly different (Table 3). The TNF- α levels were not significantly correlated with TIMI frame count (Table 4).

Discussion

CSF has been demonstrated to be more common in males, smokers, and individuals with hyperlipidemia, metabolic

Table 1. Demographic and clinical characteristics of CAD, CSF and healthy subjects.

Variable	CAD (n=16)	CSF (n=18)	Healthy (n=18)	p-value
Age (years)	58.13 \pm 8.24	53.39 \pm 10.08	44.22 \pm 8.59	<0.001 ^{a,*}
Male gender (n (%))	10 (62.50%)	11 (61.11%)	6 (33.33%)	0.148 ^b
BMI (kg/m ²)	25.13 (21.87-35.15)	24.80 (18.73-34.66)	21.38 (19.26-22.22)	<0.001 ^{c,*}
SBP (mmHg)	144.38 \pm 23.08	131.67 \pm 11.50	114.44 \pm 7.84	<0.001 ^{a,*}
DBP (mmHg)	83.13 \pm 10.14	83.06 \pm 10.73	78.33 \pm 3.83	0.186 ^a
Hemoglobin (g/dL)	13.68 \pm 1.91	13.60 \pm 2.56	12.67 \pm 1.24	0.257 ^a
Hematocrit (%)	38.59 \pm 6.28	39.10 \pm 7.08	37.90 \pm 2.75	0.817 ^a
Leukocyte (cells/ μ L)	8,115 (3,800-25,510)	7,350 (5,100-17,250)	7,905 (3,880-13,110)	0.695 ^b
Platelet (10 ³ / μ L)	258.5 (150-290)	242 (171-423)	311 (229-434)	0.005 ^b
ESR (mm/hour)	21.94 \pm 16.13	22 (2-130)	16 (1-45)	0.172 ^b
Total Cholesterol (mg/dL)	197.38 \pm 36.03	210.67 \pm 65.92	183.22 \pm 31.44	0.231 ^a
LDL cholesterol (mg/dL)	116.81 \pm 32.35	135.61 \pm 55.15	116.67 \pm 28.13	0.290 ^a
HDL cholesterol (mg/dL)	44.94 \pm 11.89	40.11 \pm 14.93	47.50 \pm 8.95	0.192 ^a
Triglyceride (mg/dL)	111 (54-416)	125.50 (35-222)	74.50 (38-194)	0.019 ^b
Urea (mg/dL)	25.50 (10-75)	29.50 (14-74)	21 (15-33)	0.026 ^b
Creatinine (mg/dL)	1.06 (0.60-2.43)	1.01 (0.60-1.80)	0.73 (0.56-1.18)	0.007 ^b
Urid Acid (mg/dL)	6.87 \pm 2.63	6.46 \pm 1.94	5.01 \pm 1.32	0.022 ^a
Fasting glucose (mg/dL)	99.50 (74-141)	99 (69-250)	89.50 (79-111)	0.250 ^b
2-hour post prandial glucose (mg/dL)	143 (84-307)	140 (86-561)	102.50 (91-152)	0.001 ^b

Data were presented as mean \pm SD, median (minimum-maximum) or n (%). BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; ESR: Erythrocyte Sedimentation Rate; LDL: Low-density Lipoprotein; HDL, High-density Lipoprotein. ^aOne-way analysis of variance (ANOVA) test with Dunnett's post-hoc; ^bChi square test; ^cKruskall-Wallis test with Mann-Whitney post-hoc; *significance at $p<0.001$.

Table 2. TIMI frame counts of CAD and CSF subjects.

Variable	CAD (n=16)	CSF (n=18)	p- value
RCA (frame counts)	28.07±8.82	26.83±3.87	0.486 ^d
LCx (frame counts)	34.4±10.34	28.37±2.76	0.003 ^d
LAD (frame counts)	47.27±10.11	42.37±7.77	0.040 ^d
cTFC LAD (frame counts)	27.80±5.96	24.67±4.81	0.029 ^d
cTFC (frame counts)	30.13±7.94	26.83±3.55	0.042 ^d
Target vessel (n (%))			
- RCA	4 (25%)	17 (94.44%)	<0.001 ^{b,*}
- LCx	4 (25%)	15 (83.33%)	0.001 ^b
- LAD	13 (81.25%)	16 (88.89%)	0.648 ^b

Data were presented as mean±SD or n (%). RCA: Right Coronary Artery; LCx: Left Circumflex Artery; LAD: Left Anterior Descending Artery; cTFC: Corrected Thrombolysis in Myocardial Infarction Frame Count. ^bChi-square test; ^dIndependent T-test; *significance at $p<0.001$.

syndrome, or obesity.(7) In our study, age, BMI dan SBP were significantly different. CSF subjects were younger than CAD subjects. Regarding BMI, our current results were in accordance with previous report. BMI was suggested as an independent predictor of CSF in the North American population.(8)

Atherosclerosis, the main cause of CAD, is an inflammatory process involving vascular wall cells along with activation of inflammation markers.(9) Vessel wall inflammation is the key trait in the initiation, progression, and terminal steps of atherosclerosis. It further leads to plaque rupture and thrombosis. A number of systemic markers of inflammation have also been examined and associated to calculate future cardiovascular events and to identify patients at risk.(3,10) They include interleukin (IL)-1, IL-6, IL-8, TNF- α , anti-inflammatory cytokines IL-1 receptor antagonist, and IL-10.(5,11) TNF- α is a potent pro-inflammatory cytokine discharged by most cell types with pleiotropic effects. Many studies reported the correlation

of TNF- α and cardiovascular disease.(12,13) Carotid atherosclerosis has also been correlated to TNF receptor levels, however, it is not associated to serum TNF- α levels. (14) A recent study did not find a correlation between atherosclerotic burden and IL-6 or TNF- α .(15) Another study have shown there was a significant correlation between TNF- α and the severity of CAD.(3) In our study, we found no significant difference in serum level of TNF- α between the CAD, CSF and healthy subjects.

Our current results showed that the most common of target vessel in CSF group was RCA. These results were in in accordance with previous report on angiographic characteristics in patient with CSF. The RCA was found as the predominant vessel involved in CSF.(16) However, another study showed the most common artery involved was LAD, followed by LCX and RCA.(17)

There were many studies indicating endothelial function is impaired in patients with CSF. TIMI frame count was correlated with endothelial dysfunction.(18)

Table 3. Comparison of TNF- α level in CAD, CSF and healthy subjects.

Group	Mean±SD (pg/mL)	Median (Min-Max) (pg/mL)	95% Confidence Interval	p- value
CAD (n= 16)	2.72 ± 2.64	1.87 (1.09-12.16)	1.70-2.56	
CSF (n=18)	1.88 ± 0.87	1.64 (1.10-4.26)	1.22-2.12	0.087 ^c
Healthy subjects (n=18)	1.64 ± 0.35	1.61 (1.03-2.14)	1.34-1.93	

^cKruskal-Wallis Test.

Table 4. Correlation between TNF- α level and TIMI frame count.

Variable	95% Confidence Interval	p-value ^f
RCA	-0.150 (-0.464-0.198)	0.397
LCx	-0.178 (-0.486-0.17)	0.314
LAD	-0.071 (-0.399-0.273)	0.689
cTFC LAD	-0.071 (-0.399-0.273)	0.689
cTFC	-0.214 (-0.514-0.133)	0.224

^fSpearman's correlation test.

In our study, there was no correlation between the TNF- α level with TIMI frame count. The results showed inverse correlation between the TNF- α level and TIMI frame count. In another study, plasma nitric oxide (NO) levels were found to be lower in patients with SCF than in healthy subjects, so that NO level were negatively correlated with TIMI frame count.(19)

Conclusion

In conclusion, the results of the present study show no significant difference of TNF- α level in CAD, CSF and healthy subjects. In addition, there was no correlation between the TNF- α level with TIMI frame count as well. Nevertheless, further clinical studies with more subjects are needed.

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