RESEARCH ARTICLE

Relations Between Atherogenic Index of Plasma, Ratio of Small Dense Low Density Lipoprotein/Lecithin Cholesterol Acyl Transferase and Ratio of Small Dense Low Density Lipoprotein/Cholesteryl Ester Transfer Protein of Controlled and Uncontrolled Type 2 Diabetes Melitus

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BACKGROUND: Patients with Diabetes Melitus are proven to be prone to atherosclerosis and coronary heart disease, especially type 2 Diabetes Melitus (T2DM) patient who have higher risk and mortality for cardiovascular risk factor. The Dyslipidemia condition is very common in T2DM as one of the risk factors. Diabetic dyslipidemia is marked by the increased triglyceride (TG), low HDL cholesterol (HDL-C), and increased small dense LDL and apolipoprotein B. Therefore the aim of this study is to assess the differential and correlation between Atherogenic Index of Plasma (AIP), ratio of small dense low density lipoprotein (sdLDL)/ lecithin cholesterol acyl transferase (LCAT) and ratio of sdLDL/cholesteryl ester transfer protein (CETP) of controlled and uncontrolled T2DM.

METHODS: This study was observational with cross sectional design. In total of 72 patients with T2DM consist of 36 controlled and 36 uncontrolled, participated in this study. The serum TG, HDL-C, sdLDL, LCAT and CETP were examined in their relationship with to T2DM risk.

RESULTS: The results of the study indicate that the AIP ($p < 0,001$) increase controlled and uncontrolled T2DM and the ratio of sdLDL/CETP ($p = 0,004$), odds ratio of AIP was 4 (95% C.I : 1,501-10,658) and odds ratio of sdLDL/CETP ratio was 4 (95% C.I : 1,501-10,658) in uncontrolled T2DM.

CONCLUSION: This study showed that the AIP and ratio of small dense LDL/CETP had a significant correlation with the uncontrolled T2DM. The AIP and ratio of small dense LDL/CETP increase was found at the uncontrolled T2DM to be 4 times greater than the controlled T2DM.

KEYWORDS: T2DM., atherosclerosis, atherogenic index of plasma, small dense LDL, LCAT, CETP, ratio of sdLDL/LCAT, ratio of sdLDL/CETP.

Introduction

In individuals with T2DM, metabolic syndrome, and the combined dyslipidemia, cardiovascular risk is increased by a clustering of risk factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL cholesterol, increased triglyceride, and an increased in small dense LDL particles. The current increase in the incidence of T2DM in the population perhaps poses the most urgent cardiovascular risk. Although insulin resistance is crucial to the pathogenesis of the disease, the associated atherogenic lipoprotein phenotype considerably enhances the risk. Hence there is an ongoing intense search for a medication capable

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of modifying the atherogenic lipid profile as well as lowering glucose. Atherogenic index of plasma (AIP) is a new sign of atherogeneity since the AIP is related directly to the atherosclerosis risk. AIP is ratio calculated as log (TG/HDL-C). Small dense LDL is related to the atherogenic lipoprotein profile. LCAT and CETP are closely related to the reverse cholesterol transport mechanism, especially in esterification and transfer of mature HDL formation.

Methods

PATIENTS

This was observational study with cross sectional design, with two groups as controlled and uncontrolled T2DM. Written consent from subjects or patient's family received before subjects were enrolled in this study.

MEASUREMENTS OF BIOCHEMICAL MARKERS

All venous blood samples, and the serum was immediately separated by centrifugation and stored at -20OC until use. Serum triglyceride levels were measured by GPO-PAP using the kits from Roche Diagnostic, Mannheim, Germany. Serum HDL cholesterol levels were measured by homogenous from Daiichi pure chemical, Tokyo, Japan. Serum sdLDL levels were measured by homogenous from Denka Seiken, Tokyo, Japan. Serum LCAT and CETP levels were measured by enzymatic from Daiichi pure chemical, Tokyo, Japan. Serum triglyceride and HDL cholesterol measurements was performed in Prodia Clinical Laboratory – Kramat Raya Jakarta Branch. Serum sdLDL, LCAT and CETP measurements was performed in Research & Development Laboratory of Prodia Clinical Laboratory. All assays were performed according to manufacturers instruction.

For each run of serum triglyceride and HDL-C, sdLDL, LCAT and CETP controls were included in the assays, and all results were within acceptable ranges. In our laboratory, the intraday imprecisions for all assays were lied between 0,35 % - 4,1 %.

STATISTICAL ANALYSIS

Statistical analysis were performed with SPSS for windows ver. 11.5. Univariate analysis were performed to calculate mean, maximum and minimum value and SD. Comparison of AIP, ratio of sdLDL/LCAT and ratio (sdLDL/CETP) levels between cases and control group were analyzed using Mann-Whitney non-parametric test. Spearman correlation statistics were used to determined the correlation among the various variables. The relationship between was AIP, ratio (sdLDL/LCAT) and ratio (sdLDL/CETP) determined using crosstabs test to obtain OR and 95% CI..

Results

Table 1 shows mean values and SDs of BMI (Body Mass Index), waist circumference, smoker, exercise and the measured biomarkers between controlled and uncontrolled T2DM. They reveal significance differences of fasting glucose ($p < 0.001$), A1C ($p < 0.001$) and triglyceride ($p < 0.001$) between controlled and uncontrolled T2DM (control). However, no significance differences of serum HDL-C, sdLDL, LCAT, CETP, (sdLDL/LCAT) ratio and (sdLDL/CETP) ratio between controlled and uncontrolled T2DM.

Tabel. 1 : Basic Characteristics of Subjects

Variables	Controlled T2DM (n=36)	Uncontrolled T2DM (n=36)	p
	Mean ± SD %	Mean ± SD %	
Age (years)	56,31 ± 10,54	60,11 ± 9,99	0,120
Height (cm)	168,19 ± 5,48	167,06 ± 6,89	0,440
Weight (kg)	69,09 ± 8,83	71,27 ± 10,24	0,337
Waist circumference (cm)	90,78 ± 8,23	91,81 ± 7,70	0,586
BMI	24,36 ± 2,40	25,5 ± 2,98	0,079
Smoker	19,4	25	0,571
Exercise	75	80,6	0,571
Fasting glucose (mg/dl)	119,25 + 30,46	189,70 ± 52,04	0,000*
A1C (%)	6,81 ± 0,88	10,42 ± 1,80	0,000*
Triglyceride (mg/dl)	124,54 ± 50,48	204,94 ± 109,68	0,000*
HDL cholesterol (mg/dl)	44,81± 9,61	40,33 ± 8,18	0,37
LCAT (U)	500,49 + 143,89	499, 76 + 144, 61	0,983
CETP (ug/ml)	2,58 + 0,65	2,42 + 0,56	0,270
sdLDL (mg/dl)	32, 14 + 22,74	42,05 + 22,97	0,07
AIP	0,42 + 0,22	0,65 + 0,28	0,000*
Ratio of (sdLDL/LCAT)	0,07 + 0,05	0,09 + 0,06	0,124
Ratio of (sdLDL/CETP)	13, 31 + 10,01	18,52 + 12,37	0,54

BMI= Body Mass Index; HDL= High Density Lipoprotein; LCAT=Lecithin Cholesterol Acyl Transferase; CETP=Cholesterol Ester Transfer Protein; sdLDL=small dense Low Density Lipoprotein; AIP=Atherogenic Index of Plasma; p=probability, * = statistically significance (p < 0,05)

Correlation analysis using Spearman statistics reveal that there is significance correlation between serum A1C levels and AIP (r = 0,428, p = < 0,001), AIP and serum sdLDL levels (r = 0,455, p = < 0,01). Inversely, it was found significance negative correlation between serum A1C levels and serum LCAT levels (r = -0.068, p = 0.570), serum A1C levels and CETP (r = -0.021, p = 0.860),

Table 2. Correlation Among Variables

Variabel	r	p
A1C – AIP	0,428**	< 0,001
A1C – sdLDL	0,191	0,109
A1C – LCAT	-0,068	0,570
A1C – CETP	-0,021	0,860
AIP – sdLDL	0,455**	< 0,001
AIP – LCAT	0,062	0,606
AIP – CETP	0,017	0,885
LCAT – sdLDL	0,122	0,308
LCAT – CETP	0,039	0,743
sdLDL - CETP	0,079	0,511

AIP=Atherogenic Index of Plasma; sdLDL-small dense Low Density Lipoprotein; LCAT=Lecithin Cholesterol Acyl Transferase; CETP=Cholesterol Ester Transfer Protein; r = coefisien of correlation, p = probability, * = statistically significance

Table 3 show the results of association analysis using crosstabs method between AIP, (sdLDL/LCAT) ratio and (sdLDL/CETP) ratio with uncontrolled T2DM.. There is evidence for significant association between AIP and ratio (sdLDL/CETP) (OR = 4; 95%; C.I : 1,501-10,658, p = 0,006) in uncontrolled T2DM.

Table 3. Association of AIP, ratio of sdLDL/LCAT and ratio of sdLDL/CETP with uncontrolled T2DM

Variabels	B	Wald	p	OR (95% C.I)
AIP	1,386	7,687	0,006	4 (1,501-10,658)
Ratio of (sdLDL/LCAT)	0,222	0,222	0,638	1,25 (0,495-3,151)
Ratio of (sdLDL/CETP)	1,386	7,687	0,006	4 (1,501-10,658)

p= probability; OR=Odds Ratio; CI=Confidence interval; AIP= Atherogenic Index of Plasma ; sdLDL=small dense Low Density Lipoprotein; LCAT : Lecythin Cholesterol Acyl Transferase; CETP : Cholesteryl Ester Transfer Protein.

Discussion

This study was conducted with 72 participants that were divided into two groups. Control group consist of 36 patients that with A1c value ¬< 8%, while 36 patients from case group had A1c value >8%.

A1c was used in the study as the control and case group determinant, since increase in A1c has strong relationship with the presence of micro-vascular and macro-vascular complication in DM patients. (1). Cohort study from United Kingdom Diabetes Study (UKPDS) on 2060 DM patients in The Atherosclerosis Risk in Communities that had been followed up for 10 years showed a 25% decline (p=0.0099) in the risk of cardiovascular and a 16 % decline in myocardial infarction (p=0.05) in intensive-treated group with A1c limit = 7% compared with conventional-treated group with A1c limit = 7.9% (1).

Comparison of AIP value between controlled and uncontrolled T2DM was 0.42 + 0.22 and 0.65 + 0.28 in mean + standard deviation. The difference of two groups was p<0.001. Similar result was obtained by a cohort study in 1433 patients with various risk of atherosclerosis including T2DM, hypertension and dyslipidemia, which showed that AIP had significant correlation (r = 0.803) (2), that the group of controlled T2DM will certainly had higher risk of atherosclerosis than the controlled T2DM group.

Ratio of sdLDL/LCAT between controlled and uncontrolled T2DM group are 0.07 + 0.05 and 0.09 + 0.06 in mean value + standard deviation. The difference of two groups ratio was not significant (p = 0.124). It meant that the ratio of sdLDL/LCAT in the uncontrolled group was higher than the controlled T2DM group.

These findings were explainable by a study of small dense LDL by Physicians Health Study with 266 male subjects with fatal and no fatal CHD for 7 years that indicated significant comparison with controlled group (p< 0.001) and relative risk of 1.38. Therefore, LDL size was a strong predictor for CHD (3), or in other words small dense LDL was pro-atherogenic. On the other hand, LCAT was anti-atherogenic and played role in the estherification of free cholesterol in the circulating plasma lipoprotein. Free LCAT was not found in plasma but it was bound to HDL Cholesterol (4). It caused the ratio of sdLDL/LCAT in the study gave no significant result.

Comparison of the ratio of sdLDL/CETP between controlled DM and uncontrolled DM group was 13.31 + 10.01 and 18.52 + 12.37 in mean value + standard deviation. The difference of two groups ratio was not significant, with p = 0.054. It meant that the ratio of sdLDL/CETP in uncontrolled group is higher than controlled T2DM. This finding was explainable by a study of small dense LDL by Quebec Cardiovascular Study which included 2,103 people for 5 years, where small dense LDL had significant correlation with CHD

by odds ratio of 3.6 (p , 0.01). After multi-variant analysis by excluding the other risk factors like TG, Apo B and HDL-C, they got odds ratio of 2.5 ($p < 0.08$) (5). Meanwhile, CETP played role in the change of LDL I/II to LDL III. Besides, CETP acted as the mediator in the transformation of cholesterol ester rich-LDL, and together with the next action by HL, induced the lipolysis of triglyceride rich-LDL to form small dense LDL (6).

Distribution of AIP value against the AIC value with statistical analysis of bivariat correlation showed correlation with $r = 0.428$ ($p = <0.001$). Whereas, using partial correlation analysis by controlling the other factors i.e. sdLDL, LCAT and CETP we got a significant correlation between A1c and AIP ($r = 0.392$, $p < 0.01$). The explanation was that hypertriglyceridemia was the primary dyslipidemia disorder in individuals with type 2 DM. Epidemiology study showed that hypertriglyceridemia was the risk factor of CVD in T2DM. It has been known that triglyceride-rich lipoprotein (VLDL and LDL) is atherogenic, however the association with CHD was caused by the presence of negative correlation between hypertriglyceridemia and decrease in HDL value, and increase in small dense LDL in T2DM patients and insulin resistance syndrome (central obesity) (7, 8).

Distribution value the ratio of sdLDL/LCAT against the A1c value. Statistical analysis of bivariat correlation showed correlation with $r = 0.213$ ($p = 0.072$). Whereas, using partial correlation analysis by controlling the other factors i.e. AIP and CETP, we obtained a weak and insignificant correlation between the ratio of sdLDL/LCAT and A1c ($r = 0.190$, $p = 0.266$). Several case-control studies indicated that small dense LDL correlated with the atherogenic lipoprotein profile, which included the elevation of triglyceride in plasma, Apo B and a decline in HDL-Cholesterol (9). High concent of small dense LDL correlated with the increase in the risk of CHD 3-7 times without taking note of total LDL level in blood circulation. High consent of small dense LDL was generally found in patients with elevated triglyceride level but low HDL-Cholesterol level. Small dense LDL was rarely found in individual with triglyceride < 1.0 mmol/l, but in triglyceride > 2.0 mmol/l (6).

LCAT facilitated the RCT process and modulated the atherosclerosis progression (4). LCAT activity declines significantly as much as 24–50% in individuals with CHD (10) but in plasma LCAT was bound to HDL (11). From the result of this study, it could be explained that LCAT couldn't directly describe the level of atherogenicity in the subjects of study, despite being ratio with small dense LDL, using both bivariat and partial correlation analysis.

Distribution of the ratio value of sdLDL/CETP against the AIC value. Bivariat correlation analysis revealed a correlation with $r = 0.169$ ($p=0.155$). Whereas, using partial correlation analysis, with AIP and LCAT as the controlling factors, we obtained a weak and insignificant correlation between the ratio of sdLDL/CETP and AIC ($r=0.011$, $p=0.926$). These results were explainable that CETP played role in LDL and HDL remodeling to be smaller particles. This activity in plasma resulted in an increase in the level of triglyceride, LDL and HDL cholesterol. But as LCAT, CETP couldn't directly illustrate the level of atherogenicity in the subjects of this investigation, despite being ratio against small dense LDL, using bivariat or partial correlation analysis.

Analysis of the correlation of AIP level with the occurrence of uncontrolled and controlled T2DM. We used the descriptive analysis of crosstabs to analyze the correlation of the level of AIP with the occurrence of uncontrolled T2DM. Before performing the crosstabs analysis, we determined a cut-off point of AIP using the frequency analysis (tertile), and we obtained the value of 0.53 as the cut-off point. This value was used to categorize the subjects into 2 groups namely high risk group ($AIP \geq 0.53$) and low risk group ($AIP < 0.53$). The analysis showed that the level of AIP correlated significantly with the uncontrolled T2DM ($p = 0.004$*).

Analysis of the correlation of the ratio of sdLDL/LCAT with the occurrence of controlled and uncontrolled T2DM. We obtained the value of 0.064 as the cut-off point, to categorize the subjects into the high risk group ratio of sdLDL/LCAT ≥ 0.064 and low risk group ratio of sdLDL/LCAT < 0.064. The results showed that the ratio of sdLDL/LCAT didn't significantly correlate with the occurrence of uncontrolled T2DM ($p = 0.637$). it could be explained that LCAT played role as the main enzyme in the estherification of free cholesterol contained in the circulating plasma lipoprotein, where LCAT in plasma bound to HDL and thus either LCAT itself or being ratio with sdLDL didn't show significant correlation with uncontrolled T2DM.

Analysis of the correlation of the ratio of sdLDL/CETP with the occurrence of uncontrolled T2DM as case and controlled type 2 DM as control. We obtained the value of 12.99 as the cut-off point, and this value was used to categorize the high risk group ratio of sdLDL/

CETP ≥ 12.99 and low risk group ratio of sdLDL/CETP < 12.99. The analysis revealed that the ratio of sdLDL/CETP correlated significantly with the occurrence of uncontrolled DM (p = 0.004*). It might be caused by the role of CETP in the transformation of LDL I/II to be LDL III. Besides, CETP acted as the mediator in the transformation of cholesterol ester rich-LDL, and together with the next action by HL, lipolysis triglyceride rich-LDL to form small dense LDL (6).

To analyze the interaction between free variables (AIP, ratio of sdLDL/LCAT and ratio of sdLDL/CETP and the occurrence of T2DM, we performed the logistics regression analysis. The results showed that AIP (p = 0.006), ratio of sdLDL/LCAT (p = 0.638) and ratio of sdLDL/CETP (p = 0.0060). The complete results could be seen in table 8, which showed that the OR value of AIP is 4 (95 % CI; 1.501-10.658), which meant that individuals with AIP ≥ 0.053 had the 4-fold risk compared to individuals with AIP < 0.053 against the occurrence of uncontrolled T2DM.

The variable ratio of sdLDL/LCAT had the OR value of 1.25 (95 % CI; 0.495 – 3.151) which meant that individuals with ratio of sdLDL/LCAT ≥ 0.069 had a 1.25-fold risk compared to individuals with ratio of sdLDL/LCAT < 0.068 against the occurrence of uncontrolled T2DM.

The variable ratio of sdLDL/CETP had the OR value of 4 (95 % CI; 1.501 – 10.658) which meant that individuals with sdLDL/ CETP ≥ 12.99 had a 4-fold risk compared to individuals with the ratio of sdLDL/ CETP < 12.99 against the occurrence of uncontrolled T2DM.

The results of logistics regression analysis showed that the variable of AIP and ratio of sdLDL/CETP had the better predictive value than the ratio of sdLDL/LCAT against the occurrence of uncontrolled T2DM.

The phenomena that AIP value ≥ 0.053 had 4-fold risk than AIP < 0.053 and the ratio of sdLDL/CETP ≥ 12.99 had 4-fold risk than the ratio < 12.99 could be explained that in the uncontrolled T2DM group, a dyslipidemia diabetes took place and was worse than in controlled T2DM. CETP could be both pro-atherogenic and anti-atherogenic. This was associated with a research where administer of synthetic CETP inhibitor would increase the HDL level and reduce atherosclerosis in experimental animals. It had also been reported that patients with CETP deficiency in Japan had a very high HDL level (12). Most experimental evidences in animals showed the pro-atherogenic role of CETP and supported the idea that the inhibition of CETP would be anti-atherogenic (13, 14). Thus, if CETP was being ratio with small dense LDL which was also pro-atherogenic, the value would show a significant correlation with the uncontrolled T2DM.

Conclusion

This study showed that the AIP and ratio of small dense LDL/CETP had a significant correlation with the uncontrolled T2DM. The AIP and ratio of small dense LDL/CETP increase was found at the uncontrolled T2DM to be 4 times greater than the controlled T2DM.

Acknowledgement:

We thank Endang Hoyaranda and The Prodia Foundation for Research and Training for their invaluable help in supporting and conducting the many procedures of this research.

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