The Ratio of 7-Ketocholesterol to Free Cholesterol on Patients of Acute Myocardial Infarction Treated in ICCU

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Abstract

Context: Coronary Heart Disease (CHD) and its complications still become the main cause of morbidity and mortality globally, including Indonesia. Previous research simultaneously investigates the conversion of oxidized lipid cholesterol biomarker inside the plasma as the predictor of acute myocardial infarction cardiovascular disease. In accordance with the background of the study, this research attempts to analyze the increasing ratios of 7-ketocholesterol to free cholesterol in the plasma, 7-ketocholesterol (7KC), free cholesterol (FC), and fasting blood glucose level (FBG) of Acute Myocardial Infarction Coronary Heart Disease (AMI-CHD) and post-Acute Myocardial Infarction Diabetes Mellitus (Post-AMI DM) patients. This research applied the cross-sectional laboratory observation design. Free cholesterol, 7-ketocholesterol and its ratio to free cholesterol were analyzed using Ultra-Fast Liquid Chromatography (UFLC). The result of Levene's bivariate t-test analysis indicates the comparison of 7-KC/FC ratio, 7-KC, F, and FBG increases with different significance ($p<0.05$) on AMI-CHD patients and Post-AMI DM ones. Pearson correlational statistics shows negative (inversed) correlation between the increasing free cholesterol level and linear correlation of 7-ketocholesterol and 7-ketocholesterol ratio on free cholesterol. The average 7-KC/FC ratio is significantly higher than the normal score. This is significantly different between AMI-CHD and Post-AMI DM.7-KC/FC ratio significantly correlates with 7-KC concentration with $r = 0.62$ on AMI-CHD patients and $r = -0.725$ on post-AMI DM ones.

Keywords: *7-KC/FC ratio; 7-Ketocholesterol; Free Cholesterol;Acut Myocardial infarctions; diabetes mellitus.*

Introduction

Atherosclerotic Coronary Heart Disease (ASCHD) places the highest rank of mortality and morbidity cause in developed countries and many developing countries. ASCHD triggers acute myocardial infarction and chronic damage. Since 1990, the mortality rate of Coronary Heart Disease (CHD) had increased from 6 million to 7 million in 1999 and had been projected to reach 9 million by 2020 [1'2'3] Although the mortality rate of ASCHD is actually decreasing in the last thmillion to 7 million in 1999 and had been projected to reach 9 million by 2020

ree deCHDes mostly caused by better life control and advanced therapy, aging process and careless-ignorant lifestyle of the affected population makes the prevalence of the disease remain high [4]. Recent type-2 diabetes epidemic may complicate this problem. National census held in 2001 indicated the deaths from cardiovascular diseases was 26.4% and becoming the main cause of early death on about 40% of middle-aged men [5].

Lipid biomarkers trigger lipotoxicity of ASCHD lesions which leads to injury and cell death caused by excessive free fatty acids and other lipid-related metabolites. The increasing free fatty acid level generates

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the accumulation of reactive oxygen species due to the decreasing endogenous antioxidant capacity.

Excessive free cholesterol, 7-ketocholesterol, and its ratio to free cholesterol complicates the identification of lipotoxic species affecting lipid regulation, inflammation, stress on endoplasmic reticulum (ER), apoptosis, and necrosis[6]. Therefore, the ratio of 7-ketocholesterol to free cholesterol in blood plasma is considered as an important target factor in preventing CHD incidence that reflects pathogenesis process of coronary heart disease; consequently, with clinical application. However, it is difficult to perceive the significance of 7-ketocholesterol and free cholesterol concentration clinically because it is hard to analyze the concentration of this metabolite accurately. In this study, the measurement system for 7-ketocholesterol and free cholesterol concentrations in blood plasma is carried out by applying high performance liquid chromatography spectrometry technique on the blood sample of AMI-CHD and Post-AMI DM patients.

Method

Study Design, Setting, and Sampling: The research design used was cross-sectional in Acute Myocardial Infarction Coronary Heart Disease (AMI-CHD) and post-Acute Myocardial Infarction Diabetes Mellitus (Post-AMI DM) patients. The population in this study was all Acute Myocardial Infarction Coronary Heart Disease (AMI-CHD) and post-Acute Myocardial Infarction Diabetes Mellitus (Post-AMI DM) patients in Dr Soetomo Surabaya Hospital. A sample of 16 28 AMI-CHD and 15 Post-AMI DM. The study was conducted on November 2016 to November 2017.

Study Variables: The independent variable is increasing ratios of 7-ketocholesterol to free cholesterol in the plasma, 7-ketocholesterol (7KC), free cholesterol (FC), and fasting blood glucose level (FBG). The dependent variable was manifestation of AMI-CHD and Post-AMI DM.The main instrument used in preparation and analysis was UFLC (Ultra-Fast Liquid Chromatography).

Data Analysis: This study uses descriptive analysis carried out using the number of frequencies and percentages for categorical data and the mean, median, and standard deviation used for numerical data. Analysis of the main data using the Levene's t-test with a significant level of $p < 0.05$.

Results

The results of the biomarkers are of 7-ketokolestertol (7-KC) plasma, and 7-KC/FC ratio, and free cholesterol (FC), in patients with AMI-CHD and Post-AMI DM are shown in Table 1. Sequentially, the increased concentrations of 7-ketocholestrol/free cholesterol ratio, 7-ketocholesterol, and free cholesterol, on AMI-CHD patients is significantly higher than those on post-AMI DM patients.

Blood plasma 7-ketocholesterol, 7-KC/FC ratio, and free cholesterol are the biomarkers with higher average score compared to normal reference value on both AMI-CHD patients and Post-AMI DM patients. The proportion of AMI-CHD patients is higher than Post-AMI DM patients with an average score above the reference value for each patient proportion and the mean level of 7-ketocholesterol biomarkers (93.79%> 46.66%; 38.48> 24.12 ng / ml), fasting blood glucose (75%> 40%; 140> 120.45mg / dl), 7-KC/FC ratio (81.25> 40%; 0.38> 0.227ng / ml), free cholesterol (87.50>46.66%; 124.90> 93.45%).

Pearson correlation test shows that high 7-KC/FC ratio has linear correlation with the increasing 7-ketocholesterol concentration on AMI-CHD patient blood plasma (95%CI; 0.37-0.85; r=0.62; p=0.01) and has significant negative (inversed) correlation with free cholesterol concentration on the blood plasma of AMI-CHD patients [95% CI; r = -0.72 (0.86 –0.37); p=0.001] and post-AMI DM patients [95% CI; r = -0.83 (0.93 – 0.58); p=0.009]. Significant inverse correlation only occurs on the increasing blood plasma 7-ketocholesterol concentration with the decreasing triglyceride concentration on post IMA DM patients (shown on Table 2).

Table 1: Results of Levene's Significance Test Among Lipid Biomarker Profiles on AMI-CHD and Post-AMI DM Subjects

No.	Research Variables	Levene's Significance Test (95% CI), AMI-CHD proportion, Post-AMI DM proportion & average value above reference	p Value
1.	7-ketokolestertol (7KC)	14.36 (5.71-23.003); (93.75; 46.66%) (38.48; 25.67)ng/ml	S (0.002)
2.	Free Cholesterol (FC)	31.45 (3.00-59.00); (87.50; 46.66%) (124.90; 93.45ng/ml)	S (0.002)
3.	7-KC/FC ratio (r7-KC/FC)	0,16 (0.014-0.31); (81.25 : 40.00%) (0.37; 0.25ng/ml)	S (0.033)

Table 2: Results of Pearson Correlation Test (↔) on the increasing biomarkers of AMI-CHD and Post-AMI DM patients

Biomarker Correlation	r (95%;CI)	p value
AMI-CHD: Ratio7-KC/FC ↔7KC	r = 0.62 (0.37 to 0.85)	(S) 0.010
Ratio7-KC/FC ↔ FC	r = -0.72 (-0.86 to -0.37)	(S) 0.001
Post-AMI DM: Ratio7-KC/FC↔FC	r = -0.83 (-0.93 to -0.58)	(S) 0.007
7KC↔TG	r = -0.53 (-0.83 to -0.20)	(S) 0.041

Discussion

The results showed that the IVUS study indicates that 7-KC reflects fragile coronary plaques that cannot be detected by coronary angiography. 7-KC excess in atherosclerotic plaques further contributes to the development of atherosclerosis, triggers apoptosis and inhibits smooth muscle cell migration [7,8]. The finding of previous study suggests the accumulation of 7-KC may reduce the number of cells and make atherosclerotic plaques unstable.

AMI-CHD patients with plasma r7-KC/FC levels above the normal reference score (81.25%) is larger than Post-AMI DM patients (40.00%), Levene's Test ($p = 0.009$); there were significant differences plasma r7KK / KB levels that increase in PJK-IMA patients and post IMA-DM.

Related to the findings of previous research concerning free cholesterol, r7-KC/FC increases ≈10 times in mice aorta, but not in the lung. The concentration of r7-KC/FC in LDL is ≈ twice higher on mice exposed to PM25 pollutant [9]. Brown's research shows 7KK molar quantity and KB to be esterified into 7-ketocolesteryl ester (7KKE) and cholesteryl ester (KE) in mouse cells peritoneal macrophages (MPM) and J774A, which contains oxidized LDL (ox-LDL) and acetylated LDL (ac-LDL) indicating the ratio of 7KKE:7KC is higher than the ratio of KE:FC on the both types of cell[10].

The increasing plasma r7KK / KB levels in patients with AMI-CHD are influenced by high levels of 7KC and auto-oxidation of FC, KE, and 7KKE accumulated in ruptured plaque lesions. When plaque ruptures, the accumulated cholesteryl ester, oxysterol 7-ketocholesteryl ester in LDL, cellular waste, calcium, and other lipid substances stored in the intima wall of this artery breaks and overflows the circulation stream. However, the low concentration of r7-KC/FC on post AMI-DM after 2-3 months' period may be the effect of healing detoxification process at the level of organelle-cellular caused by expression of cytosolic sulfotransferase of mRNA activation (Sulfonate steroid-sterol), an enzyme that protects the cytotoxic accumulation of cardiovascular disorders [6].

The increasing blood plasma 7-KC concentration on AMI-CHD patients is the effect of ruptured plaque. Ruptured plaque releases a large number of dead cell remnants and other products of advanced oxidative stress that triggers the increasing lipotoxic 7KC and other oxysterol inside blood circulation [11].

Hence, blood plasma 7KC is important not only in reflecting the progression of atherosclerosis, but also in causing plaque rupture as the result of the most severe complication of coronary artery atherosclerosis that triggers AMI-CHD[12]. This metabolite can be reduced to 7-β hydroxolesterol by steroldehydrogenase 11β-hydroxy type 1 (11β-HSD-1). Oxysterol 7-cholesterol, 5.6 α epoxy cholesterol, 5.6 β epoxicholesterol, 7-α hydroxycholesterol, 7-β hydrocholesterol and 27-hydroxycholesterolemia will detoxify at the organelle-cellular level by expression of

cytosolic sulfotransferase activation (Sulfonate steroid-sterols) protective enzymes for the accumulation of cytotoxic cardiovascular disorders.

Consistent with detoxification mechanism explained above, it is reasonable that the level of 7-KC on post-DM AMI CHD patient bleed plasma is lower than 7-KC level of AMI-CHD patients after 2-3 months' recovery period. However, the value is still higher than the normal reference score due to the effect of lipotoxicity and chronic glucotoxicity. Non-esterified fatty acids (NEFAs) secreted by adipose tissues on obese people may generate a new hypothesis that insulin resistance and β cell pankreas dysfunction are most likely related. In angiography study, the concentration of 7-KC on AMI CHD subjects is significantly higher than NCA; multi-logistic regression analysis reveals that 7-KC is chosen as independent factor for AMI incidence as the default factor[12]. The high plasma 7-KC concentration is a different biomarker of blood cholesterol even though 7-KC is a product of advanced cholesterol oxidation. Blood plasma 7-KC oxysterol can be used as a predictor for AMI CHD incidence that cannot be detected by conventional lipid profiles.

The high blood plasma 7-ketocholesterol concentration has negative (inversed) correlation with the low triglyceride concentration on post AMI CHD-DM patients. The data indicates the imbalance FC esterification in SBMF lysosome, causing most of FC to oxidize and increases 7-KC concentration [11].

The mean value of plasma KB level of on AMI-CHD patients (124.90 ± 48.54) is higher than the post-AMI DM CHD (93.45 ± 24), and above the normal score <91 ng/ ml plasma. Disruption in the pathway of free cholesterol efflux foam cell artery walls greatly affects the increasing free cholesterol on AMI-CHD patient blood plasma.

Macrophage foam cells has four efflux pathways of non-esterified cholesterol, to extracellular HDL acceptor to catabolism and elimination in the liver. Risearch to test on macrophages have been incubated with sera from participants with and without coronary heart disease and strikingly, cholesterol efluks capacity is found to be a strong inverse predictor of the accurrense of disease [13]. The increased intracellular free cholesterol crystals may destruct the cells by physically damages intracellular structure. The intracellular free cholesterol accumulation of lysosomes that is proven to be very difficult to mobilize out of the lysosome foam cell macrophages will increase the cholesterol oxidation stress further into oxysterols, some of which are very cytotoxic [11].

Finally, overloading free cholesterol in foam cell macrophages can trigger a series of apoptotic pathways. Excessive cholesterol on membrane may disrupt the function of signaling proteins and affect certain membrane integral proteins that require conformation flexibility to perform right functions. These functions will be disrupted by high ratio of cholesterol/phospholipid [14].

The model of free cholesterol culture macrophage has revealed cellular responses that attract free cholesterol accumulation and final consequence of free cholesterol loading that is relevant to atherosclerosis development and complication. Macrophage is usually protected from excessive free cholesterol accumulation through esterification mediated by ACAT-1 and by disintegrating cholesterol. In addition, the results of free cholesterol hydrolysis stored by neutral hydrolase of CE usually do not exceed cell capacity to efflux or re-esterify this cholesterol collection. A study that measures cholesterol in the aorta and tissue of rat lungs exposed to pollutant particles PM-25 and FA has failed to estimate the different cholesterol level on the same tissue [9].

Plasma free cholesterol in patients with post AMI-DM interval of 2-3 months leads to recovery of cholesterol metabolism which can be converted to 27-hydroxycholesterol in adipocytes, 4β-hydroxycholesterol in liver-adipocytes, and conversion of FC to 7α-hydroxycholesterol for secretion in the liver [6].

Higher FC concentration on Post-AMI CHD patient blood plasma compared to normal reference score is affected by multi factors, such as the effect of insulin resistance and chronic glucose toxicity is believed to slow the recovery of atherogenic dyslipidemia[15]. Example the synthesis of advanced glycation end products (AGEs) in AMI-DM CHD complications can regulate cholesterol metabolism by reducing the expression of ABCA-1 and ABCG-1, that disrupts the transportation of cholesterol from arterial wall artery lesions to the liver which allows its excretion to bile and feces [16].

Xiaoquan has conducted a research to measure free cholesterol levels in rat aortic, liver and lung tissue, but they failed to detect differences in free cholesterol content between PM2,5 and FA exposure groups in the aorta, liver and lung[9]. The instability of

cholesterol molecules that is vulnerable to enzymatic conversion, ozonolysis and auto-oxidation. Therefore, it is reasonable if the research team of Xiaoquan found some difficulties in measuring the proportion of free cholesterol in circulation, adipocyte tissue, and rat liver HFC and ND[9].

Conclusion

Lipid biomarkers that significantly increased on AMI-CHD and post-AMI DM incidence are 7-KC/FC ratio, 7-ketocholesterol, and free cholesterol. High concentration of 7-ketocholesterol/free cholesterol ratio has linear correlation with 7-ketochole- sterol on AMI-CHD patients and has negative (inversed) correlation with free cholesterol on both AMI-CHD and post-AMI DM patients.

Ethical Clearance: The ethical approval for this study was granted by the IRB committee of the Dr Soetomo Surabaya in 2016.

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Conflict of Interest: None

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