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Major Determinants of the Carotid Intima-Media Thickness in Type 2 Diabetic Patients: Age and Body Mass Index

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Abstract. The present study has been designed to quantify and compare right and left carotid intima-media thicknesses (IMT) in type 2 diabetics and healthy controls. It was also intended to investigate the effects of various risk factors on the carotid IMT in these subjects. A total of 122 subjects; 70 patients with type 2 diabetes and 52 non-diabetic subjects as controls, were recruited for the study. Right and left common carotid artery stiffness indices were assessed with ultrasonography in both groups. Age, body mass index (BMI), duration of diabetes, cigarette smoking, lipid profile including lipoprotein a, Chlamydia pneumonia seropositivity, glycemic indices, fasting insulin levels, serum fibrinogen levels and presence of hypertension, coronary artery disease, degenerative complications of diabetes mellitus were all assessed in order to define their role as determinants of carotid artery IMT. The difference between the groups regarding mean carotid IMT was statistically significant for the left carotid arteries ($p=0.028$) and borderline significance was found for the right carotid arteries ($p=0.055$). Age has a very strong association with carotid IMT in diabetic patients ($p<0.0001$) with univariate analysis. According to the results of multivariate analysis, age and BMI were found to be the most important independent determinants of carotid IMT for both sides. When age was excluded from the model, BMI and coronary artery disease were found to have strong association with IMT on the right ($p=0.0036$ and 0.0249) and BMI was the only significant determinant for the left side ($p=0.0025$). This study shows that carotid IMT is greater in diabetic subjects compared with healthy controls. For the diabetic subjects, age, BMI and presence of coronary heart disease have a strong influence on the atherosclerotic process of the carotid arteries.

Key words: Carotid intima-media thickness, Body mass index, Diabetes mellitus

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DIABETES mellitus is an established independent risk factor for cardiovascular mortality [1]. Moreover, multiple risk factors for macrovascular disease are frequently found in individuals with diabetes, especially patients with type 2 diabetes. The reasons and mechanisms for the macrovascular disease in subjects with type 2 diabetes are not sufficiently known [2]. Several lines of evidence suggest that a common mechanism for the impairment of endothelial function may be effective. In addition to disturbed endothelium-dependent relaxation, activation of coagulation pathways, platelet hypersensitivity and depressed fibrinolysis cause a prothrombotic state, as do the changes in vascular architecture and matrix composition [3].

Investigation of the function of the arterial wall in living subjects has been difficult. Modern ultrasound technology has permitted noninvasive and repeatable assessment of arterial stiffness [1]. Intima-media thickness (IMT) measured using this noninvasive technique has been validated against histological specimens of the carotid artery [4]. Ultrasonographic measurements of the combined thickness of the carotid intima and media are useful to examine early stages of atherosclerosis [5]. B-mode ultrasonography is a valid and reliable tool for the

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measurement of subclinical atherosclerosis in population studies.

In most previous studies, analysis of carotid IMT was performed using the mean or maximal value of right and left IMT. It was documented before by Crawford *et al.* (1969) that left common carotid artery was found to be more susceptible to atherosclerosis with respect to the right [6]. In this study, we tried to determine any difference in susceptibility to atherosclerosis in diabetic patients between right and left carotid arteries.

The aim of our study was to quantify right and left IMT separately in type 2 diabetic patients and to investigate the relationship between the various risk factors for atherosclerotic disease in these subjects.

Subjects and Methods

A population of 70 consecutive patients with type 2 diabetes mellitus who were being followed in the outpatient diabetes mellitus clinic in Hacettepe University Faculty of Medicine, Department of Endocrinology were enrolled for the study. Subjects with type 1 diabetes mellitus as well as subjects with secondary diabetes, thyroid diseases, alcoholism, renal insufficiency of nondiabetes etiology, chronic liver disease, overt carcinoma and those treated with insulin were considered not to be eligible for the study. The healthy control group for the study population constituted the volunteers from the hospital staff with no known disease (n=52). The following variables were specifically recorded: General demographic details, years of diabetes duration, chronic complications of diabetes, and smoking. All the participants were given informed consent to participate in the study in accordance with the Helsinki declaration.

Diabetic subjects were treated with diet alone or with oral antidiabetic agents. We performed a cross-sectional study of the relation of carotid artery intima-media thickness with age, body mass index (BMI), diabetes duration, history of cigarette smoking, presence of hypertension, coronary artery disease (CAD), lipid profile including lipoprotein a, Chlamydia pneumonia (C. Pneumonia) seropositivity, glycemic indices, fasting and postprandial insulin levels, serum fibrinogen levels and with degenerative complications of diabetes mellitus.

The diagnosis of diabetes was made in the clinical setting and according to the diagnostic criteria of the World Health Organisation Expert Committee on Diabetes Mellitus [7]. BMI was calculated as weight (in kilograms) divided by height (in square meters). Blood pressure was calculated as the mean of the blood pressure in the right and left arms, measured while the patient was in sitting position after a 5-minute rest. Hypertension was defined as systolic blood pressure of ≥ 140 and diastolic blood pressure of ≥ 90 mmHg and/or history of antihypertensive drug treatment. Information regarding smoking history was obtained by interviews.

Diagnosis of CAD was based on clinical evidence of myocardial infarction, angina pectoris, electrocardiography and coronary angiography findings. Peripheral vascular disease was clinically defined by the presence of intermittent claudication, absent or weakened peripheral pulses or both. Previously defined evidence of stroke was considered as macrovascular complication of diabetes mellitus.

Retinopathy was documented by standard fundus examination in all the diabetic patients by the same experienced ophthalmologist, and diagnosed on the observation of microaneurysms, venous dilation, cotton-wool spots, neovascularization or hemorrhages.

Clinical neuropathy was defined by an abnormal neurological examination, consistent with the presence of peripheral sensorimotor neuropathy (altered vibration threshold and altered tendon reflexes), plus abnormal nerve conduction in at least two peripheral nerves with temperature controlled and the patient lying down.

Microalbuminuria was defined as urinary albumin excretion between 30–300 mg/day or 20–200 μg/min. Advanced nephropathy was defined by the presence of urinary albumin excretion more than 300 mg/day and a creatinine clearance of less than 70 ml/minute.

Blood was collected after an overnight fasting of at least 10 hours. Triglycerides and cholesterol were measured by commercial colorimetric assay (GPO-PAP and CHOP-PAP kit, respectively, Boehringer-Mannheim, Mannheim, Germany). HDL-cholesterol in plasma was determined by a precipitation-based method with phosphotungustic acid [8]. LDL-cholesterol was calculated by Friedewald formula [9]. Plasma glucose determinations were obtained from venous sampling after 12 hours of overnight

fasting, by glucose-oxidase method (Boehringer-Mannheim, Mannheim, Germany). Plasma fibrinogen determination was made by the clotting method of Clauss (STA compact analyser) [10]. Lipoprotein a concentrations were measured using ELISA method (Boehringer-Mannheim kit), the detection limit which was 0.5 mg/dl. The intra- and interassay coefficients of variation were 5–12% and 2–6%, respectively.

C. Pneumonia immunoglobulin G (C. Pneumonia Ig G) was detected by using indirect immunofluorescence technique (Euroimmun, Frankfurt, Germany). Ig G titers with dilutions $\geq 1/100$ were considered as seropositive.

Twenty-four hour urinary albumin excretion was measured by radioimmunoassay method (Diagnostic Product Corp., Los Angeles, CA, USA). The intra-assay and interasssay coefficients of variation were 3.5% and 5%, respectively. Serum insulin determination was made by radioimmunoassay (Diagnostic Systems Laboratories Inc., DSA-1600 Insulin RIA, Webster, TX, USA). The minimal detection limit of this assay with 95% confidence interval was 1.3 μIU/ml. The intraassay and interasssay coefficients of variation were 4.5% and 9.9%, respectively.

Hemoglobin A_{1C} (Hb A_{1C}) determinations were made from the same venous sample by colorimetric method (Stanbio, San Antonio, TX). Normal limits were 6–8%. Postprandial plasma glucose and insulin determinations were made from postprandial second hour venous plasma samples.

Ultrasonography

Subjects were examined in supine position with the head turned 45°C away from the side being scanned. The ultrasound system used was a Toshiba Sonolayer SSA 270 A (Toshiba Medical Systems, Tokyo, Japan), equipped with a 7.5 MHz linear array transducer. The left and right common carotid arteries were examined in anterolateral, posterolateral, and mediolateral directions, respectively. Measurement of the intima media thickness was performed from the near wall of the common carotid artery at its segment 1 cm proximal to the bifurcation at each direction, so a total of six measurements (three on each side) were obtained for one patient. The IMT as defined by Pignoli *et al.* was measured as the distance from inner echogenic line which represents the lumen-intimal interface to the outer echogenic line which represents the collagen containing layer of the tunica adventitia or the media-adventitia interface [11, 12]. All scanning was conducted in a blind fashion by one radiologist who was experienced in carotid Doppler imaging. The variability of the arterial wall thickness measurements was examined by means of repeated scans and readings on volunteer participants with observed reliability coefficients of 0.76 [13].

Statistical analysis

Differences between patient groups were assessed for statistical significance using the Student's t test [14] and the Chi-square [15]. Univariate analysis within the DM-group regarding right intima-media thickness (IMTR) and left intima-media thickness (IMTL) was performed using the Student's t test and the Spearman correlation where appropriate. Pathological threshold levels for biochemical parameters were used as cut-offs to define groups within with DM-group. Multivariate analysis within the DM-group regarding IMTR and IMTL was performed using the multiple linear regression model with forward stepwise selection [16]. All variables with a significance level of ≤ 0.25 were entered into the model. All results were expressed as means$\pm$SD unless otherwise indicated. Statistical analysis was performed using the SPSS for Windows software package, Release 6.0. Statistical significance was considered when a P value was 0.05 or below.

Results

Table 1 gives the clinical and biochemical characteristics of the type 2 diabetic patients and the healthy control group. In the diabetic and healthy group the male/female ratios were 16/54 and 8/44, respectively. The mean age of the diabetic group was 58$\pm$11.2 (range 18–80 years) and of the controls' was 51.65$\pm$12.69 (range 30–74 years). The mean diabetes duration was 8.3$\pm$4.82 years (range 4.5–11.2 years).

Mean IMT of the right carotid artery was 0.72$\pm$0.19 mm in the diabetic group and 0.54$\pm$0.14 mm

Table 1. Clinical and biochemical characteristics of the diabetic patients and the control group

	Type 2 diabetics	Non-diabetics
n	70	52
Age (years)	58.17±11.2	51.65±12.29
Sex (M/F)	16/54	8/44
Duration of diabetes (years)	8.3±4.82	—
HbA_{1C} (%)	7.09±1.45	—
Basal insulin	14.54±10.43	—
Postprandial insulin	33.14±21.79	—
Total cholesterol	226.64±46.70	219.35±52.07
Triglyceride	166.7±66.30	152.90±71.68
HDL cholesterol	45.67±14.03	49.33±13.45
VLDL	33.30±13.1	31.48±14.77
LDL cholesterol	160.13±129.78	138.94±44.30
LDL cholesterol/HDL cholesterol	3.5	2.8
BMI (kg/m^2)	28.59±5.26	29.17±4.18
Lipoprotein A	361.26±242.31	321.58±216.536
Fibrinogen	373.6±87.93	323.52±99.88
Hypertension	32	4
Cigarette smoking	12	9
History of macrovascular disease	15	3
Diabetic retinopathy	10	—
Diabetic autonomic neuropathy	6	—
Diabetic peripheric neuropathy	6	—
Diabetic nephropathy	—	—
Microalbuminuria	10	—
Chlamydia Pneumonia Ig G seropositivity	34	31

for the non-diabetic subjects. For the left carotid artery mean IMT was 0.74±0.21 mm in the diabetic patients and 0.56±0.16 mm in the non-diabetic subjects. Difference between the right and left IMT was statistically significant in the nondiabetic group ($p=0.022$) while the difference was not statistically significant in the diabetic group. The difference between the groups regarding mean IMT was statistically significant for the left carotid arteries ($p=0.028$) and borderline significance was found for the right carotid arteries ($p=0.055$).

There was no difference between the diabetic and non-diabetic groups regarding age, sex, BMI, fibrinogen, cigarette smoking and C. Pneumonia Ig G seropositivity. Although the atherogenic lipoprotein levels of the diabetic group were higher when compared to the controls, statistically there was no difference between the diabetic and non-diabetic groups regarding HDL-cholesterol (45.67±14.03 and 49.33±13.45, respectively), LDL-cholesterol (160.13 ±129.78 and 138.94±44.30, respectively), VLDL-cholesterol (33.30±13.1 and 31.48±14.77, respectively), total cholesterol (226.64±46.70 and 219.35 ±52.07, respectively), triglycerides (166.70±66.3 and 152.90±71.68, respectively) and lipoprotein a (361.26±242.31 and 321.58±216.54, respectively) levels.

CAD and hypertension were encountered with significantly higher proportions in the diabetic group than in the non-diabetic group ($p=0.016$ and $p<0.00001$, respectively).

Univariate analysis within the DM-group regarding IMTR and IMTL (Table 2) revealed significances with age ($p<0.0001$, Pearson=0.52); CAD ($p=0.04$) for IMTR and age ($p<0.0001$, Pearson=0.51); basal insulin levels ($p=0.05$, Pearson=−0.23); retinopathy ($p=0.03$) for IMTL. Age, BMI, basal insulin, fibrinogen, duration of diabetes, total cholesterol, triglycerides, presence of hypertension, coronary artery disease, retinopathy and microalbuminuria were taken into the regression model as determinants of mean IMTR. Age, BMI, basal insulin, duration

Table 2. Univariate analysis results of the diabetic group

DIABETIC GROUP UNIVARIATE ANALYSIS RESULTS				
PARAMETER	IMTR		IMTL	
	p	Pearson	p	Pearson
Basal Insulin	0.25	−0.13	0.05	−0.23
BMI	0.11	0.19	0.09	0.21
Fibrinogen	0.07	0.22	0.45	0.09
HbA_{1C}	0.65	−0.05	0.52	−0.08
HDL	0.56	−0.07	0.33	−0.12
Cholesterol	0.17	0.17	0.34	0.12
LDL	0.64	0.06	0.44	0.09
TG	0.23	0.14	0.31	0.12
VLDL	0.32	0.12	0.44	0.09
Lipo. A	0.88	0.02	0.36	0.11
PP Insulin	0.69	−0.05	0.54	−0.07
DM duration	0.12	0.19	0.22	0.15
Age	<0.0001	0.52	<0.0001	0.51
Sex	0.81	—	0.52	—
HT	0.20	—	0.18	—
CAD	0.04	—	0.20	—
Chl IgG	0.31	—	0.92	—
Smoking	0.63	—	0.48	—
Microalbuminuri	0.25	—	0.50	—
Neuropathy	0.30	—	0.43	—
Retinopathy	0.17	—	0.03	—

of diabetes, presence of hypertension, CAD and retinopathy were taken into the regression model as determinants of mean IMTL. Age has a very strong association with carotid IMT in diabetic patients ($p<0.0001$, Table 2) as duration of diabetes and other diabetic complications increase with age. Advanced age seems to be the most important determinant of increased carotid intima-media thickness in the diabetic patients when taken into the regression model. Therefore, in order to get rid of the masking

Table 3. Results of the multivariate analysis with logistic regression model

	IMTR				IMTL			
Parameter	B	SE of B	T	p	B	SE of B	T	p
Age	**0.15**	**0.04**	**3.78**	**0.003**	**0.14**	**0.05**	**3.01**	**0.0037**
Basal Ins.	—	—	−0.29	0.77	—	—	−1.53	0.13
BMI	**0.15**	**0.05**	**3.14**	**0.0026**	**0.18**	**0.05**	**3.32**	**0.0015**
Fibrinogen	—	—	1.08	0.28	—	—	—	—
HT	—	—	−0.22	0.83	—	—	0.03	0.97
CAD	—	—	0.92	0.36	—	—	0.28	0.78
Cholesterol	—	—	0.81	0.42	—	—	—	—
Microalb.	—	—	0.98	0.33	—	—	—	—
Duration	—	—	1.19	0.24	—	—	0.89	0.38
TG	—	—	1.28	0.21	—	—	—	—
Retinopath.	—	—	0.96	0.34	—	—	1.88	0.06
R^2	0.26				0.23			

Table 4. Results of the multivariate analysis with age being excluded from the regression model

	IMTR				IMTL			
Parameter	B	SE of B	T	p	B	SE of B	T	p
Basal	—	—	−0.94	0.35	—	—	−1.86	0.07
BMI	**0.15**	**0.05**	**3.02**	**0.0036**	**0.18**	**0.06**	**3.14**	**0.0025**
Fibrinogen	—	—	0.97	0.34	—	—	—	—
HT	—	—	0.83	0.41	—	—	0.87	0.39
CAD	**0.12**	**0.05**	**2.29**	**0.0249**	—	—	1.46	0.15
Cholesterol	—	—	1.09	0.28	—	—	—	—
Microalb.	—	—	1.02	0.31	—	—	—	—
Duration	—	—	1.58	0.12	—	—	1.53	0.13
TG	—	—	0.59	0.56	—	—	—	—
Retinopath.	—	—	1.14	0.26	—	—	1.95	0.06
R^2	0.17				0.13			

effect of age on other determinants of carotid IMT, it was kept out of the final regression model.

According to the regression model when age was included, age of the patient and BMI were found to be the most influential independent determinants of increased IMT of both right and left carotid arteries (Table 3). When the age parameter was excluded from the model, increased IMTR has significant association with BMI and presence of CAD independently (Table 4). With the same exclusion, increased IMTL was found to have significant association with BMI, whereas basal insulin levels and retinopathy reached borderline significance after the suppressing effect of age had been removed (Table 4).

For nondiabetic controls age was found to have significant correlation with IMTR and IMTL ($p<0.001$) while BMI did not reveal any correlation with IMT of either side. Presence of CAD, on the other hand, revealed borderline significance with respect to IMT ($p=0.055$ for IMTL and $p=0.052$ for IMTR).

Discussion

Both type 1 and type 2 diabetes are established independent risk factors for cardiovascular disease [3]. Moreover multiple risk factors for macrovascular disease are frequently found in individuals with type 2 diabetes [1]. Insulin resistance and high insulin concentrations that are associated with high triglyceride and low HDL cholesterol concentrations and increased tendency to hypertension may play a role in atherosclerosis [2].

Our study confirms earlier reports showing that mean carotid IMT is greater in patients with type 2 diabetes mellitus than in non-diabetic subjects. There was no significant difference between the diabetic and non-diabetic groups regarding age, sex, BMI, fibrinogen, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, total cholesterol, triglycerides, lipoprotein a, cigarette smoking and Chlamydia immunoglobulin G seropositivity. CAD and hypertension were encountered with significantly higher proportions in the diabetic group than in the non-diabetic group.

Recent studies have suggested that chronic infections with C. Pneumonia may be associated with cardiovascular disease. Despite the fact that infectious diseases are more common in diabetic than in non-diabetic patients, available data are not sufficient as to the prevalence of chlamydial infections and their role in the development of cardiovascular disease in diabetic subjects [17]. C. Pneumonia is difficult to culture and confirmation of infection often requires identifying systemic antibody responses [17–19]. In our study we found no statistically significant difference between diabetic and nondiabetic groups with regard to C. Pneumonia seropositivity. In the prospective study of Melnick *et al.* [20] past infection by C. Pneumonia was found to be significantly correlated with the asymptomatic atherosclerosis and they concluded a causal relationship with the infection and atherosclerosis.

Although more direct investigation of the bacteria in the atherosclerotic plaques is needed to conclude the impact of the organism on the wall of the artery, according to our results diabetes mellitus does not seem to have a causal relationship with the prevalence of C. Pneumonia infection.

Age has a very strong association with carotid IMT in diabetic patients and in nondiabetic controls. The two groups, diabetic and non-diabetic subjects were matched with respect to age in our study and the oldest patient in the study group was an 80 year old diabetic man. Age, directly influencing the duration of diabetes and metabolic consequences of diabetes, modifies atherosclerotic process. As age has a very strong effect on atherosclerosis it was kept out of the final regression model in order to get rid of the masking effect on other risk factors of atherosclerosis.

Having CAD and retinopathy was significantly associated with increased IMT in diabetic patients. It was shown in previous prospective studies that IMT is a significant predictor of new coronary heart disease events and interventions that slow IMT progression result in concomitant reductions in coronary heart disease events [4]. It is not surprising to find greater IMT in diabetic patients with documented CAD. It is known that microalbuminuria is associated with excess cardiovascular mortality in both diabetic and non-diabetic subjects [21, 22]. In the present study, presence of microalbuminuria was not found to be associated independently with IMT. This may be due to the limited number of diabetic subjects with microalbuminuria. Retinopathy is a microvascular complication of diabetes mellitus found to be significantly associated with carotid IMT in univariate analysis. The number of diabetic patients with retinopathy is also small and the duration of diabetes in these subjects is relatively long (data not given). So we cannot conclude that there is any direct correlation between retinopathy and carotid artery IMT based on the present data although the statistical analysis reveals a correlation between presence of retinopathy and increased carotid artery stiffness.

According to the regression analyses, age, BMI and CAD were found to have significant association with increased IMT. In our study age and BMI were the strongest determinants of IMT. It was shown in previous studies that obesity, especially the upper body obesity is associated with CAD, hyperinsulinemia and hypertriglyceridemia [23]. Although body fat distribution of the participants of the study was not clarified with regard to waist-hip circumference ratio and subscapular skin-fold thickness, our data reveal strong association of BMI with increased carotid IMT. Therefore, routine determination of carotid IMT in obese type 2 diabetic patients may be a beneficial noninvasive tool to determine existing subclinical atherosclerosis.

It is interesting that our results showed greater difference in mean carotid IMT in the left carotid artery than in the right where the significance was borderline with regard to the non-diabetic subjects ($p=0.055$). In the study of Niskanen *et al.* [5], the carotid IMT was found significantly greater at the bifurcation of carotid relative to the common carotid. It was stressed that the hemodynamic factors related to the oscillatory and turbulent blood flow at the bifurcation, albeit with relatively low shear stress, favors the formation of atherosclerotic process at the bifurcation site. Particular susceptibility of the carotid bifurcation is most likely due to the hemodynamic conditions at this location. Plaque formation is accelerated within areas of low flow velocity and inhibited in areas with high flow velocity and elevated shear stress [24]. In the study of Crawford *et al.* left common carotid artery was found to be more susceptible to atherosclerosis with respect to the right [6]. In this study, we obtained similar findings and observed statistically significant difference between right and left IMT in the control group.

Although there are few references [5, 6, 24, 25] to provide a satisfactory explanation and scientific basis for unilateral carotid intima-media thickness, our current study provides some important preliminary clues regarding macrovascular complications in diabetic patients. It can be suggested that in the diabetic patients left and right common carotid arteries are affected differently by various risk factors. Although no firm scientific and objective explanation can be drawn yet to explain the different susceptibility profile of carotid arteries, differences in hemodynamic stresses and susceptibility due to the anatomical location and distance from the heart, which may result in minute differences in exposure potential to atherogenic factors and possible undefined molecular differences in endothelial function,

may play a role. More detailed studies are needed to define these yet undefined and complicated interacting mechanisms underlying the differences in right and left carotid artery susceptibility profile.

In addition to the mentioned significant differences, some of the established risk factors for atherosclerotic disease, e.g. fibrinogen, total and LDL-cholesterol, were found to differ at borderline significance. The study population (n = 122) was not small, but increasing its size may increase the significance of borderline variables, also providing stronger evidence for unilateral carotid intima-media thickness.

In conclusion, we demonstrated that carotid artery IMT is greater in diabetic subjects compared with sex and age matched healthy subjects. For the diabetic subjects, age, BMI and presence of CAD have strong influence on the IMT. It should also be stressed that left and right carotid artery is likely to be affected by the various factors differently in patients with diabetes. The present study, being a preliminary one regarding differences in right and left carotid IMT both in diabetic patients and nondiabetics, provides a basis for future clinical investigations. Still further studies with larger groups of patients are needed to ascertain the risk factors of the atherosclerosis in diabetic subjects in order to define clinical approaches and reduce the increased risk of cardiovascular burden.

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