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Carotid intima-media thickness among normoglycemia and normotension first-degree relatives of type 2 diabetes mellitus

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Introduction: Theoretically, first-degree relatives (FDRs) of type 2 diabetes mellitus (T2DM) are predisposed to have earlier and more severe atherosclerosis than non-FDR due to hereditary insulin resistance. A previous study reported that atherosclerotic plaques were found in 45.2% of young adults FDR of T2DM, but the study did not include non-FDR as control group. The aim of this study was to compare subclinical atherosclerosis (carotid intima-media thickness, CIMT) between FDR of T2DM and non-FDR.

Method: This was a cross-sectional study involving 16 FDR subjects and 16 age-sex matched non-FDR subjects, aged 19–40 years, with normal glucose tolerance and no hypertension. Collected data included demographic characteristic, anthropometric measurement (BMI and waist circumference), laboratory analysis (fasting blood glucose, HbA1c, lipid profile), and CIMT examination (using B-mode ultrasound).

Results: The mean of CIMT in the FDR group was higher than that in the non-FDR group (0.44 mm vs 0.38 mm, p=0.005). After adjusting for waist circumference, BMI, low-density lipoprotein cholesterol, and triglyceride, CIMT maintained significant difference between FDR and non-FDR subjects. BMI and waist circumference showed moderate correlation with CIMT.

Conclusion: CIMT in young adult FDR of T2DM is thicker than that in age-and sex-matched non-FDR population.

Keywords: first-degree relatives, type 2 diabetes mellitus, subclinical atherosclerosis, carotid intima-media thickness

Introduction

Cardiovascular disease (CVD) remains a leading cause of death globally. Recent data in 2013 showed that approximately 17.3 million of the total 54 million deaths per year in the world were caused by CVD.[1] According to WHO data in 2014, CVD is the leading cause of death in Indonesia, comprising 37% of all causes of death.[2]

Atherosclerosis is a major underlying cause of CVD, including myocardial infarction, stroke, heart failure, and peripheral artery disease. This condition begins early in childhood and is progressive. Atherosclerosis is often asymptomatic for several decades before manifesting clinically, termed as subclinical atherosclerosis.[3,4] Cardiovascular risk factors such as hypertension, hyperglycemia, dyslipidemia, and obesity not only play a role in the development of the atherosclerosis process, but also serve as components of insulin-resistance syndrome.[5]

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One of the risk factors for developing insulin resistance is first-degree relatives (FDRs) who suffer from type 2 diabetes mellitus (T2DM). The FDR group shows the tendency of insulin resistance and pancreatic beta cell function impairment, even in adolescence.[6] A study showed that normoglycemic FDR of T2DM patients had higher insulin levels, more fat accumulation in muscle, and lower peripheral glucose uptake than in the non-FDR population.[7] The insulin-resistance FDR group also proved to have impaired coronary artery blood flow and coronary elasticity.[8] A previous study in Indonesia showed that atherosclerotic lesions were found in 45.2% normoglycemic and normotensive young adults from FDR population.[9] However, the study did not include the non-FDR group; therefore, the difference in subclinical atherosclerotic lesions between the two groups was unknown.

Method

This study was conducted between June and September 2018, involving 16 subjects of FDR T2DM and 16 subjects with non-FDR T2DM, who were matched for age and gender. Sample collection was performed using consecutive sampling method. The FDR subjects were recruited through direct invitation by diagnosed T2DM patients of the Endocrinology Outpatient Clinic at Cipto Mangunkusumo National General Hospital (RSCM). Consecutively, all T2DM patients were asked to allow their offsprings to participate in the study. All of the candidates were given information regarding the study and were screened based on the study criterion. Informed consent was obtained from those who fulfilled the criteria. Other inclusion criteria for the FDR group included men and women aged 19–40 years and who were normoglycemic and normotensive (HbA1c <5.7%, blood pressure <140/90 mmHg). For the control group, we recruited non-medical workers at RSCM, who did not have a family history of T2DM and had similar inclusion criteria with that of the FDR group. Exclusion criteria for both groups were as follows: 1) smoking; 2) history of coronary heart disease, heart failure, arrhythmia, anemia, stroke, transient ischemic attack, peripheral arterial disease, history of hypertension, and diabetes mellitus; 3) taking hypertension drugs, oral contraceptives, or other drugs that might affect lipid and/or glucose metabolism; 4) history of liver disease, kidney disease, and other chronic diseases; and (5) history of acute bleeding or a history of repeated blood transfusions. All subjects signed a written informed consent to be included on a voluntary basis in the study. The study was approved by the Ethics Committee of the Faculty Medicine, University of Indonesia (No:0242/UN2.F1/ETIK/2018). The written informed consent process was in accordance with the Declaration of Helsinki. Blood pressure measurement was performed using the Riester® sphygmomanometer, which has been calibrated, and a Litmann® stethoscope. Blood pressure was measured by a cuff looped on the upper arm with its center in the brachial artery. During blood pressure measurement, the cuff was inflated until the pulse of the artery disappears and then slowly deflated 3–4 mmHg per second until the arterial pulse returns.

Following that, the subjects underwent anthropometric examination and lipid profile examination after fasting for 12 hrs. BMI was calculated using the following formula: weight(kg)/height^2(m^2). Weight measurement was measured using the calibrated Shoenle® scales. Height measurement was performed using microtoise height measuring instruments. Waist circumference was measured in order to determine the presence of central obesity (≥80 cm in women and ≥90 cm in men, based on NCEP ATP III criteria Asia Pacific modification). Waist circumference measurement was performed by using “Butterfly” measuring tape. This was based on the method determined by WHO. Triglyceride levels, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol in the patient’s blood were evaluated after fasting for at least 12 hrs, using the Roche Cobas 501 MMPA tool with an enzymatic colorimetric method.

The carotid intima-media thickness (CIMT) was measured using a B-mode ultrasound. A single operator, who was an internal medicine specialist, carried out the CIMT examination. CIMT was determined by the average result of the measurement of the thickness of the tunica intima-media on the right common carotid artery (RCCA-IMT) and left common carotid artery (LCCA-IMT), which appeared as a double-line sign longitudinally on the walls of the common carotid artery (CCA). This measurement was using the B-mode ultrasound of the Eccoson E 66D brand.

Statistical analyses were performed using SPSS version 20.0. The researcher set a two-way hypothesis with the value of α 1% and β 10%. A p-value <0.01 was considered statistically significant. Subject characteristics are shown in Table 1 and grouped between subjects with FDR T2DM and non-FDR T2DM. Numerical variables with normal distribution were displayed in the form of mean and SD. Numerical data with abnormal distribution were presented

Table 1 Demographic and anthropometric characteristics of subjects

Variable	FDR (n=16)	Non-FDR (n=16)	p-value
Age (years), average (SD)	28.81 (6.18)	28.81 (6.18)	1.00
Sex, n(%)			1.00
Male	6 (37.5)	6 (37.5)	
Female	10 (62.5)	10 (62.5)	
Family history of diabetes, n (%)			<0.01
Father	4 (25)	0 (0)	
Mother	9 (56.3)	0 (0)	
Both	3 (18.8)	0 (0)	
No family history	0 (0)	16 (100)	
Height (m), average (SD)	1.59 (0.10)	1.62 (0.07)	0.31
Weight (kg), average (SD)	59.24 (9.96)	58.24 (11.34)	0.79
Body mass index (kg/m^2), average (SD)	23.24 (3.22)	21.90 (3.46)	0.27
SBP (mmHg), median (min–max)	110 (90–120)	103 (90–123)	0.04
DBP (mmHg), median (min–max)	70 (60–80)	70 (60–82)	0.49
Waist circumference (cm), average (SD)	78.35 (9.86)	77.69 (10.45)	0.85

Note: The mean difference was considered statistically significant if p<0.01.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; FDR, first-degree relative.

in median and minimum–maximum values. Shapiro–Wilk test was conducted to assess the normality of the data distribution due to the number of subjects (<50). The distribution of data was considered normal if p>0.05. Bivariate analysis was conducted between confounding variables and the dependent variable using the Pearson test if the data distribution is normal or Spearman test if the data distribution is not normal. Variables from bivariate analysis that showed p<0.25 were assessed by multivariate analysis using linear regression. The results of bivariate and multivariate analysis were considered significant if the value showed p<0.05.

Results

The mean age of the subjects in both groups was 28.81 years, each with a SD of 6.18 years. Most subjects were female (62.5%) (Table 1). Our results showed that 9 subjects (56.3%) had mothers with T2DM, 4 subjects (25%) had fathers with T2DM, and 3 subjects (18.8%) had both parents with T2DM (Table 1). The average BMI of FDR subjects was 23.24 kg/m^2 (Table 1). When compared with FDR subjects, non-FDR subjects had a lower average BMI than those with FDR (21.9 kg/m^2) (Table 1). FDR subjects had higher waist circumference than that of non-FDR {78.35 (SD 9.86) cm vs 77.69 (SD 10.45) cm} (Table 1).

Because the distribution of HbA1c data is not normal, the average HbA1c cannot be shown. The average fasting blood glucose in the FDR subject is 87.44 (SD 9.19) g/dl. The mean LDL cholesterol in this study was 126.06 (SD 44.41) mg/dL on the subject of FDR. Non-FDR subjects had a lower LDL cholesterol. The mean HDL cholesterol in this study was 52.06 (SD 10.36) mg/dl in the FDR group. While the non-FDR group have lower HDL cholesterol mean. Triglyceride levels in this study in both FDR and non-FDR subjects did not have normal data distribution so that triglyceride levels could not be shown. The characteristics of the research subject based on the results of laboratory examinations can be seen in Table 2.

The mean thickness of tunica intima-media of the left carotid artery (LCCA-IMT) in the subject of FDR was 0.45 (SD 0.08) mm. The mean LCCA intima-media thickness in non-FDR subjects was 0.38 (SD ±0.06) mm. There was no statistically significant difference between the mean LCCA-IMT in FDR subjects compared to non-FDR subjects (p=0.015) (Table 3).

Table 2 Lipid and glycemic profile among FDR and non-FDR of T2DM

Variable	FDR (n=16)	Non-FDR (n=16)	p-value
HbA1c (%), median (min–max)	5.15 (4.60–5.50)	5.20 (4.00–5.60)	0.58
Fasting blood glucose (mg/dl), average (SD)	87.44 (9.19)	83.81 (7.96)	0.34
Total cholesterol (mg/dL), average (SD)	190.81 (46.70)	185.38 (25.43)	0.69
LDL cholesterol (mg/dL), average (SD)	126.06 (44.41)	124.75 (23.40)	0.92
HDL cholesterol (mg/dL), average (SD)	52.06 (10.36)	50.25 (12.26)	0.66
Triglyceride, median (min–max)	74 (42–221)	78.50 (54–167)	0.49

Note: The mean difference was considered statistically significant if p<0.01.
Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; FDR, first-degree relative.

Table 3 Results of carotid intima-media thickness examination

Examination	FDR (n=16)	Non-FDR (n=16)	p
LCCA-IMT (mm), average (SD)	0.45 (0.08)	0.38 (0.06)	p=0.015
RCCA-IMT (mm), median (min–max)	0.42 (0.33–0.59)	0.37 (0.32–0.44)	p=0.015
CIMT (mm), average (SD)	0.44 (0.06)	0.38 (0.05)	**p=0.005**

Notes: The mean difference was considered statistically significant if p<0.01. Bold value indicates the difference of CIMT between 2 groups is statistically significant.
Abbreviations: LCCA-IMT, left common carotid artery-intima media thickness; RCCA-IMT, right common carotid artery-intima media thickness; CIMT, carotid intima-media thickness (LCCA-IMT+RCCA-IMT/2); FDR, first-degree relative.

Table 4 Correlation of CIMT with confounding variables

Variable	CIMT	
	r	p-*value*
BMI	0.479	0.006
LDL cholesterol	0.342	0.056
HDL cholesterol	−0.144	0.433
Waist circumference	0.539	0.001
Triglyceride	0.258	0.155

Abbreviations: CIMT, carotid intima-media thickness; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

The median thickness of the right intima-media of the carotid artery (RCCA-IMT) in the FDR subject was 0.42 mm, ranging from 0.33 to 0.59 mm. The median RCCA-IMT in non-FDR subjects was 0.37 mm, ranging from 0.32 to 0.44 mm. There were no statistically significant differences between the mean RCCA-IMT in FDR subjects compared to non-FDR subjects (p=0.015) (Table 3).

The mean thickness of the carotid artery intima-media (CCA-IMT) on FDR subjects was 0.44 (SD 0.06) mm. The mean CCA-IMT in non-FDR subjects was 0.38 (SD 0.05) mm. A statistically significant difference was found between the mean CCA-IMT in FDR subjects compared to non-FDR subjects (p=0.005) (Table 3).

Several factors that were estimated as confounding variables were BMI, waist circumference, LDL cholesterol, HDL cholesterol, and triglyceride (Table 4).

Variables with p<0.25 in bivariate analysis were included in the multivariate analysis. A statistically significant difference was found in the mean CIMT between FDR and non-FDR (p<0.01), after the addition of confounding variables, which were waist circumference, BMI, LDL cholesterol, and triglycerides.

Discussion

This study was performed to a specific population, which was a healthy population with a family history of T2DM. The FDR T2DM population had a greater cardiovascular risk factor when compared to the population without a family history of T2DM; therefore, this study might provide a beneficial data to emphasize the early prevention of the onset of T2DM and other cardiovascular complications.

The subjects involved were native Indonesians of indigenous Indonesian descent to minimize the influence of ethnical variations on the assessed parameters, which were CIMT. Ideally, subject screening should consider tribal factors, but considering the heterogenous population that resides in Jakarta, it was difficult to find subjects from one ethical group.

Most of the study participants were female, with as many as 10 women (62.5%) out of a total of 16 subjects in each group. This might be due to the reason that many male subjects were excluded due to smoking. In addition, most of the male subjects were working; therefore, it was challenging to participate in the study. This difference in gender proportion also occurred in similar studies in Brazil and Italy where female subjects had higher representation than male subjects.[10,11]

The mean age of this research subject was 28.81 (SD ± 6.18) years. This study was performed to people <40 years with the consideration that age is one of the factors that influence the thickness of the intima-media tunica of the carotid artery; therefore, it is expected that in young adults the age factor is not major. In addition, with the recognition of atherosclerotic lesions at a younger age is expected to be a consideration for early screening; therefore, an intervention can be initiated early. The mean age in this study was similar to that in the Pannacciulli study, in 2003, which include FDR and non-FDR group whose age around 30 and 29.4 years, respectively,.[10] Similar studies in India in 2006 and 2017 also showed similar age range in FDR and non-FDR group (age 27–30.84 years and 28.21–28.30 years, respectively).[12,13]

The average BMI of FDR subjects in this study was 23.24 (SD ± 3.22) kg/m^2. Non-FDR subjects had a mean BMI of 21.90 (SD ± 3.46) kg/m^2. Our finding regarding BMI was similar to the study published by Kumar, which reported that the mean BMI in FDR subjects was 23.28 kg/m^2 with a SD of 3.48 kg/m^2.[13] Meanwhile, similar studies conducted outside of Asia reported that the average BMI of FDR subjects was greater than that in this study, which was around 25.04 (SD ± 3.24) kg/m^2 and 32.1 (SD ± 0.63) kg/m^2.[10,11]

BMI and body fat percentage vary between ethnic groups globally. With the same age, gender, and body fat percentage, it showed that the African American had a lower BMI of 1.3 kg/m^2 and the Polynesian ethnic group had a lower BMI of 4.5 kg/m^2 when compared to Caucasians. Chinese ethnicity, Ethiopian, Indonesian, and Thai had a BMI of 1.9 kg/m^2, 4.6 kg/m^2, 3.2 kg/m^2, and 2.9 kg/m^2 lower than Caucasians, respectively. Differences in body fat percentage and BMI are also found between Caucasians in Europe and America, where Europeans have a body fat percentage of 3.8% greater than that of Americans on the same BMI. This difference might be caused by energy intakes, body compositions, and energy expenditure that might differ between these ethnicities.[14] Inter-ethnicity in Asia also has variations in the relationship between body fat percentage and BMI. Within the same BMI, age, and gender, India had a greater body fat percentage than that of Malay.[15]

Waist circumference was used as another anthropometric parameter to assess the risk of insulin resistance. In our study, FDR subjects had bigger waist circumference than that of non-FDR {78.35 (SD 9.86) cm vs 77.69 (SD 10.45) cm, respectively}. Besides BMI, several studies outside Asia also showed agreater waist circumference, both in FDR and in non-FDR subjects, than that in Asia. A study in Italy reported waist circumferences among FDR and non-FDR of T2DM were 100.3 (SD 1.54) cm and 97.7 (SD 1.37) cm, respectively.[10]

Measurements of the intima-media thickness of the CCA is useful due to the high reproducibility because the atherosclerotic process appears as diffuse thickening due to proliferation of smooth muscle cells and extracellular matrix deposits in this segment of the vessel, compared to the internal carotid artery where the manifestation of the atherosclerotic process appears as plaque (focal atherosclerosis).[11]

In our study, the mean of CIMT in the FDR group was significantly higher than that in the non-FDR group {0.44 (SD 0.06) mm vs 0.38 (SD 0.05) mm, p=0.005}. The results of this study were in line with previous studies that examined the tunica-intima thickness of the carotid artery media on FDR subjects.[10–13]

There are a limited number of studies that measure the thickness of the tunica intima-media of the carotid artery, especially in high-risk populations in Indonesia. Previous studies in Indonesia conducted by Purnamasari in 2011 reported a thick median of the tunica intima-media of the carotid artery in 62 FDR subjects was 0.56 mm with arange of 0.42–1.50 mm.[9] When compared with the study, the carotid tunica intima-media thickness in this study population was smaller. This difference may be due to the extensive age range of the subjects in this study (19–40 years) and because the exclusion criteria were more strict in this study. Third, this difference might be influenced by the different techniques used by operators. However, the thickness of the average tunica intima-media of the carotid artery in this study was relatively similar when compared to the study by Maarifat in Jakarta, involving 96 healthy subjects with or without cardiovascular risk. This study reported that the mean thickness of the right and left tunica intima-media of the carotid artery in the population of 20–30 years is 0.4 mm (0.04 cm).[16]

Factors that correlate to thickness of the carotid artery intima-media in this study included waist circumference and BMI. BMI and waist circumference have a relationship with insulin resistance. In obese subjects who were still sensitive to insulin, insulin plays a role in inhibiting upregulation of inflammatory markers circulating in the blood. Conversely, insulin-resistant obese subjects have high levels of inflammatory markers due to impaired insulin inhibitory effects.[17] Acute-phase protein increases as a result of insulin resistance, which will result in endothelial dysfunction and atherogenesis.[18]

In our study, after multivariate analysis was carried out to eliminate the effects of confounding variables such as BMI, waist circumference, LDL cholesterol, and triglycerides, there was still a difference in thickness of the carotid artery intima-media which was statistically significant between FDR subjects and non-FDR subjects. This indicates that DM genetic factors still have a major role in the occurrence of subclinical atherosclerosis.

A point mutation from mitochondrial DNA (mtDNA) at nucleotide position 3243 A to G is a pathogenetic factor for diabetes.[19] Damage to mtDNA by reactive oxygen species (ROS) will result in ATP production dysfunction and cause an increase in ROS which will result in subsequent mtDNA mutations.[20] Mitochondrial DNA polymorphism is also associated with the development of atherosclerosis, which is thought to have an effect on the process of oxidative phosphorylation and production of ROS in mitochondria.[21] In diabetic patients, both hyperglycemia and hyperinsulinemia can increase oxidative stress so that diabetes itself will increase mtDNA damage due to ROS.[22] In vivo and invitro studies explain that oxidative stress due to hyperglycemia can result in atherogenesis.[23]

Several studies report many polymorphisms associated with atherosclerosis, one of which is the insertion/deletion polymorphism of angiotensin-converting enzyme (ACE I/D).[24] A previous study showed that there were no differences in atherosclerotic or carotid artery intima-media thickness in the three ACE gene genotypes; in other words, there was no relationship between ACE gene I/D polymorphism and early atherosclerosis in the FDR population of type 2 DM subjects.[9] The study showed that in the atherosclerosis process, the influence of risk factors other than renin angiotensin aldosterone (RAA) system may be more dominant; therefore more studies are still needed to assess the genetic polymorphism in FDR T2DM subjects on the incidence of atherosclerosis. In addition to the ACE I/D polymorphism, the researcher also found p22phox polymorphism, the subunit of NAD(P)H oxidase associated with atherosclerosis.[25] However, as far as the knowledge of the researcher, the study examining this polymorphism in the FDR T2DM population is still very limited.

To the best of our knowledge, this is the first study in Indonesia to examine subclinical atherosclerosis in a young adult FDR of T2DM patients. Although there were several studies outside of Indonesia that showed the differences in the thickness of the carotid artery intima-media tunica in FDR and non-FDR subjects, this study still has an important role to detect subclinical atherosclerosis in FDR populations in Indonesia, especially young adults who are still normoglycemic and normotensive.

There were several limitations of the study. First, our sample size is small which limits generalization of our results. Second, for the recruitment of non-FDR subjects, to exclude family history of diabetes, we heavily relied on the interview. This study did not directly assess the level of HbA1c of both parents of the subjects, due to the limited funds and difficulties in presenting both parents of the subjects therefore signify a recall bias. In addition, B-mode ultrasound to measure CIMT was assessed by using a manual measurement. However, possible errors can be minimized by the same operator to measure CIMT blindly between FDR and non-FDR subjects. In this study, we did not examine inflammatory markers in order to see the role of inflammation in the pathogenesis of subclinical atherosclerosis.

Conclusion

The tunica intima-media of the carotid artery in the young adult FDR of T2DM who are normoglycemic and normotensive is thicker than that in non-FDR T2DM.

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Disclosure

The authors report no conflicts of interest in this work.

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