Homocystein Levels and Lipid Profile on Non-DM and DM Individuals with and without Cardiovascular Complications

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ABSTRACT

Background: Homocysteine is suspected to increase the risk of diabetes mellitus (DM) complications and is associated with CV disease. While dyslipidemia and DM are risk factors for death due to CV disease, studies on relationship between homocysteine and glycemic control were inconsistent. **Objective**: To analyze the difference of Hcy level and lipid profile on non-DM (Group I), DM with CV (II) and without CV (III). **Methods:** This crosssectional study was conducted in Dr. Kariadi Hospital Semarang from April to October 2016. Samples were recruited consecutively, of which group I consists of 26 persons, group II (30) and III (30). All of the samples aged 30-75 years old, with long DM duration of more than 5 years. Fasting and 2 hours PP blood glucose, lipid profile was analyzed with *auto analyzer*, while Hcy was analyzed with ELISA. Data were analyzed using independent t test. Significance is expressed at $p < 0.05$. **Results:** A significant difference on Hcy level was found between group I and III (p=0.000), but not between group I and II or II and III. No significant difference was observed on total cholesterol (TC) and LDLC in all groups. Significant differences were found on HDLC level between group I and II (p=0.009); II and III (p=0.000); I and III (p=0.033). Triglyceride level on group I was significantly different compared to group II and III (p=0.030 and 0.013 respectively), but was not significantly different compared to group II and III. **Conclusion:** The highest Hcy level, the lowest HDL-C and the highest triglyceride level were found in DM patients with cardiovascular complication.

Keywords: DM, Homocysteine, Lipid Profile, CHD

WHO estimated that the number of DM patients in worldwide scale will increase to as much as 194 million people in the year 2003, and about two-thirds of these people lived in developing countries.(1)(2) [14,16] DM will have an impact on the quality of human resources and a substantial increase in health costs.(3)

DM individuals are two to four times more likely to develop vascular disease than non-DM. Homocysteine (Hcy) is an interesting topic on arteriosclerosis and cardiovascular disease in recent years.(4) Homocysteine is an amino acid (AA) with AA derivatives of essential sulfhydryl group of methionine and source of animal protein.(5) Hyperhomocysteinemia/HHcy is predicted to increase the risk of DM complications and is often associated with retinopathy, nephropathy and cardiovascular disease (CV).(6)

Hypotheses on rising levels of Hcy is due to the acceleration of glucose as a trigger of oxidative stress on endothelial cells have been proven in animal study, it is evidenced that Hcy is a more real trigger of endothelial dysfunction in diabetes than in non-DM.(5) The mechanism on how Hcy can increase the risk of vascular disease is not clear yet. In one of the studies, it is mentioned that HHcy stimulates the growth of vascular smooth muscle cells, causing intimal thickening of the artery walls, inhibiting oxygenation of the vascular wall and increasing oxygen-free radicals with tissue damage. Reduction of 2 O_2 molecules on Hcy oxidation will produce free radicals and hydrogen peroxide and cause endothelial cell damage. Hyperhomocysteinemia may increase oxidative stress and, alongside with other mechanisms, induce vascular endothelial dysfunction and vascular smooth muscle cell proliferation, atherogenesis then occurs and increases the risk of vascular thromboembolic disease. (4)

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Dyslipidemia is a risk factor of death due to CV disease, this risk will increase in patients with type 2 DM (DMT2). Hyperglycemia, diabetic dyslipidemia, insulin resistance and hypertension will produce atherogenic environment in circulation. However, atherosclerosis also occur in patients without CV dyslipidemia risk factors, smoking, diabetes or hypertension, and this led us to think towards the other risk factors such as Hcy. Low HDL-Cholesterol (HDL-C) levels in DMT2 indicate an inverse relationship between HDL-C and the incidence of cardiac abnormalities. Increased level of LDL-C has been known as an independent risk factor. The increase in LDL-C spur increased oxidized LDL which plays a role in heart vascular. Atherogenic. Hyperglycemia in DMT2 increases the risk of coronary arterial disease (CAD) incident. Increased levels of triglycerides will cause an increase in VLDL-C and LDL-C independently, and causes atherogenity LDL-C through triglycerides oxidized lipoproteins. The ratio of total cholesterol/HDL-C was higher in DMT2 and is stronger predictor of CAD than total cholesterol or HDL-C in single.(7) Recent studies have examined Hcy as a cardiovascular risk factor in DM patients, but the results of research on the association of Hcy levels with glycemic control are still inconsistent and require further investigation.(8)

The purpose of this study is to analyze differences in homocysteine levels and lipid profiles in non-DM individuals, as well as DM patients, with and without cardiovascular complications

MATERIALS AND METHODS

The research was conducted after obtaining research permission from Health Research Ethics Commission Faculty of Medicine UNDIP/ Dr Kariadi Hospital Semarang. Before the study was conducted, all subjects were requested written informed consent.

This is a descriptive analytic study with cross sectional approach. It was conducted in Dr. Kariadi Hospital Semarang, from April to October 2016. The subjects for DM patients with and without CV complication were taken in DM and Cardiology clinic (30 patients each) and for non-DM subject were students of Faculty of Medicine (26 respondents). The inclusion criteria were age 30-75 years old, not DM (for the non-DM group), suffering DM > 5 years (for DM group without CV complication), or already suffering from DM over 5 years and had complications CHD based on minimal electrocardiography (ECG) examination (for DM group with cardiovascular complications), not taking any medications for lowering lipids, and willing to take part in this study. Patients with acute myocardial infarction were rejected to participate in this study. Blood were taken from cubital vein (5 ml) and analyzed for fasting and 2 hours PP glucose levels using auto analyzer methods. Serum Hcy levels were analyzed using ELISA were analyzed using SPSS version 21.0. Each numerical variable was tested for normality, on which abnormal data were transformed to search for a normal distribution, and parametric test was done by using independent t test. Significance is expressed at $p<0.05$.

RESULTS

Description of result

The subjects of this study consisted of non-DM individual groups: 26 persons, consisting of 12 men (46.2%) and 14 women (53.8%). DM subjects without CV complications were 30 persons, consisting of 10 men (33.3%) and 20 women (66.7%) and DM subjects with CV were 30 persons, consisting of 23 men (76,7%) and women 7 people (23.3%) as can be seen in table 1.

The mean age of non-DM group is 32.7 ± 6.3 years, DM without CV complication 56.2 ± 9.7 years and DM group with CV is 59.3 ± 7.8 years. Subject smokers were more common in the DM group with complications of CV counted 19 people (63.3%). Mean body mass index DM group with CV was higher than the other group that is 25.6 ± 2.2 µml/dl. The mean duration of DM was longer in the DM group with CV complications (9.0 ± 3.5 years) than in the DM without CV (6.9 ± 3.2 years). Mean fasting blood glucose level of non-DM group was 84.1 ± 9.1 mg/dl, DM group without CV was 167.7 ± 52.4 mg/dl and DM with CV complication was 186.9 ± 90.0 mg/dl. However, the mean of 2 hours PP glucose levels of both DM groups with and without CV complications were similar. (See table 1).

The mean Hcy levels were the lowest in control group (7.7 ± 1.7 mg / dl). Groups of DM with and without CV complications were similar, 13.1 ± 3.7 vs. 13.1 ± 15.7 mg / dl, respectively.

The mean of total cholesterol levels of the non-DM and DM groups with no similar CV complications were (183.2 ± 32.2 mg / dl and 183.2 ± 34.9 mg / dl). DM group with lower CV complications (165.0 ± 50.5 mg / dl). The highest mean triglyceride rate was found in the DM group with CV complications, followed by DM without the complications of CV and the lowest in the non-DM group (202.5 ± 178.9 vs 183.2 ± 34.9 vs. 104.6 ± 78.2 mg/dl).

The mean of LDL cholesterol level was highest in the DM group without complications of

CV, followed by the non-DM group and the DM group with CV complications (115.2 ± 34.9 vs 109.7 ± 30.2 and 105.7 ± 47.4 mg/dl). The highest HDL cholesterol average level was found in the non-DM group, followed by DM without CV complications and DM group with CV complications (42.4 ± 6.3 vs 37.6 ± 7.0 vs. 34.3 ± 4.1mg / dl).

Table 1. Description of the subject

Parameter	Mean±SD Min-maks	Control (n=26)	DM without CV (n=30)	DM with CV (n=30)
Age(years)	Mean±SD	32.7 ± 6,3	56.2 ± 9.7	59.3 ± 7.8
	Min-maks	22-51	36-75	40-73
Sexuality (total/in %)	Man	12 (46.2)	10 (33.3)	23 (76.7)
	Woman	14 (53.8)	20 (66.7)	7 (23.3)
Smoking (total/in %)	Yes	7 (26.9)	6 (20.0)	19 (63.3)
	No	19 (73.1)	24 (80.1)	11 (36.7)
BMI	Mean±SD	23.3 ± 4.5	22.8 ± 2.5	25.6 ± 2.2
	Min-maks	17.6-39.8	18.4-27.6	22.4-29.8
Long suffering from	Mean±SD		6.9 ± 3.2	9.0 ± 3.5
DM(years)	Min-maks		3-15	5-20
Fasting blood glucose(mg/dl)	Mean ±SD	84.1 ± 9.1	167.7 ± 52.4	186.9 ± 90.0
	Min-maks	69-110	100-285	65-451
2 hours Post Prandial glucose	Mean±SD	89.4 ± 19.1	259.8 ± 69.3	258.2 ± 117.0
(mg/dl)	Min-maks	57-152	132-434	118-605
Total Cholesterol(mg/dl)	Mean±SD	183.2 ± 32.2	183.2 ± 34.9	165.0 ± 50.5
	Min-mak	145-298	110-267	110-267
Triglyseride (mg/dl)	Mean±SD	104.6 ± 78.2	151.2 ± 78.4	202.5 ± 178.9
	Min-maks	47-451	62-419	62-419
LDL (mg/dl)	Mean±SD	109.7 ± 30.2	115.2 ± 34.9	105.7 ± 47.4
	Min-maks	77-223	49-207	39-205
HDL (mg/dl)	Mean±SD	42.4 ± 6.3	37.6 ± 7.0	34.3 ± 4.1
	Min-maks	35-63	27-66	24-45
Homocysteine(µml/dl)	Mean±SD	7.7 ± 1.7	13.1 ± 15.7	13.1 ± 3.7
	Min-maks	5.0-13.0	4.0-93.9	6.4-26

Table 2 Differences in levels of homocysteine and lipid profiles between groups

Group	Hcy	Cholesterol	LDL-C	HDL-C	Triglyseride
Non-DM and DM without CV complication	0.086	0.999	0.531	0.009*	0.030*
Non-DM and DM wth CV complication	0.000*	0.111	0.710	0.000*	0.013*
DM without and with CV complication	0.993	0.111	0.383	0.033*	0.156

Differences in levels of Homocysteine and Lipid Profiles between Groups

Difference test for each variable between groups were done by using independent t test for Hcy level and lipid profile, and can be seen in Table 2.

Homocysteine

The results showed that there were significant difference on Hcy levels between non-DM group and DM group with CV complications of (p=0.000). However, there was no significant difference between non-DM group and DM group without CV complication (p=0.086), as well as between DM group without and with CV complication (p=0.993).

Lipid profile

The results of data analysis in this study showed that there is no significant difference between total cholesterol and LDL cholesterol in the non-DM group and DM without CV

complications (p=0.999 and p=0.531); non-DM and DM with CV complication (p=0.111 and p=0.710); DM without and with complications (p=0.111 and p=0.383)

We found there were significant differences in HDL cholesterol levels between the non-DM group and the DM group without CV complication (p=0.009), between the non-DM group and the DM group with CV complications (p=0.000), as well asq between the DM group without and with CV complications (p=0.033). In this study there was a significant difference in triglyceride levels between the non-DM group and the DM group without CV complications P=0.030) and with CV complication (p = 0.013). There was no significant difference of triglyceride level between the DM group without and with CV complication (p=0.156)

DISCUSSION

Obesity, DM, and duration of DM were the risk factors for cardiovascular disease, the mean duration of DM in this study was longer in the DM group with CV complications. The mean fasting blood glucose level was higher in the group with CV complications (186.9 ± 90.0 mg/Dl) compared to group without CV complications (167.7 ± 52.4 mg/dl), but the mean 2 hours PP blood glucose levels of both two groups were similar.

High triglyceride levels and low HDL levels are risk factors for cardiovascular disease. High Hcy levels are also risk factor for cardiovascular disease as found in the DM group with complications of CV.

Homocysteine

The majority of diabetes mellitus subjects with cardiovascular complication were: males (76.7%), the oldest age among the three groups, the highest fasting glucose level compared to the other two groups, smoking (63.3%), the highest BMI and longer suffering from DM (table 1). All of these factors are risk factors for cardiovascular disease. The risk factors of hyperhomocysteinemia encountered in this group are male, higher BMI, smoking and DM. As we know increasing levels of Hcy also a risk factor for cardiovascular disease. There was significant difference in Hcy levels between the non DM group and the DM with cardiovascular complication, eventhough still in the normal range.

Homocysteine is an amino acid which is a metabolic intermediate in the metabolism of essential amino acids methionine.[6] Homocysteine is found in daily diets, and high levels are common in atherosclerotic DM and CHD. Thus, Hcy is strong independent predictors of CHD occurrence. Association between Hcy and atherosclerosis remains unclear, suspected to be associated with free radicals, smooth muscle cell stimulation and changes in platelets and hemostasis. Hyperhomocysteinemia is a risk factor for endothelial dysfunction. Homocysteine levels are higher in patients DM and is a risk of CHD.(9)

The results of various studies on the effect of glycemic control on plasma Hcy were inconsistent. Previous studies suggested that there was no correlation between Hcy and glycemic control. Hoogoven et al reported that there is no association between Hcy and HbA1c. Another study conducted by Aghamohammadi et al, examined the association between Hcy levels and glycemic control in 70 men with type 2 DM and concluded that there was no correlation between Hcy on glycemic control.(10) Diabetes has a CVD risk of 2 to 4 times compared to non-diabetes and atherosclerotic also develops despite mild glucose tolerance disorder.

Homocysteine is an interesting topic in vascular disease in recent years, elevated Hcy levels can increase CVD and mortality in the population. Increased Hcy levels are also present in DM patients but their association with CVD remains unclear.

Studies in humans showed that Hcy spurred atherosclerosis, it is characterized by platelet accumulation and thrombus formation in the endothelial area of the lesion. Hcy stimulates endothelial injury in sub-endothelial matrices that will stimulate platelet activation.(11) Other researchers pointed out that Hcy is a risk factor for type 2 DM not only for coronary events but also deaths from cardiovascular, retinopathy and microalbuminuria.(12)

Several cross-sectional studies and case control studies showed that moderate HHcy increased risk factors for atherosclerosis and cardiovascular disease (CVD). Some researchers found significant Hcy effect on CVD. Recent studies mention that HHcy contribute 10% to the risk of Coronary artery diseases (CAD). Although possible mechanism explaining the relationship of plasma homocysteine level and CVD is inconclusive. The most possible hypothesis that elevated homocysteine levels is endothelium dysfunction due to enhanced oxidative stress and reduced the production and bioavailability of nitric oxide (a strong relaxing factor) of the endothelium .(13)

Lipid Profile

Abnormal lipids are often primarily found in DM type 2. The prevalence of dyslipidemia in DM

is 95%. Dyslipidemia is a major risk factor for CHD which is a cause of morbidity and mortality in DM patients due to elevated serum triglycerides (69%), cholesterol (56%), LDL-C (77%) and HDL-C decrease (71%). Hyperlipidemia is the most common complication of DM and predisposes to atherosclerosis and macrovascular complications. The most common abnormal lipids in DM are elevated triglycerides, LDL-C and decreased HDL-C. Good glycemic control will prevent the development and progression of abnormal lipids in DM patients.(14)

Several factors can affect lipid levels in patients with DM, such as interrelation between carbohydrates (CH) and lipid metabolism. Therefore, any interference on CH metabolism will cause disruption of lipid metabolism and vice versa. Insulin resistance is a primary defect in majority of DM type 2 and in non-DM insulin resistance and hyperinsulinism is a predictor factor of progression to DM type 2. Some studies indicate that insulin affects apolipoprotein production by the liver, regulates activity of enzyme lipoprotein lipase and cholesterol ester transfer protein (CETP) causing dyslipidemia in DM. In addition, insulin deficiency decreases hepatic lipase activity and some of the production stages of biologically active lipase protein.(15)

This study obtained no significant difference on Total Cholesterol and LDL-C between each group, this is probably due in DM patients with CV complications has been aware to keep his diet and is probably already taking oral diabetic medications and anti-lipidemia, despite trying dug this questioners. It is evidenced by mean cholesterol and LDL-C levels were higher in the DM group without CV complications compared with DM group with CV complication.

Pakard et al reported a decrease in HDL-C is a strong predictor of CHD, while Goldberg reported hyperglycemia progressive increase transfer of cholesterol esters from HDL-C to particles VLDL-C, then the LDL-C particle solids will acquire the majority of HDL ester and will reduce levels of HDL-C. Improved glycemic control will increase HDL-C, poor glycemic control will lower lipoprotein levels. Poor insulinization will increase lipolysis in adipose tissue which will increase fatty acid (FA) transport to the liver and increase VLDL-C. Insulin directly degrades Apo B (the main protein VLDL-C) and increases Apo B secretion.(15)

The highest level of HDL-C was found in Non DM group, followed by DM group without CV complication and the lowest in DM group with CV complication.

Decreased HDL-C levels are associated with an increased risk of CHD. HDL-C is cardio-protective. Decreased HDL-C is often accompanied by increased levels of trigliceride.(16) HDL-C decreases in DM due to decreased lipoprotein activity because the rate of HDL2 formation depends on the flux rate of the surface component of triglyceride rich lipoprotein mediated in part by lipoprotein lipase. When VLDL-C mediated by LPL catabolism is efficient, the availability of surface components for HDL-C transfer is increased, when undisturbed VLDL-C lipolysis will result in a decrease in HDL-C. Increased HDL-C catabolism due to hypertriglyceridemia, triglyceride transfer rate to HDL2 high and will result in triglyceride-rich HDL2 susceptible to catabolism by hepatic triglyceride lipase. Increased activity of cholesterol - ester transfer proteins (CETP), which modify pathological lipid composition of the sub-population of apoprotein B containing lipoproteins to form atherogenic b-like VLDL particles, so the increased activity of CETP are atherogenik.(3) Descriptive data in this study showed that the lowest average of fasting glucose level in the non-DM group and the highest in the DM group with CV complications, the same case was found in the triglyceride level, the highest level was found in the DM group with CV complications (Table 1).

Dyslipidemia increased in patients with elevated blood glucose levels. Hypertriglyceridemia is the most common lipid abnormality found in type 2 diabetes mellitus (73.3%)(15)(12)Other researchers attributed high triglyceride levels to poor glycemic control in patients with diabetes mellitus and obesity, thought to be associated with decreased lipoprotein lipase activity in muscle and adipose tissue.(15)

DMT2 is associated with elevated triglyceride and VLDL-C levels due to elevated glucose and FFA, VLDL-C and triglyceride clearance disorders resulting from decreased lipase activity of lipase proteins especially in moderate-severe hypertriglyceridemia indicating both deficiency and insulin resistance. In diabetes due to hypertriglyceridemia, large particles of triglyceride rich VLDL are secreted. Changes in VLDL composition have implications for increasing the propensity for atherosclerosis.(17)

Evidence of the relationship between plasma triglyceride levels and the risk of coronary arterial disease (CAD) is broadly based on epidemiological studies. Meta-analysis of 7 populations based on prospective studies found that each 1 mmol/ L increase in triglyceride levels increased the risk of 32% of coronary disease in men and 76% in women after corrected with HDL-C effects to 14% in men and 37% in women. The

direct atherogenic effects of triglyceride rich particle especially IDL-C and the remnant lipoprotein may independently contribute to plasma triglyceride levels as cardiovascular risk factors. It is said that dyslipidemia is associated with insulin resistance in DMT2 and is strongly associated with increased cardiovascular risk.(13)

CONCLUSION

The highest Hcy level, the lowest HDLC and the highest Triglyceride level found on DM with CV complication.

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